ADVANCES IN TOMATO AND TOMATO COMPOUNDS RESEARCH AND TECHNOLOGY

EDITED BY: José Pinela, Lillian Barros and
Spyridon Alexandros Petropoulos

PUBLISHED IN: Frontiers in Nutrition

frontiers Research Topics

Frontiers eBook Copyright Statement

The copyright in the text of individual articles in this eBook is the property of their respective authors or their respective institutions or funders. The copyright in graphics and images within each article may be subject to copyright of other parties. In both cases this is subject to a license granted to Frontiers.

The compilation of articles constituting this eBook is the property of Frontiers.

Each article within this eBook, and the eBook itself, are published under the most recent version of the Creative Commons CC-BY licence. The version current at the date of publication of this eBook is CC-BY 4.0. If the CC-BY licence is updated, the licence granted by Frontiers is automatically updated to the new version.

When exercising any right under the CC-BY licence, Frontiers must be attributed as the original publisher of the article or eBook, as applicable.

Authors have the responsibility of ensuring that any graphics or other materials which are the property of others may be included in the CC-BY licence, but this should be checked before relying on the CC-BY licence to reproduce those materials. Any copyright notices relating to those materials must be complied with.

Copyright and source acknowledgement notices may not be removed and must be displayed in any copy, derivative work or partial copy which includes the elements in question.

All copyright, and all rights therein, are protected by national and international copyright laws. The above represents a summary only. For further information please read Frontiers' Conditions for Website Use and Copyright Statement, and the applicable CC-BY licence.

ISSN 1664-8714
ISBN 978-2-83250-217-4
DOI 10.3389/978-2-83250-217-4

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

ADVANCES IN TOMATO AND TOMATO COMPOUNDS RESEARCH AND TECHNOLOGY

Topic Editors:
José Pinela, Instituto Politécnico de Bragança, Portugal
Lillian Barros, Polytechnic Institute of Bragança (IPB), Portugal
Spyridon Alexandros Petropoulos, University of Thessaly, Greece

Citation: Pinela, J., Barros, L., Petropoulos, S. A., eds. (2022). Advances in Tomato and Tomato Compounds Research and Technology. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83250-217-4

Table of Contents

frontiers | Frontiers in Nutrition

TYPE Editorial
PUBLISHED 31 August 2022
DOI 10.3389/fnut.2022.1018498

Check for updates

OPEN ACCESS

EDITED AND REVIEWED BY
Elena Ibañez,
Institute of Food Science Research (CSIC), Spain

*CORRESPONDENCE
José Pinela
jpinela@ipb.pt

SPECIALTY SECTION
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

RECEIVED 13 August 2022
ACCEPTED 19 August 2022
PUBLISHED 31 August 2022

CITATION
Pinela J, Petropoulos SA and Barros L (2022) Editorial: Advances in tomato and tomato compounds research and technology. *Front. Nutr.* 9:1018498. doi: 10.3389/fnut.2022.1018498

COPYRIGHT
© 2022 Pinela, Petropoulos and Barros. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Editorial: Advances in tomato and tomato compounds research and technology

José Pinela[1,2]*, Spyridon A. Petropoulos[3] and Lillian Barros[1,2]

[1]Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal, [2]Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Bragança, Portugal, [3]Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Volos, Greece

KEYWORDS

***Solanum lycopersicum* L., nutritional quality, flavor quality, processing, lycopene, phenolic compounds, bioaccessibility, health benefits**

Editorial on the Research Topic
Advances in tomato and tomato compounds research and technology

Tomato is the fruit of *Solanum lycopersicum* L., a Solanaceae crop of worldwide economic importance. Today, there are a large number of tomato cultivars and local varieties with different morphological and sensory characteristics, as well as a wide range of tomato-based foods. These are great dietary sources of micronutrients and bioactive compounds, such as lycopene, vitamins, minerals, and phenolic compounds, which have been linked to many health-promoting effects (1). Several pre- and postharvest efforts have been made to improve the quality of tomato fruit and derived food products, as both tomato production and processing are being carried out under more sustainable and innovative practices. This Research Topic features 12 papers covering relevant subjects, including the production and processing of tomatoes and tomato-based foods and ingredients, as well as the bioaccessibility and health-promoting effects of tomato bioactive compounds.

Traditional varieties represent an important component of agricultural biodiversity and play a vital role in the sustainability and security of the agri-food system (2). In this sense, Raigón et al. characterized morphological, nutritional, and chemical characteristics of two Malacara tomato cultivars (with red and yellow fruits) grown under organic farming conditions. This type of cultivars ("Cuelga") originates from Sierra de Cádiz, Spain, is cultivated and harvested during the summer and tomato trusses are hung from beams in the farmhouses for consumption during the winter; hence the name "Cuelga" which stands for hanging. The main differences among these small, pallid tomatoes were mainly related to morphological parameters, but also to fiber, minerals (Fe, Mg, Ca), and lycopene contents. 2-Phenylethanol was detected in both Malacara cultivars, and the low concentration of aldehydes in this varietal type could be related to its long shelf-life.

The effect of different production systems on tomato quality was also addressed in this Research Topic. Ilahy et al. investigated the impact of pre-harvest treatments with saline water and spent engine oil on nutritional quality of ripe tomatoes. Moderate salinity stress promoted an increase in soluble solids, lycopene, total phenolics, and radical scavenging activity compared with the control treatment (untreated plants). In turn, the flavonoid content decreased when plants received the treatment of 0.5% spent engine oil. Interestingly, the correlation of the redness/yellowness ratio with β-carotene, lycopene, vitamin C, tocopherols, and radical scavenging activity was suggested as a possible indicator of tomato fruit quality in areas inflicted by such agro-environmental restrictions. In another study, Erika et al. analyzed sensorial properties and volatile organic compounds (VOCs) associated with tomato flavor under organic low-input production systems. Salad and cocktail cultivars showed a wide range of variation for the studied traits, with the exception of specific VOCs. Twelve VOCs were correlated with sensorial attributes and allowed the differentiation of the cultivars depending on their fruit types, namely salad and cocktail cultivars. Among these, phenylethyl alcohol and benzyl alcohol were positively correlated with the acceptability of cocktail cultivars, whereas 2-isobuthylthiazole and 6-methyl-5-hepten-2-ol negatively was correlated with the acceptability of salad cultivars. Therefore, organic breeders were recommended to use cultivars from a wide range of breeding programs to improve important tomato quality and agronomic traits and compromise the trade-off of high yield and quality.

Light-emitting diode (LED) lamps are increasingly being used in tomato production systems. Alsina et al. evaluated the effect of additional lighting of different quality used in greenhouse cropping systems on the accumulation of bioactive compounds in tomatoes. High-pressure sodium lamps (HPSL) stimulated the accumulation of primary metabolites; the soluble solids content was higher compared to other lighting sources. Since LED and induction lamps emit about 20% blue-violet light, the obtained results suggested that blue-violet light of the spectrum stimulates the accumulation of phenolic compounds in tomatoes when additional lighting from these lights sources is implemented. Moreover, red fruit varieties tend to synthesize more β-carotene under these light sources, compared to HPSL, while the increase of blue light promoted the synthesis of lycopene, phenolics, and flavonoids and decreased soluble solids content. In the same context, Wang et al. studied the suitability of red and blue LED for supplementing light on tomato plants for different time periods in the morning and evening. The accumulation of vitamin C, organic acids, amino acids, carotenoids, phenolic acids, and other health-promoting compounds in fruit was promoted when plants were treated with light supplementation in the morning, while light supplementation in the evening increased the contents of sugars, flavonoids, and aromatic compounds. Thus, it could be suggested that morning light supplementation may improve the nutritional quality of tomato fruit, while evening treatments are beneficial to their flavor-related parameters.

The bioactive constituents of tomato fruit are affected by several factors, including genetic features, environmental conditions, maturation degree, and postharvest treatments. In this sense, Lima et al. performed a literature review aiming to investigate how pre- and postharvest factors may influence the content of bioactive compounds in tomatoes (with a particular focus on phenolic compounds, carotenoids, and biogenic amines) and how some heat processing methods may change the antioxidant status of food products. The potential for reintroducing tomato by-products into the value cycle was also addressed in this mini-review.

This Research Topic also covered important findings for the tomato processing and trade sectors. A non-destructive method for estimating soluble solids and lycopene contents in tomato fruit or for rapid analysis of tomato homogenates during raw material quality assessment was developed by Égei et al. using visible and near-infrared (Vis-NIR) absorbance and reflectance data. In turn, tomatoes at the mature-red and mature-green stages are prone to chilling injury when stored at temperatures below 5 and 10°C, respectively, leading to a decline in quality and shelf-life, thus restricting trade flexibility. Zhao et al. reported that the silencing of Sly-miR171e enhanced the expression of *GRAS24* (the target gene of miR171), increased the gibberellic acid content and the expression of *CBF1* and *COR* genes, and by which chilling injury of tomato fruit was alleviated. In the study by Zhang et al., lycopene was successfully encapsulated in polyelectrolyte complex nanoparticles made with a negatively charged polysaccharide and positively charged sodium caseinate. These stable nanoparticles exhibited improved water-solubility, powerful antioxidant capacity, and controlled release ability through a simulated gastrointestinal tract when compared with free lycopene. Furthermore, these biocompatible nanoparticles increased cell viability, prevented apoptosis and protected cells from oxidative damage, thus constituting a potential health supplement or nutraceutical to improve human health. In a study with canned tomatoes, Izzo et al. showed that a noticeable percentage of rutin, naringenin, chlorogenic acid, and lycopene remains bioaccessible after simulated gastrointestinal digestion, thus evidencing which compounds may exert beneficial effects on consumers' health.

Regarding the health benefits of tomato fruit and tomato compounds, Cámara et al. revised the scientific evidence regarding the beneficial effects of tomato products on both cardiovascular disease prevention and antiplatelet aggregation, as well as the European Food Safety Authority health claims for tomato products. Curiously, only one health claim has been approved so far for a water-soluble concentrated extract of tomato, namely "helping to maintain normal platelet aggregation, which contributes to healthy blood flow." Finally, Huang et al. concluded that lycopene can effectively alleviate

liver steatosis induced by a high-fat diet and could be used as a possible dietary strategy for the control and treatment of non-alcoholic fatty liver disease. This beneficial effect was related to the fact that lycopene increased the expression of genes related to liver lipid metabolic process.

Overall, this Research Topic showed that tomato is a functional food which remains in the spotlight of many researchers who focus on different nutritional/nutraceutical quality issues, ranging from its production to the final impact on consumers' health. The findings compiled in the present Research Topic highlight the importance of scientific evidence regarding the health effects of tomato fruit and food products and light up new directions for further research.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021) and to FCT for the contracts of JP (CEECIND/01011/2018) and LB (CEEC Institutional).

Acknowledgments

The authors thank all the peer reviewers who took time to review for this Research Topic.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Pinela J, Oliveira MBPP, Ferreira ICFR. Bioactive compounds of tomatoes as health promoters. In: Silva LR da, Silva BM, editors, *Natural Bioactive Compounds from Fruits and Vegetables as Health Promoters, Part II*. Sharjah: Bentham Science Publishers (2016). p. 48–91.

2. Johns T, Powell B, Maundu P, Eyzaguirre PB. Agricultural biodiversity as a link between traditional food systems and contemporary development, social integrity and ecological health. *J Sci Food Agri.* (2013) 93:3433–42. doi: 10.1002/JSFA.6351

frontiers in Nutrition

ORIGINAL RESEARCH
published: 02 November 2021
doi: 10.3389/fnut.2021.727785

Translatomics Probes Into the Role of Lycopene on Improving Hepatic Steatosis Induced by High-Fat Diet

*Tengda Huang[2], Jingsu Yu[2], Zeqiang Ma[2], Qinghua Fu[2], Siqi Liu[2], Zupeng Luo[2], Kang Liu[2], Lin Yu[2], Weiwei Miao[2], Dongling Yu[3], Ziyi Song[2], Yixing Li[2], Lei Zhou[2] and Gaoxiao Xu[1]**

[1] Key Laboratory of Embryo Development and Reproductive Regulation of Anhui Province, Fuyang Normal University, Fuyang, China, [2] State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, College of Animal Science and Technology, Guangxi University, Nanning, China, [3] Teaching and Research Section of Biotechnology, Nanning University, Nanning, China

Liver is an important organ for fat metabolism. Excessive intake of a high-fat/energy diet is a major cause of hepatic steatosis and its complications such as non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Supplementation with lycopene, a natural compound, is effective in lowering triglyceride levels in the liver, although the underlying mechanism at the translational level is unclear. In this study, mice were fed a high-fat diet (HFD) to induce hepatic steatosis and treated with or without lycopene. Translation omics and transcriptome sequencing were performed on the liver to explore the regulatory mechanism of lycopene in liver steatosis induced by HFD, and identify differentially expressed genes (DEGs). We identified 1,358 DEGs at the translational level. Through transcriptomics and translatomics joint analysis, we narrowed the range of functional genes to 112 DEGs and found that lycopene may affect lipid metabolism by regulating the expression of *LPIN1* at the transcriptional and translational levels. This study provides a powerful tool for translatome and transcriptome integration and a new strategy for the screening of candidate genes.

Keywords: translatomics, Ribo-seq, lycopene, high fat diet, NAFLD

OPEN ACCESS

Edited by:
José Pinela, Polytechnic Institute of Bragança (IPB), Portugal

Reviewed by:
Altino Branco Choupina, Polytechnic Institute of Bragança (IPB), Portugal
Yuxiang Zhang, Boston Children's Hospital, United States

****Correspondence:***
Gaoxiao Xu
xgx138@126.com

Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *19 June 2021*
Accepted: *29 September 2021*
Published: *02 November 2021*

Citation:
Huang T, Yu J, Ma Z, Fu Q, Liu S, Luo Z, Liu K, Yu L, Miao W, Yu D, Song Z, Li Y, Zhou L and Xu G (2021) Translatomics Probes Into the Role of Lycopene on Improving Hepatic Steatosis Induced by High-Fat Diet. Front. Nutr. 8:727785. doi: 10.3389/fnut.2021.727785

INTRODUCTION

The liver is one of the vital metabolic organs and the main area of lipid metabolism. The liver not only utilizes lipids to produce energy, but also secretes lipids in the form of very low-density lipoproteins (VLDLs) (1). However, high-fat/energy diet can cause lipid deposits in the liver (2), which may increase the risk of non-alcoholic fatty liver disease (NAFLD). A recent epidemiological study has shown that 12–38% of adults are suffering from NAFLD (3). Due to the high incidence of NAFLD in the population, its hazard to health, and limited treatment options (4, 5), there is an urgent need to develop new drugs and therapeutic strategies.

Lycopene (ψ,ψ-carotene) is a polyene, mainly found in ripe tomatoes, watermelon, guava, rose hips, papaya and grapefruit (6, 7). During recent decades, studies on the function of lycopene have mainly focused on its antioxidative, lipid-lowering, anti-inflammatory and antitumor effects (8–11). Some research has shown that lycopene can inhibit hepatic steatosis by inducing downregulation of fatty acid binding protein 7 through regulating miRNA-21 (12). In another study, the lycopene metabolite, apo-10'-lycopenoic acid, protects against the development of

steatosis in ob/ob mice by upregulating SIRT1 gene expression and activity (13). Metabolomics research has revealed that lycopene can increase the levels of metabolites related to the antioxidant response, to alleviate steatosis induced by high-fat diet (HFD) (14). The regulatory effect of lycopene on lipid metabolism has been reported in miRNA (12), RNA (13), and metabolomics (14) studies, but the lack of studies at the level of gene translation has limited our further understanding of the lipid-lowering effect of lycopene.

Proteins are deeply involved in all aspects of cellular, physiological and developmental processes, such as cell growth and division, organogenesis, and reproductivity (15–17). Therefore, researchers have focused on protein expression. However, proteomics has some shortcomings. The sensitivity of protein profile detection is low, and it is difficult to detect low-abundance proteins, which may lead to some proteins with important biological functions, but low expression not being detected (18). RNA sequencing (RNA-seq) has the advantages of high throughput, high sensitivity and low cost; therefore, scientists usually use RNA-seq to detect the abundance of gene transcriptional expression to evaluate the abundance of protein expression (19). However, in recent years, it has been recognized that there is a poor correlation between mRNA and protein abundance. Gygi et al. found that there were 20–30-fold differences in mRNA and protein expression levels after comparing 106 yeast proteins (20), which indicated that it is inappropriate to regard gene transcriptional abundance as protein abundance (21). From RNA to protein, there are many processes such as RNA degradation, splicing, non-coding RNA regulation, epigenetic modification, and protein processing. Translatomics can fill the long omics gap between RNA and protein quantification and facilitate the study of gene expression regulation more directly. Ribosome footprint sequencing (Ribo-seq) is a recently developed method that can measure translational activity in a genome-wide and quantitative manner by base pair resolution (22), which opens a new way to understand biological problems.

This study depicted the molecular portrait of mice liver with or without lycopene treatment from translatome, which fills the gap in the research on the translation level of the lipid-lowering effect of lycopene. The purpose of the present study was to explain the regulatory mechanism of lycopene in liver lipid metabolism and identify differently expressed genes (DEGs) through the joint analysis of translatomics and transcriptomics. The results can provide a method for screening candidate genes and further understanding the mechanism of lycopene for the treatment of NAFLD.

Abbreviations: HFD, high fat diet; LYC, high-fat feed containing lycopene; DEGs, differentially expressed genes; VLDLs, very low-density lipoproteins; NAFLD, non-alcoholic fatty liver disease; RNA-seq, RNA sequencing; Ribo-seq, ribosome footprint sequencing; OAPA, oleic and palmitic acids; TG, triglyceride; qPCR, quantitative polymerase chain reaction; RFPs, ribosome footprints; RPF, ibosome protected fragment; RPKM, reads per kilobase per million reads; GO, gene ontology; BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

MATERIALS AND METHODS

Animals and Feeds

The C57/BL6 mice used in the experiment were purchased from Guangxi Medical University. Mice were randomly divided into three groups ($n = 8$ per group) and fed with different diets from week 8: CK: standard feed (10% kcal, **Supplementary Table 1**), HFD: high-fat feed (45% kcal, **Supplementary Table 1**), and LYC: high-fat feed containing lycopene (0.33% w/w) (23, 24). The feed for mice was purchased from Jiangsu Medicience Biopharmaceutical Co. Ltd., and the lycopene mixed into the feed was purchased from Xian Baichuan Biotechnology Co. Ltd. Animals were housed in a pathogen-free barrier environment, on a 12/12-h dark/light cycle throughout the study, and were supplied water *ad libitum*. After 8 weeks on different diets, the mice were euthanized.

Oil Red O Staining

Liver tissue was prepared in frozen sections, which were stained with Oil Red O solution in 60% isopropanol for 10 min, and then counter-stained with hematoxylin for 1 min. The slides were viewed at 200× magnification.

Cell Culture

HepG2 cells were purchased from the Cell Bank of the Chinese Academy of Sciences Shanghai Institute of Cell Biology (Shanghai, China) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum under an atmosphere of 5% CO_2 at 37°C. At 80% confluence, the DMEM was changed to DMEM supplemented with oleic (200 μM) and palmitic (100 μM) acids (OAPA), simulating a high-fat environment. Lycopene (98%) was purchased from the China Beijing Solarbio Science & Technology Co., Ltd., dissolved in tetrahydrofuran containing 250 ppm butylated hydroxytoluene (99%; Shanghai Industrial Co., Ltd., Shanghai, China) and diluted to 10 μ M with fetal bovine serum. The cells were collected after 24 h of incubation.

Triglyceride (TG) Measurement

TG content of the cells and tissues were measured using the TG assay kit (Pulilai, Beijing, China), as described previously (25).

RNA Extraction and Quantitative Polymerase Chain Reaction (qPCR)

Total RNA from tissues and cells was extracted using TRIzol reagent and cDNA was synthesized using PCR conditions of 95°C for 3 min, followed by 40 cycles of 95°C for 10 s, 60°C for 1 min, and 72°C for 10 s. Gene expression levels were measured by quantitative PCR using the $2^{-\Delta\Delta Ct}$ method with *β-actin* as an internal control. The forward and reverse primers were as follows: *LPIN1*: TAAACGGAGCCGACACCTTGGA and CCGT TGTCACTGGCTTGTTTGG; *β-actin*: AACAGTCCGCCTAGA AGCAC and CGTTGACATCCGTAAAGACC.

Western Blot

Tissue was lysed in RIPA lysis buffer (Solarbio, Beijing, China) containing 1 mM PMSF. The total protein concentration was determined using a BCA protein assay kit (Beyotime,

Shanghai, China). The centrifuged supernatant was boiled and SDS-PAGE electrophoresis was performed (Mini-PROTEAN Tetra System, Bio-Rad), followed by transfer to a PVDF membrane. Subsequently, the primary antibodies anti-LPIN1 rabbit polyclonal antibody (1:1,000; D163631, Sangon Biotech, Shanghai, China), β-tubulin antibody (1:1,000; 2146s, Cell Signaling Technology, Inc., Shanghai, China) were incubated overnight at 4°C. The Image Lab (Universal Hood II, Bio-Rad) was used to detect chemiluminescent signals after the secondary antibody incubation.

Ribo-seq

Total ribosome footprints (RFPs) extraction from liver tissue of mice was performed as previously described (26). Liver was pre-treated with 100 mg/ml cycloheximide for 15 min, washed with pre-chilled phosphate-buffered saline, then treated with cell lysis buffer {1% Triton X-100 in ribosome buffer [RB buffer, 20 mM HEPES–KOH (pH 7.4), 15 mM $MgCl_2$, 200 mM KCl, 100 μg/ml cycloheximide and 2 mM dithiothreitol]}. Cell debris was removed by centrifugation at 16,200 × g for 10 min at 4°C. Supernatants were transferred into new pre-chilled 1.5-ml tubes with the addition of 2 μl Ribolock RNase Inhibitor (40 U/μl, Fermentas) in each tube. RNase I (10 U/μl, Fermentas) was added at 0.2 μl per tube, followed by incubation at 37°C for 15 min and reaction termination with 1% sodium dodecyl sulfate (1/10 volume per tube). The digested samples were pooled and layered on the surface of 15 ml sucrose buffer (30% sucrose in RB buffer). The ribosomes were pelleted by ultracentrifugation at 185,000 × g for 5 h at 4°C. Ribosome protected fragment (RPF) extraction was performed using the TRIzol method and rRNA was depleted using the Ribo-Zero rRNA Removal Kit (Mouse) (Epicenter).

RNA-seq

Total RNA was isolated using TRIzol reagent. Equal amounts of total RNA or RFPs from the HFD and LYC groups were prepared for subsequent library construction and high-throughput sequencing. RNA libraries were prepared according to the protocol of the VAHTS mRNA-seq v.3 Library Prep Kit for Illumina (Vazyme Biotech Co. Ltd., Nanjing, Jiangsu, China), and the raw sequencing reads were generated on an Illumina HiSeqX Ten sequencer.

Sequence Analysis

We used cutadapt and bowtie2 software to process raw data to obtain high-quality clean data. The clean data were compared to the reference genome (GRCm38/mm10) using STAR software. The FANSE2 series algorithm (27, 28) was used for quantitative genetic analysis. The mRNA and RPFs in each sample were normalized using reads per kilobase per million reads (RPKM) (29). Differential gene calculation was carried out for the identified genes by edgeR (30) software, and the screening threshold was |log2 fold change|>1 and $p < 0.001$. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed using the OmicShare tools; a free online platform for data analysis (http://www.omicshare.com/tools).

Statistical Analysis

The results were analyzed using GraphPad Prism 8 and presented as the mean ± standard error of the mean. The student's *t*-test was used to determine the significance of the difference between two groups, and the differences were considered statistically significant if $p < 0.05$.

RESULTS

Lycopene Alleviates TG Deposition Induced by High-Fat Diet

Figure 1 shows the schematic of the study design and high-throughput sequencing. After 8 weeks of feeding, the liver TG level under HFD was significantly increased ($p < 0.001$, **Figure 2A**), but the liver TG level of mice fed HFD supplemented with lycopene (LYC) was significantly lower than in those fed HFD alone ($p < 0.001$, **Figure 2A**). We also studied the effect of lycopene on lipid metabolism in HepG2 cells by adding OA and PA to DMEM to simulate a high lipid environment. The results showed a 25% decrease in intracellular TG level in the OAPA + lycopene group compared to the OAPA group ($p < 0.01$, **Figure 2B**). Oil Red O staining showed that a large number of lipid droplets accumulated in the liver of the HFD group, while the number of lipid droplets was significantly reduced in the LYC group (**Figure 2C**).

Overview of Ribo-seq Results

To investigate the underlying mechanism of lycopene on improving hepatic steatosis, we used a translatome analysis to identify the liver DEGs between the HFD and LYC groups. Six cDNA libraries, including three biological replicates from the HFD group (HFD1, HFD2, and HFD3) and three biological replicates from the LYC group (LYC1, LYC2, and LYC3) were constructed and analyzed by high-throughput sequencing. The principal component analysis of six samples was calculated and showed high-level repeatability of intraclass samples (**Figure 3A**). The overlapping genes in the HFD and LYC groups were counted, and there were 11,580 genes detected in both groups (**Figure 3B**). The abundance of gene in the HFD and LYC groups had a high correlation ($R^2 = 0.7814$, **Figure 3C**), which indicated that these two groups can be analyzed together. Subsequently, 1,358 DEGs of Ribo-seq were identified by $|\log_2(\text{fold change})| > 1$, $p < 0.01$, among which 505 were upregulated and 853 downregulated (**Figure 3D** and **Supplementary Table 2**). The 1,358 DEGs of Ribo-seq were demonstrated in a heat map based on gene expression levels (**Figure 3E**).

GO and KEGG Functional Enrichment Analysis

GO and KEGG analyses were conducted to examine the functional pathways for DEGs. The top 20 GO terms, classified by $-\log_{10}(p\text{-value})$, were significantly enriched in DEGs of Ribo-seq compared to the genome background GO. The top 20 GO terms included eight biological process (BP), 11 cellular component (CC) and one molecular function (MF). The BP terms were enriched in cellular metabolic process, metabolic process, organic substance metabolic process (**Figure 4A**,

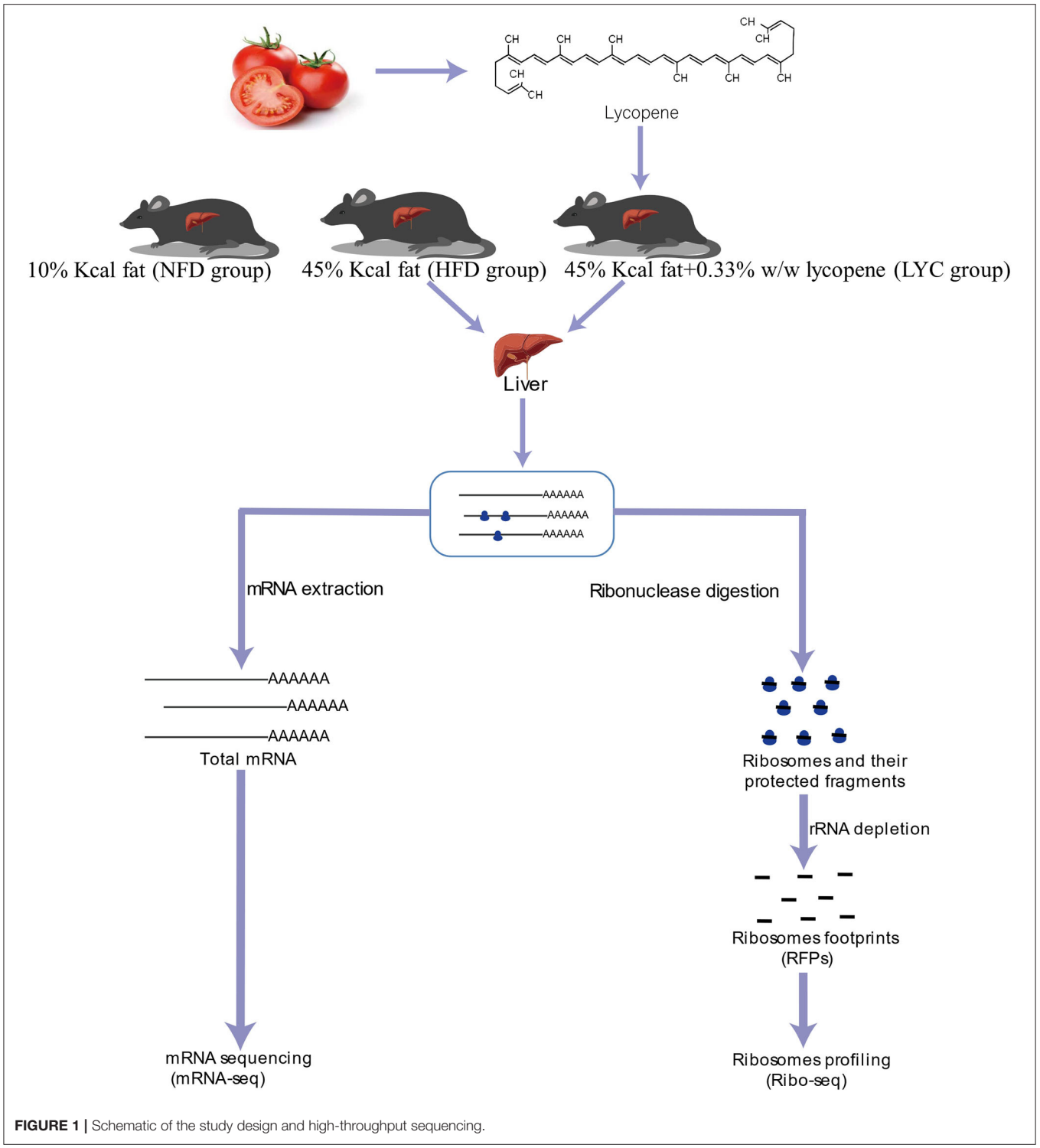


FIGURE 1 | Schematic of the study design and high-throughput sequencing.

Supplementary Table 3). The functional enrichment cycle diagram displayed the top 20 KEGG pathways, classified by $-\log_{10}(p\text{-value})$, which revealed that the DEGs of Ribo-seq were mainly enriched in five KEGG A classes, including metabolism, genetic information processing, cellular processes, organismal systems and human diseases. Among these pathways, metabolism included oxidative phosphorylation (ko00190) and glycerophospholipid metabolism (ko00564); genetic information processing included protein processing in endoplasmic reticulum (ko04141) and spliceosome (ko03040); cellular processes included ferroptosis (ko04216); organismal systems included thermogenesis (ko04714); human diseases included NAFLD

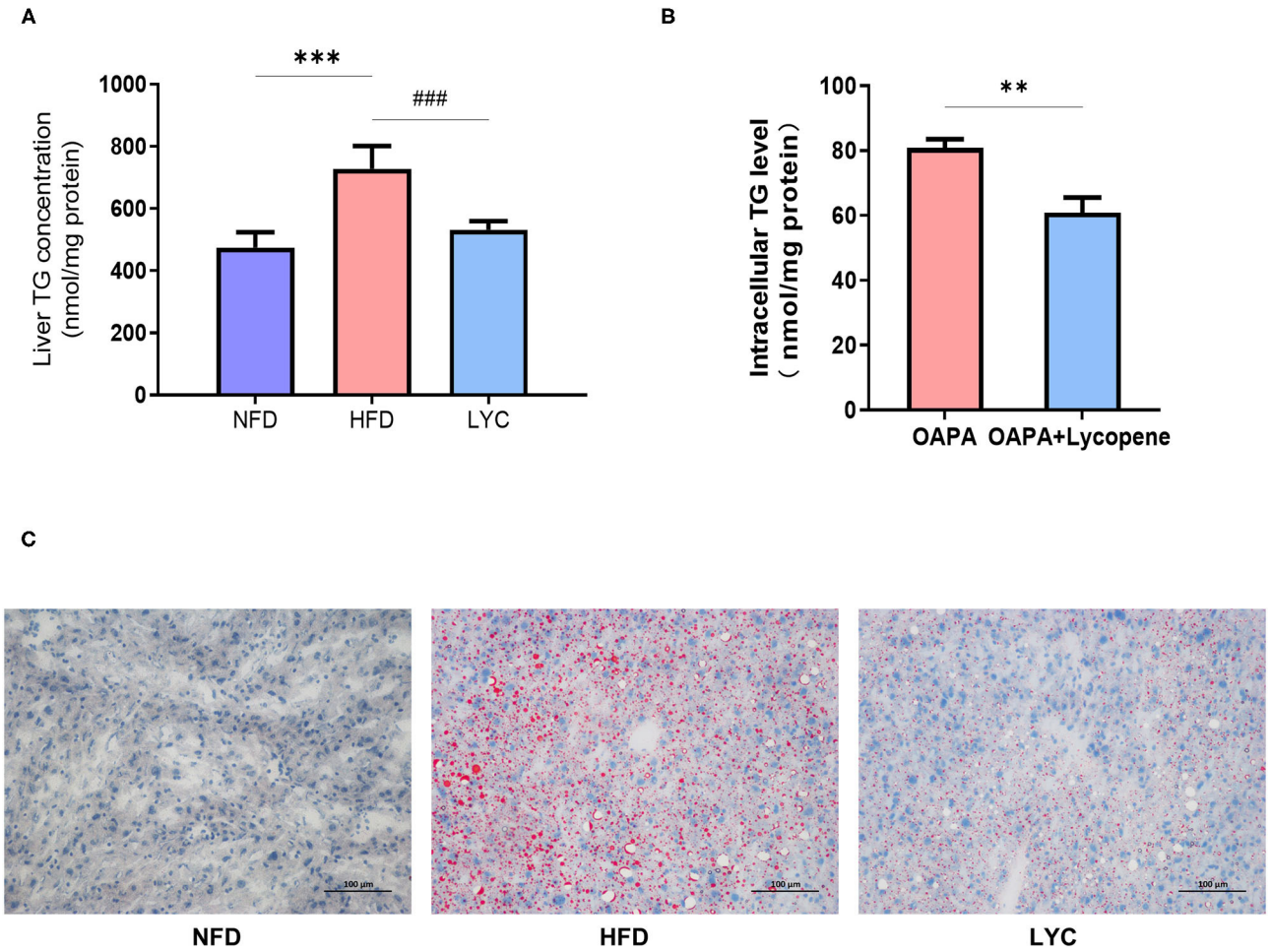


FIGURE 2 | Lycopene alleviates liver fatty deposition induced by HFD. **(A)** Mouse liver TG level. **(B)** Oil Red O staining (×200 magnification) of the liver in different groups. Scale bar = 100 μm. **(C)** HepG2 cell TG level. Data are expressed as the mean ± SD. $^{**}p < 0.01$, $^{***}p$ or $^{\#\#\#}p < 0.001$. NFD, normal fat diet group; HFD, high fat diet group; LYC: lycopene group.

(ko04932) (**Figure 4B**, **Supplementary Table 4**). As shown in **Supplementary Figures 1A,B**, the KEGG network diagram, respectively, showed 24 DEGs of Ribo-seq enriched in the oxidative phosphorylation pathway and 25 DEGs of Ribo-seq enriched in the NAFLD pathway. In conclusion, our data indicated that lycopene can regulate lipid metabolism by effecting translational level, thus alleviating NAFLD.

Lycopene Altered Gene Expression at Transcriptional and Translational Levels

To further investigate the role of lycopene in regulating hepatic steatosis induced by HFD, we performed RNA-seq using the liver of mice in the HFD and LYC groups. In the RNA-seq data, there was a good correlation of biological duplications within the two groups (**Supplementary Figure 2**). The volcano map shows 1,127 DEGs in RNA-seq (**Supplementary Figure 3** and **Supplementary Table 5**). Due to the high correlation of gene expression abundance between transcriptome and translatome, the two omics can be combined for analysis ($R^2 = 0.7225$ and 0.5372, respectively; **Supplementary Figure 4**). We detected 695 upregulated and 432 downregulated genes at the transcriptional level, and 505 upregulated and 853 downregulated genes at the translational level (**Figure 5A**). Only 51 DEGs were upregulated in both the transcriptome and translatome, and only 61 DEGs were downregulated in both the transcriptome and translatome (**Figure 5B**). Based on the fold change of RPKM ($|\log_2$(fold change)$| > 1$ and $p < 0.01$ as cutoff), genes were classified into nine categories (**Figure 5C** and **Supplementary Table 6**). Further analysis revealed that 1.03% of the responsive genes (112 genes) were among the accordant groups (quadrant C and G), which were co-regulated with their expression increased or decreased to a similar extent at the transcriptional and translational levels. Meanwhile, 16.61% of the responsive genes (1,799 genes) were located in the other six discordantly regulated groups (classes A, B, D, F, H, and I; **Figure 5C**). By combining RNA-seq and Ribo-seq, we were able to narrow our focus to the 112 DEGs (C and G quadrants) that are regulated in the same direction as transcription and translation.

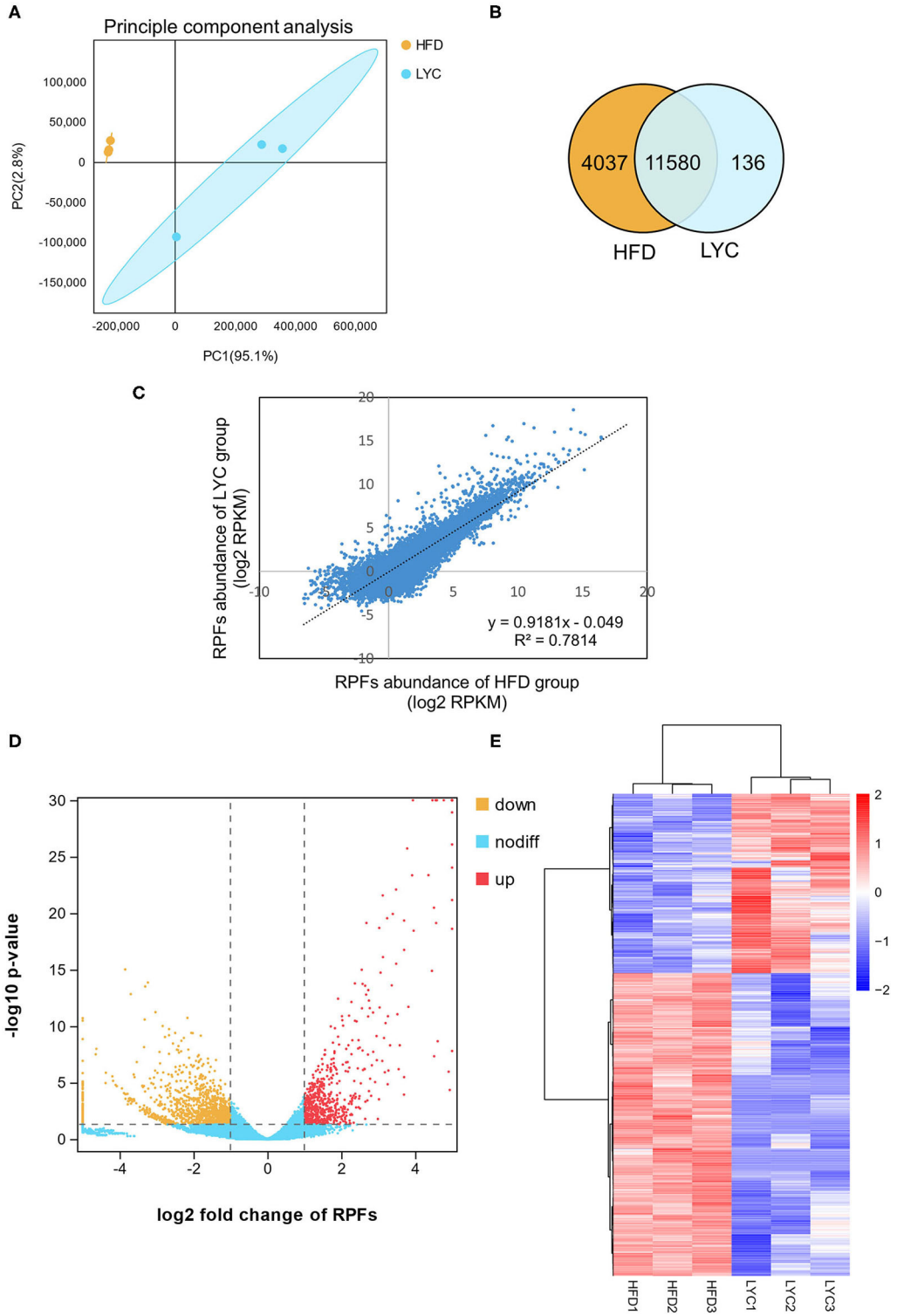
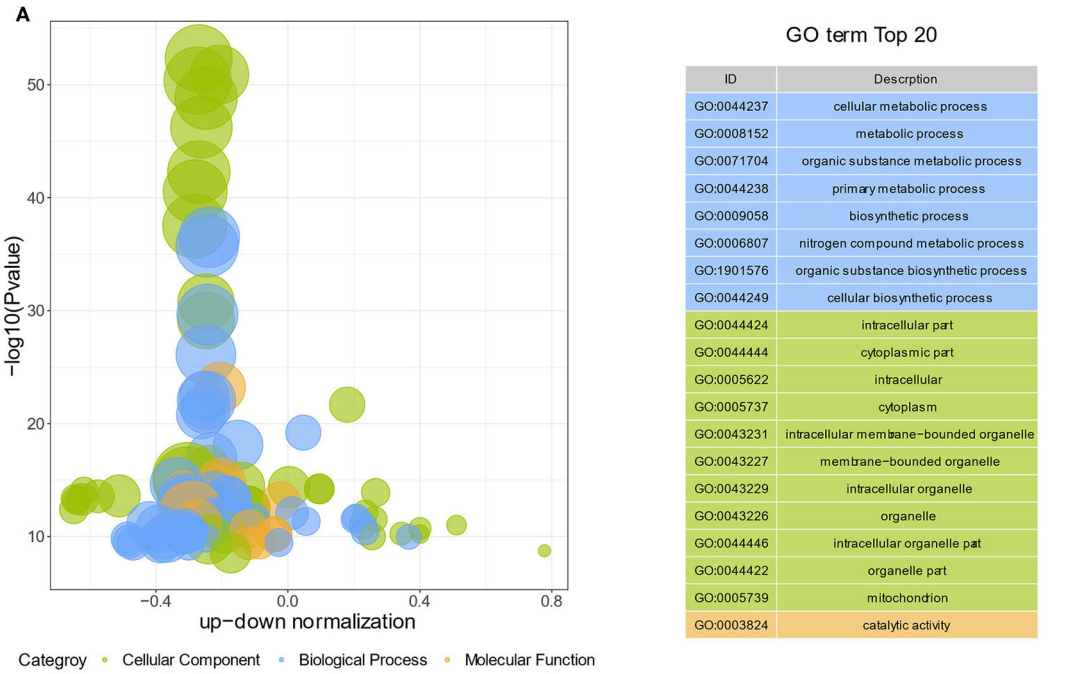


FIGURE 3 | Overview of Ribo-seq results in HFD and LYC mice livers. **(A)** Principal component analysis of Ribo-seq. **(B)** Venn diagram showing number of common genes among the two groups. **(C)** RPFs abundance correlation scatter plot between HFD and LYC livers. **(D)** Volcano plots indicate the directionality of significant DEGs. Genes upregulated (red) or downregulated (orange) by supplement lycopene correspond to a 1.0 decrease or increase in $\log_2$ fold change with $p < 0.01$. **(E)** A total of 1,358 DEGs were clearly distinguished based on their Ribo-seq abundance. The color key (from blue to red) of Z-score value (−2–2) indicated low to high expression levels. HFD1, HFD2, and HFD3 represent group HFD biological repetition 1, 2, and 3. LYC1, LYC2, and LYC3 represent group LYC biological repetition 1, 2, and 3.



GO term Top 20

ID	Descrption
GO:0044237	cellular metabolic process
GO:0008152	metabolic process
GO:0071704	organic substance metabolic process
GO:0044238	primary metabolic process
GO:0009058	biosynthetic process
GO:0006807	nitrogen compound metabolic process
GO:1901576	organic substance biosynthetic process
GO:0044249	cellular biosynthetic process
GO:0044424	intracellular part
GO:0044444	cytoplasmic part
GO:0005622	intracellular
GO:0005737	cytoplasm
GO:0043231	intracellular membrane−bounded organelle
GO:0043227	membrane−bounded organelle
GO:0043229	intracellular organelle
GO:0043226	organelle
GO:0044446	intracellular organelle part
GO:0044422	organelle part
GO:0005739	mitochondrion
GO:0003824	catalytic activity

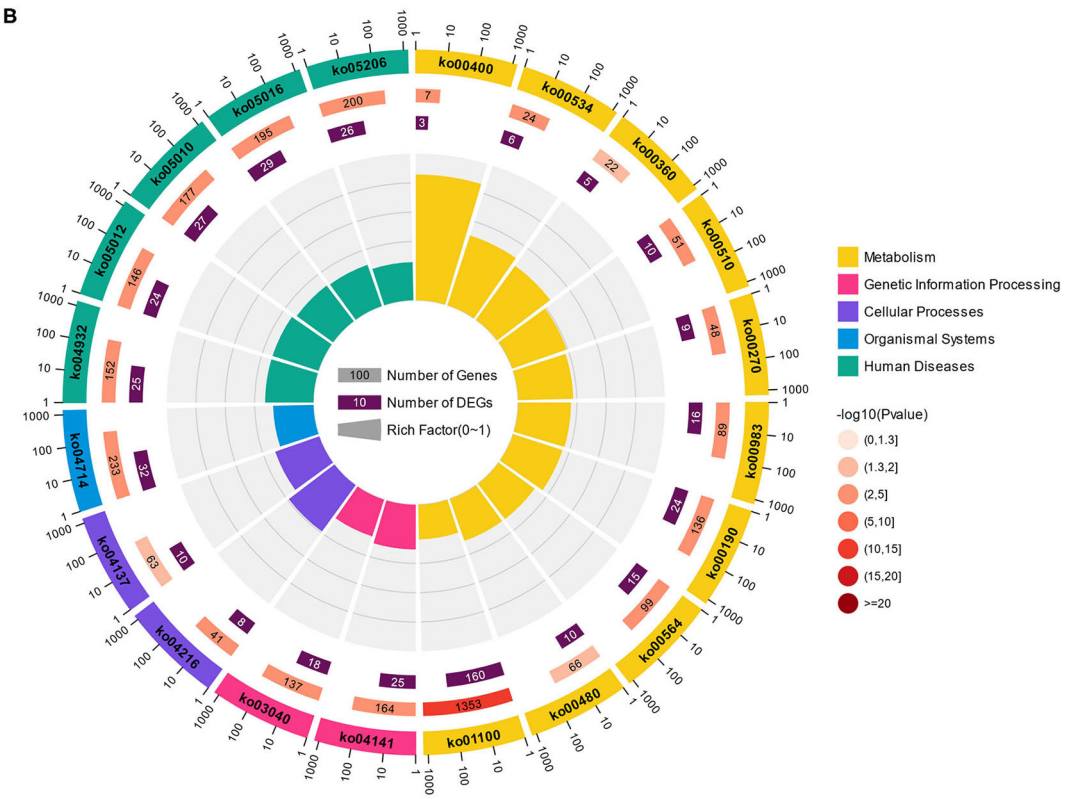


FIGURE 4 | GO and KEGG analysis of DEGs in Ribo-seq. **(A)** GO bubble diagram showing the top 20 enriched GO terms. Three colors represent three GO categories, including cellular component, biological process and molecular function. **(B)** The enrichment circle diagram shows the KEGG analysis of the top 20 pathways. Four circles from the outside to the inside. First circle: the classification of enrichment, outside the circle is the scale of the number of genes. Different colors represent different categories. Second circle: number and *p*-values of the classification in the background genes. The more genes, the longer the bars, the smaller the value, the redder the color. Third circle: bar chart of the total number of DEGs. Fourth circle: rich factor value of each classification (number of DEGs in this classification divided by the number of background genes). Each cell of the background helper line represents 0.1.

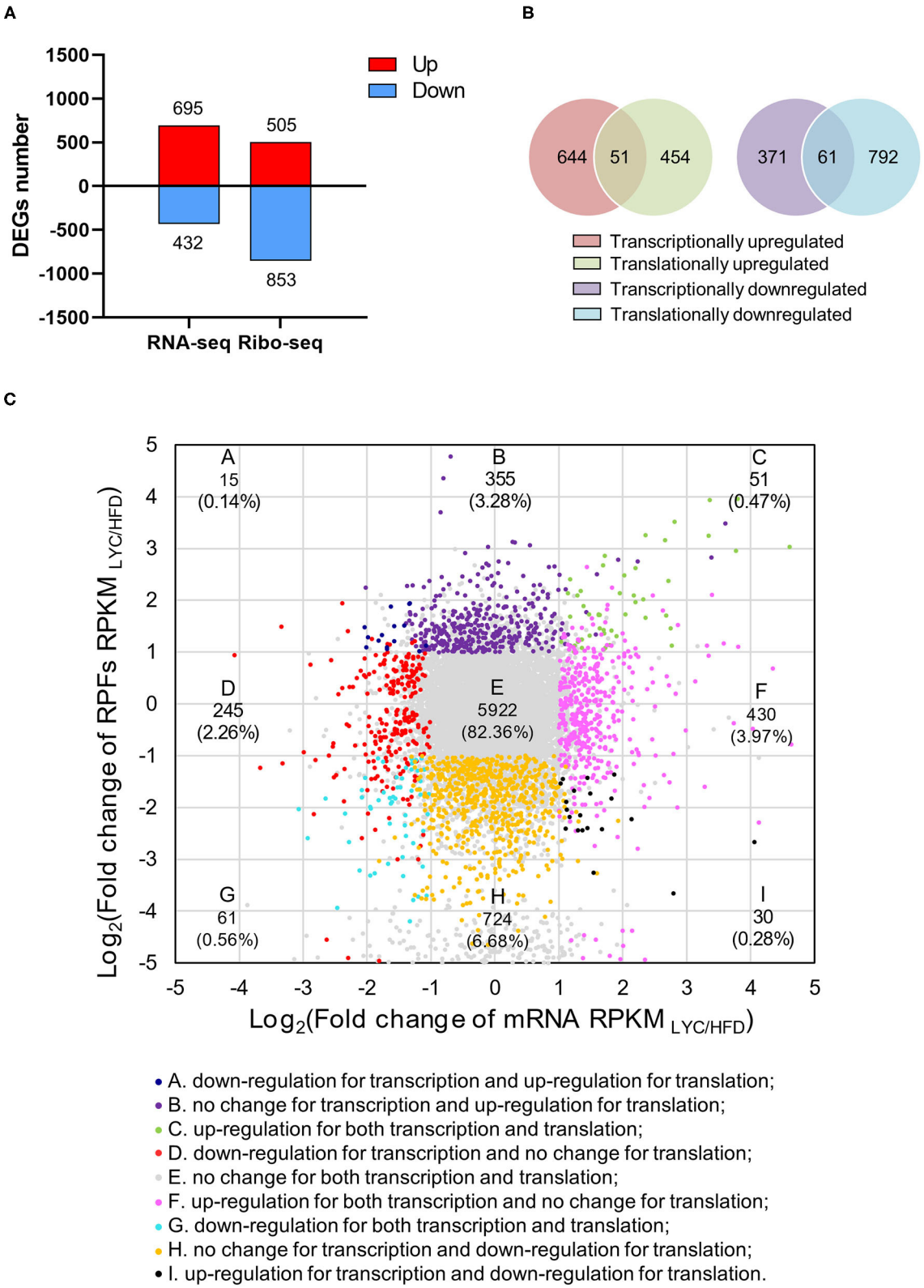


FIGURE 5 | Lycopene altered gene expression at both transcriptional and translational levels. **(A)** Number of DEGs ($|\log_2$ fold change$| \geq 1$ and $p < 0.01$) at transcriptional or translational levels. The red and blue bars refer to the number of upregulated and downregulated genes, respectively. **(B)** Venn diagram showing the relationship between transcriptome and translatome. **(C)** Fold changes of RPKMs at transcriptional and translational levels. Nine squares in different colors indicate nine responsive groups ($|\log_2$fold change$| \geq 1$ and $p < 0.01$).

Functional Enrichment Analysis of Protein–Protein Interaction Networks

The functional protein association networks were conducted using the online STRING website (https://string-db.org/) and the interactions of the coding proteins of the 112 DEGs (quadrants C and G, **Figure 5C**) were analyzed to identify the important genes. The results showed *LPIN1* had most crossover nodes in TG metabolic process, lipid metabolic process and cellular lipid metabolic process (**Figure 6A**). According to real-time fluorescent quantitative PCR, western blot, RNA-seq and Ribo-seq data, compared with the HFD group, *LPIN1* was significantly increased in the LYC group (**Figure 6B**). *LPIN1* promoted TG metabolism. These results suggest that lycopene may play a lipid-lowering role by regulating the expression of genes related to TG metabolism.

DISCUSSION

Currently, there is no definitive treatment for NAFLD, and therefore, finding new drugs and treatments is valuable. In our research, liver TG levels were significantly increased in mice fed a HFD (**Figure 2A**), indicating successful construction of the NAFLD model. In addition, lycopene can improve liver fat accumulation induced by HFD (**Figure 2A**). This is consistent with previous reports (31–33). *In vitro*, lycopene also significantly alleviated TG accumulation in HepG2 cells induced by OA and PA. Our previous study (20) has shown that different concentrations of lycopene are non-toxic and promoted cell proliferation. In addition, the mice fed with lycopene had no adverse effect, and the body weight increased and the body fat rate decreased significantly. It is suggested that lycopene can be used as a harmless natural dietary supplement.

Translational regulation is a main element of gene expression regulation. Omics measurements proved that translational regulation is obligated to more than half of all regulatory magnitudes (34, 35). Translatome, as a novel technology of omics research in recent years, may provide important information on many biological problems and can detect lncRNA (36, 37), circRNA (38) and pri-miRNA (39), which encode polypeptide. Besides, compared with the transcriptome, the translatome is more able to reflect the changes in expression of the proteome. Schafer et al. reported that in rat liver and heart tissues, ribosomal footprint abundance was better associated with genome-wide protein abundance than RNA-seq data was (40). The low correlation between RNA and protein can be attributed to mRNA half-life, protein folding, degradation and post-translational modification (41, 42). Our study showed a high correlation between mRNA and RPFs abundance (**Supplementary Figure 4**). Therefore, it is speculated that lycopene may participate in TG metabolism by participating in the regulation of gene transcription and translation.

Firstly, we performed RNA-seq on the livers of mice fed HFD and HFD supplemented with lycopene, and identified 695 upregulated and 432 downregulated DEGs of RNA-seq (**Supplementary Figure 3**). To narrow the focus of the

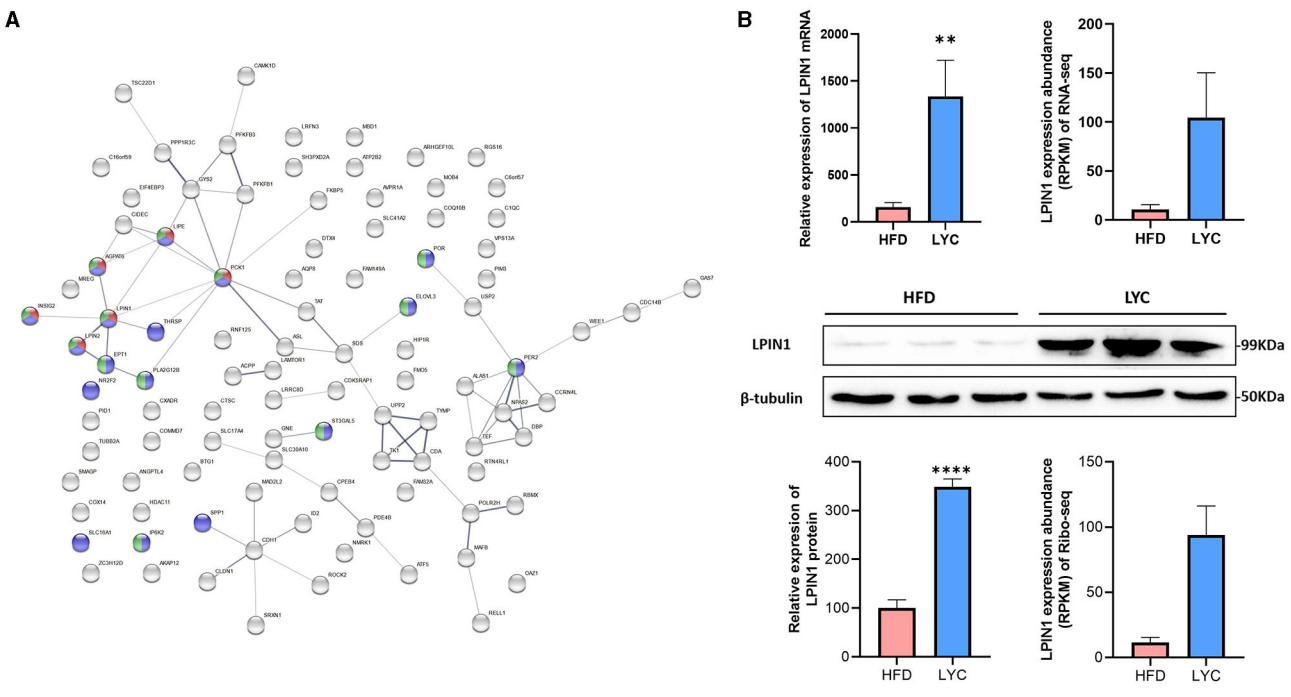


FIGURE 6 | Protein–Protein Interaction Networks. **(A)** Interactive map encoded by differential genes. Nodes represent the proteins. Red: TG metabolic process; Green: lipid metabolic process; purple: cellular lipid metabolic process. **(B)** *LPIN1* expression level base on RT-qPCR, western blot, RNA-seq and Ribo-seq data. $^{**}p < 0.01$. $^{****}p < 0.0001$.

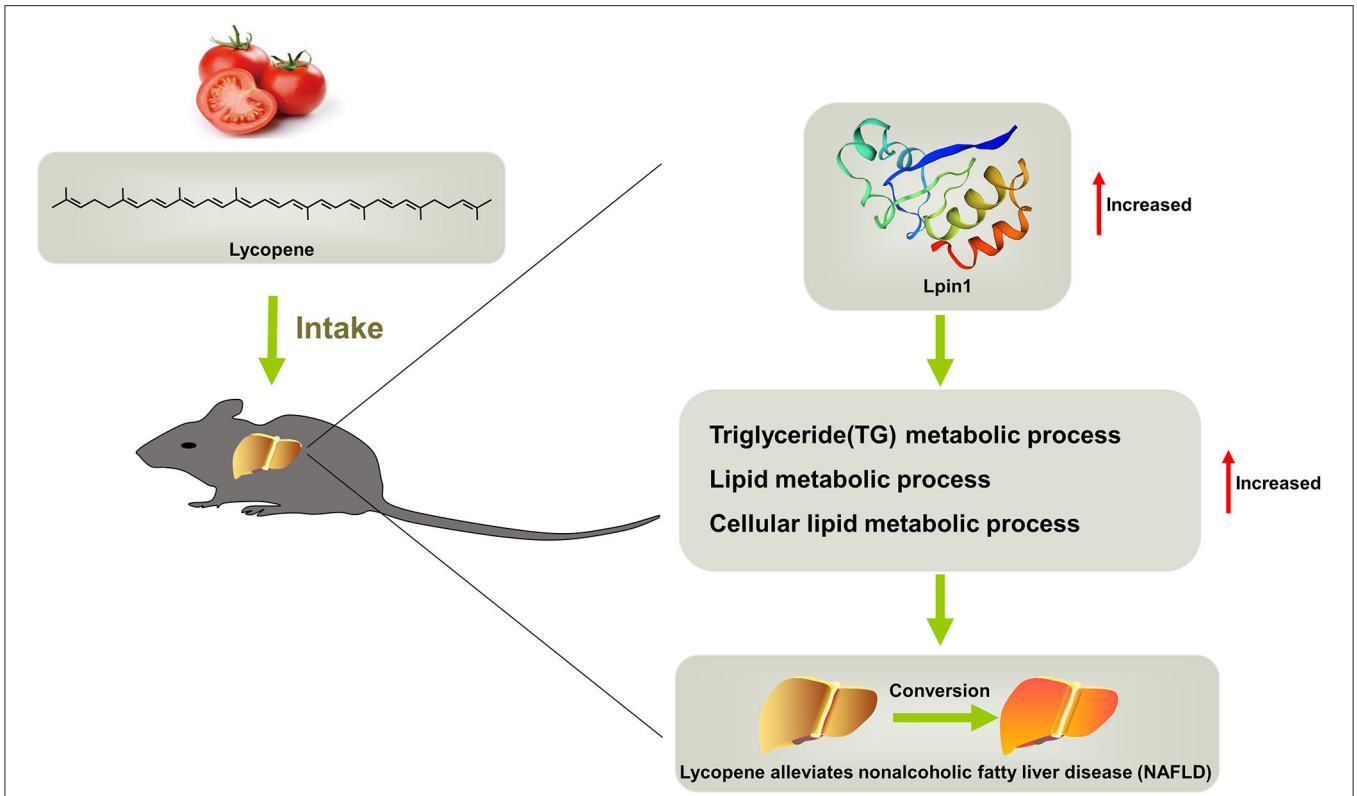


FIGURE 7 | Schematic representation of the mechanisms that mediate lycopene-provided protection against NAFLD.

functional genes, Ribo-seq was performed, and 505 upregulated and 853 downregulated DEGs were identified (**Figure 3D**). KEGG enrichment analysis of the 1,358 DEGs from Ribo-seq showed that the top 20 pathways included the NAFLD pathway (**Figure 4B**), suggesting that lycopene plays a role in the improvement of NAFLD by affecting the translational expression of related genes. By combining 1,127 DEGs in transcriptomics with 1,358 DEGs in translatomics, the functional genes of interest were narrowed down to 112 DEGs that regulated in the same direction at transcriptional and translational levels (**Figure 5C**). Translational responses contribute to the establishment of complex genetic regulation, which cannot be achieved by controlling transcription alone (43).

Protein–protein interaction analysis indicated that lycopene improved hepatic steatosis by regulating the TG metabolic process and lipid metabolic process, mainly regulating the expression of LPIN1 (**Figure 6A**). LPIN1 can activate mitochondrial fatty acid oxidative metabolism by inducing the expression of the nuclear peroxisome proliferator-activated receptor α (44). In addition, the protein encoded by LPIN1 is involved in the regulation of lipid metabolism in the liver and is associated with the phenotype of fatty liver dystrophy mice (45). Free fatty acids are the major sources of TG stored in the liver. The imbalance of fatty acid absorption and processing is a key factor leading to fat accumulation in the liver (46). Lycopene may play a lipid-lowering role by promoting triglyceride metabolism.

CONCLUSIONS

Based on the results, we concluded that lycopene can effectively alleviate liver steatosis induced by HFD, and can be used as a possible dietary strategy for the control and treatment of NAFLD. This beneficial lipid-lowering effect is due to lycopene increasing the expression of genes related to liver lipid metabolic process at transcriptional and translational levels (**Figure 7**). Our study fills the gap in the translation profiling of lycopene in the process of alleviating hepatic steatosis, which provides a reasonable molecular regulatory mechanism for this phenotype. However, it has not yet formed an in-depth and systematic regulatory network, which is worth exploring in the next step. In addition, our combined analysis based on transcriptomics and translatomics provides a new way to narrow the range of key functional genes, contributing to a better understanding of the mechanisms involved. This provides a new direction for the further research of the relationship between lycopene and lipid metabolism.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE178322, GSE178322.

ETHICS STATEMENT

The animal study was reviewed and approved by the Committee on the Ethics of Animal Experiments of Guangxi University (No. GXU2019-063).

AUTHOR CONTRIBUTIONS

TH, JY, LZ, and GX conceived the project and design the protocol. ZM, SL, ZL, WM, and LY performed the experiments. TH, KL, DY, ZS, and YL performed the data analysis. TH, QF, LZ, and GX wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by grants from the National Key R&D Program of China (2018YFD0500402), Guangxi Science Foundation for Distinguished Young Scholars (2020GXNSFFA297008), Guangxi Science and Technology Base and Talents Project (AD18281085), Guangxi Natural Science Foundation (2019GXNSFDA245029), Scientific Research and Technology Development Major Project of Nanning (20192004-1), Scientific Research and Technological Development Program of Yongning District of Nanning City (20180101B), State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources (SKLCUSA-a202006), and Training Project of High-level Professional and Technical Talents of Guangxi University.

ACKNOWLEDGMENTS

We would like to thank Shenzhen Chi-Biotech Co. Ltd. for invaluable support on the RFPs extraction experiment in terms of method optimization and for their assistance on Ribo-seq data analysis.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.727785/full#supplementary-material

Supplementary Figure 1 | KEGG pathway diagram, **(A)** oxidative phosphorylation pathway. **(B)** Non-alcoholic fatty liver disease pathway. Red represents DEGs.

Supplementary Figure 2 | Principal component analysis (PCA) in RNA-seq.

Supplementary Figure 3 | Volcano plots of DEGs in RNA-seq, $|\log_2$ fold change$|>1$ and $p < 0.01$.

Supplementary Figure 4 | The scatter plot of correlation between mRNA abundance and RPF abundance. Red: HFD; blue: LYC.

Supplementary Table 1 | Nutritional composition of mice feed.

Supplementary Table 2 | Differentially expressed genes in Ribo-seq.

Supplementary Table 3 | GO biological process of DEGs in Ribo-seq.

Supplementary Table 4 | KEGG pathways of DEGs in Ribo-seq.

Supplementary Table 5 | Differentially expressed genes in RNA-seq.

Supplementary Table 6 | The gene information in different quadrants.

REFERENCES

1. Nguyen P, Leray V, Diez M, Serisier S, Le Bloc'H J, Siliart B, et al. Liver lipid metabolism. *J Anim Physiol Anim Nutr.* (2008) 92:272–83. doi: 10.1111/j.1439-0396.2007.00752.x
2. Jian T, Yu C, Ding X, Chen J, Li J, Zuo Y, et al. Hepatoprotective effect of seed coat ofeuryale ferox extract in non-alcoholic fatty liver disease induced by high-fat diet in mice by increasing IRs-1 and inhibiting CYP2E1. *J Oleo Sci.* (2019) 68:581–9. doi: 10.5650/jos.ess19018
3. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther.* (2011) 34:274–85. doi: 10.1111/j.1365-2036.2011.04724.x
4. Komine S, Akiyama K, Warabi E, Oh S, Kuga K, Ishige K, et al. Exercise training enhances *in vivo* clearance of endotoxin and attenuates inflammatory responses by potentiating Kupffer cell phagocytosis. *Sci Rep.* (2017) 7:11977. doi: 10.1038/s41598-017-12358-8
5. Moore MP, Cunningham RP, Dashek RJ, Mucinski JM, Rector RS. A Fad too Far? Dietary strategies for the prevention and treatment of NAFLD. *Obesity.* (2020) 28:1843–52. doi: 10.1002/oby.22964
6. Grabowska M, Wawrzyniak D, Rolle K, Chomczyński P, Oziewicz S, Jurga S, et al. Let food be your medicine: nutraceutical properties of lycopene. *Food Funct.* (2019) 10:3090–102. doi: 10.1039/C9FO00580C
7. Li N, Wu X, Zhuang W, Xia L, Chen Y, Wu C, et al. Tomato and lycopene and multiple health outcomes: umbrella review. *Food Chem.* (2021) 343:128396. doi: 10.1016/j.foodchem.2020.128396
8. Kawata A, Murakami Y, Suzuki S, Fujisawa S. Anti-inflammatory activity of β-carotene, lycopene and Tri-n-butylborane, a scavenger of reactive oxygen species. *In vivo.* (2018) 32:255–64. doi: 10.21873/invivo.11232
9. Zeng Z, He W, Jia Z, Hao S. Lycopene improves insulin sensitivity through inhibition of STAT3/Srebp-1c-mediated lipid accumulation and inflammation in mice fed a high-fat diet. *Exp Clin Endocrinol Diabetes.* (2017) 125:610–7. doi: 10.1055/s-0043-101919
10. Jhou BY, Song TY, Lee I, Hu ML, Yang NC. Lycopene inhibits metastasis of human liver adenocarcinoma SK-Hep-1 cells by downregulation of NADPH oxidase 4 protein expression. *J Agric Food Chem.* (2017) 65:6893–903. doi: 10.1021/acs.jafc.7b03036
11. Lindshield BL, Canene-Adams K, Erdman JJ. Lycopenoids: are lycopene metabolites bioactive? *Arch Biochem Biophys.* (2007) 458:136–40. doi: 10.1016/j.abb.2006.09.012
12. Ahn J, Lee H, Jung CH, Ha T. Lycopene inhibits hepatic steatosis via microRNA-21-induced downregulation of fatty acid-binding protein 7 in mice fed a high-fat diet. *Mol Nutr Food Res.* (2012) 56:1665–74. doi: 10.1002/mnfr.201200182
13. Chung J, Koo K, Lian F, Hu KQ, Ernst H, Wang XD. Apo-10'-lycopenoic acid, a lycopene metabolite, increases sirtuin 1 mRNA and protein levels and decreases hepatic fat accumulation in ob/ob mice. *J Nutr.* (2012) 142:405–10. doi: 10.3945/jn.111.150052
14. Elvira-Torales LI, Navarro-González I, González-Barrio R, Martín-Pozuelo G, Doménech G, Seva J, et al. Tomato juice supplementation influences the gene expression related to steatosis in rats. *Nutrients.* (2018) 10:1215. doi: 10.3390/nu10091215
15. Sousa MJ, Liu X, Oke A, Arora R, Franciosi F, Viville S, et al. DAZL and CPEB1 regulate mRNA translation synergistically during oocyte maturation. *J Cell Sci.* (2016) 129:1271–82. doi: 10.1242/jcs.179218
16. Miettinen TP, Kang JH, Yang LF, Manalis SR. Mammalian cell growth dynamics in mitosis. *Elife.* (2019) 8:e44700. doi: 10.7554/eLife.44700

17. Fujii K, Shi Z, Zhulyn O, Denans N, Barna M. Pervasive translational regulation of the cell signalling circuitry underlies mammalian development. *Nat Commun.* (2017) 8:14443. doi: 10.1038/ncomms14443
18. Liu M, Ge R, Liu W, Liu Q, Xia X, Lai M, et al. Differential proteomics profiling identifies LDPs and biological functions in high-fat diet-induced fatty livers. *J Lipid Res.* (2017) 58:681–94. doi: 10.1194/jlr.M071407
19. Lu Y, Shao M, Xiang H, Zheng P, Wu T, Ji G. Integrative transcriptomics and metabolomics explore the mechanism of kaempferol on improving nonalcoholic steatohepatitis. *Food Funct.* (2020) 11:10058–69. doi: 10.1039/D0FO02123G
20. Gygi SP, Rochon Y, Franza BR, Aebersold R. Correlation between protein and mRNA abundance in yeast. *Mol Cell Biol.* (1999) 19:1720–30. doi: 10.1128/MCB.19.3.1720
21. Maier T, Güell M, Serrano L. Correlation of mRNA and protein in complex biological samples. *FEBS Lett.* (2009) 583:3966–73. doi: 10.1016/j.febslet.2009.10.036
22. Ingolia NT, Ghaemmaghami S, Newman JR, Weissman JS. Genome-wide analysis *in vivo* of translation with nucleotide resolution using ribosome profiling. *Science.* (2009) 324:218–23. doi: 10.1126/science.1168978
23. Liu S, Yang D, Yu L, Aluo Z, Zhang Z, Qi Y, et al. Effects of lycopene on skeletal muscle-fiber type and high-fat diet-induced oxidative stress. *J Nutr Biochem.* (2021) 87:108523. doi: 10.1016/j.jnutbio.2020.108523
24. Zhao B, Liu H, Wang J, Liu P, Tan X, Ren B, et al. Lycopene supplementation attenuates oxidative stress, neuroinflammation, and cognitive impairment in aged CD-1 mice. *J Agric Food Chem.* (2018) 66:3127–36. doi: 10.1021/acs.jafc.7b05770
25. Qi Y, Zhang Z, Liu S, Aluo Z, Zhang L, Yu L, et al. Zinc supplementation alleviates lipid and glucose metabolic disorders induced by a high-fat diet. *J Agric Food Chem.* (2020) 68:5189–200. doi: 10.1021/acs.jafc.0c01103
26. Lian X, Guo J, Gu W, Cui Y, Zhong J, Jin J, et al. Genome-wide and experimental resolution of relative translation elongation speed at individual gene level in human cells. *PLoS Genet.* (2016) 12:e1005901. doi: 10.1371/journal.pgen.1005901
27. Xiao CL, Mai ZB, Lian XL, Zhong JY, Jin JJ, He QY, et al. FANSe2: a robust and cost-efficient alignment tool for quantitative next-generation sequencing applications. *PLoS ONE.* (2014) 9:e94250. doi: 10.1371/journal.pone.0094250
28. Huang T, Yu J, Luo Z, Yu L, Liu S, Wang P, et al. Translatome analysis reveals the regulatory role of betaine in high fat diet (HFD)-induced hepatic steatosis. *Biochem Bioph Res Commun.* (2021) 575:20–7. doi: 10.1016/j.bbrc.2021.08.058
29. Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat Methods.* (2008) 5:621–8. doi: 10.1038/nmeth.1226
30. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics.* (2010) 26:139–40. doi: 10.1093/bioinformatics/btp616
31. Wan X, Yang Z, Ji H, Li N, Yang Z, Xu L, et al. Effects of lycopene on abdominal fat deposition, serum lipids levels and hepatic lipid metabolism-related enzymes in broiler chickens. *Anim Biosci.* (2021) 34:385–92. doi: 10.5713/ajas.20.0432
32. Ota T. Prevention of NAFLD/NASH by Astaxanthin and β-Cryptoxanthin. *Adv Exp Med Biol.* (2021) 1261:231–8. doi: 10.1007/978-981-15-7360-6_21
33. Lee Y, Hu S, Park YK, Lee JY. Health benefits of carotenoids: a role of carotenoids in the prevention of non-alcoholic fatty liver disease. *Prev Nutr Food Sci.* (2019) 24:103–13. doi: 10.3746/pnf.2019.24.2.103
34. Zhao J, Qin B, Nikolay R, Spahn C, Zhang G. Translatomics: the global view of translation. *Int J Mol Sci.* (2019) 20:212. doi: 10.3390/ijms20010212
35. Ingolia NT, Brar GA, Rouskin S, McGeachy AM, Weissman JS. The ribosome profiling strategy for monitoring translation *in vivo* by deep sequencing of ribosome-protected mRNA fragments. *Nat Protoc.* (2012) 7:1534–50. doi: 10.1038/nprot.2012.086
36. Kondo T, Plaza S, Zanet J, Benrabah E, Valenti P, Hashimoto Y, et al. Small peptides switch the transcriptional activity of Shavenbaby during Drosophila embryogenesis. *Science.* (2010) 329:336–9. doi: 10.1126/science.1188158
37. Anderson DM, Anderson KM, Chang CL, Makarewich CA, Nelson BR, McAnally JR, et al. A micropeptide encoded by a putative long noncoding RNA regulates muscle performance. *Cell.* (2015) 160:595–606. doi: 10.1016/j.cell.2015.01.009
38. Zhang M, Huang N, Yang X, Luo J, Yan S, Xiao F, et al. A novel protein encoded by the circular form of the SHPRH gene suppresses glioma tumorigenesis. *Oncogene.* (2018) 37:1805–14. doi: 10.1038/s41388-017-0019-9
39. Lauressergues D, Couzigou JM, Clemente HS, Martinez Y, Dunand C, Bécard G, et al. Primary transcripts of microRNAs encode regulatory peptides. *Nature.* (2015) 520:90–3. doi: 10.1038/nature14346
40. Schafer S, Adami E, Heinig M, Rodrigues K, Kreuchwig F, Silhavy J, et al. Translational regulation shapes the molecular landscape of complex disease phenotypes. *Nat Commun.* (2015) 6:7200. doi: 10.1038/ncomms8200
41. Schwanhäusser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, et al. Global quantification of mammalian gene expression control. *Nature.* (2011) 473:337–42. doi: 10.1038/nature10098
42. Nedialkova DD, Leidel SA. Optimization of codon translation rates via tRNA modifications maintains proteome integrity. *Cell.* (2015) 161:1606–18. doi: 10.1016/j.cell.2015.05.022
43. Loya CM, Van Vactor D, Fulga TA. Understanding neuronal connectivity through the post-transcriptional toolkit. *Genes Dev.* (2010) 24:625–35. doi: 10.1101/gad.1907710
44. Finck BN, Gropler MC, Chen Z, Leone TC, Croce MA, Harris TE, et al. Lipin 1 is an inducible amplifier of the hepatic PGC-1alpha/PPARalpha regulatory pathway. *Cell Metab.* (2006) 4:199–210. doi: 10.1016/j.cmet.2006.08.005
45. Péterfy M, Phan J, Xu P, Reue K. Lipodystrophy in the fld mouse results from mutation of a new gene encoding a nuclear protein, lipin. *Nat Genet.* (2001) 27:121–4. doi: 10.1038/83685
46. Musso G, Gambino R, Cassader M. Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD). *Prog Lipid Res.* (2009) 48:1–26. doi: 10.1016/j.plipres.2008.08.001

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Huang, Yu, Ma, Fu, Liu, Luo, Liu, Yu, Miao, Yu, Song, Li, Zhou and Xu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 11 January 2022
doi: 10.3389/fnut.2021.810812

Nutritional Characterization of a Traditional Cultivar of Tomato Grown Under Organic Conditions—cv. "Malacara"

María D. Raigón[1]*, *María D. García-Martínez*[1] and *Octavian P. Chiriac*[2]

[1] *Instituto de Conservación y Mejora de la Agrobiodiversidad Valenciana/Departamento de Química, Universitat Politècnica de València, Valencia, Spain,* [2] *Department of Agricultural, Forest and Food Sciences, University of Turin, Turin, Italy*

OPEN ACCESS

Edited by:
José Pinela, Polytechnic Institute of Bragança (IPB), Portugal

Reviewed by:
Cristina Patanè, National Research Council (CNR), Italy
László Csambalik, Hungarian University of Agriculture and Life Sciences, Hungary

***Correspondence:**
María D. Raigón
mdraigon@qim.upv.es

Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *07 November 2021*
Accepted: *09 December 2021*
Published: *11 January 2022*

Citation:
Raigón MD, García-Martínez MD and Chiriac OP (2022) Nutritional Characterization of a Traditional Cultivar of Tomato Grown Under Organic Conditions—cv. "Malacara". Front. Nutr. 8:810812. doi: 10.3389/fnut.2021.810812

The loss of genetic diversity due to the replacement of local tomato (*Solanum lycopersicum* L.) varieties by improved cultivars has been mitigated in many cases by the good work of organic farmers in maintaining local agricultural biodiversity. In parallel to these initiatives, in recent years, consumers have developed an increasing awareness of both food-related health, environmental issues, and food demand to recover the flavors of the past. In the case of tomatoes, these attributes (nutritional, organoleptic, social, and environmental) are closely related to organic production using local varieties. "Malacara" tomato is an example of a local variety. Coming from Sierra de Cádiz, it is a varietal type called "Cuelga" ("for hanging," because the tomato trusses are hung from beams in the farmhouses). Cultivated and harvested in the open air during the summer months, these tomatoes are commercialized and consumed in the winter. Historically, this variety has enabled the fresh consumption of tomatoes during the winter, without the need to force cultivation. It is highly appreciated in the local cuisine and is the basis for sauces figuring in typical dishes. Its characteristic traits are small, pallid fruits, and long shelf life. The main objective of this work has been to typify two Malacara tomato cultivars (red and yellow color) grown under organic farming conditions, through the characterization of morphological, nutritional, and volatile parameters. The main differences are due to morphological parameters (fruit weight and color of the exocarp and endocarp). Other characteristics such as the content of ash, fiber, moisture, the concentration of iron, magnesium, and calcium, and content of lycopene are different between both cultivars. This study provides information on the nutritional and aromatic composition of two Malacara tomato cultivars, differentiated by their color and grown under organic farming conditions. The results add value to the native horticultural heritage and can aid in the selection of tomato varieties suitable for a sustainable production system and to produce tomatoes with high nutritional value and rich in aroma.

Keywords: biodiversity, flavors of the past, for hanging, red exocarp, yellow exocarp

INTRODUCTION

Plant genetic resources for food and agriculture, and specifically, traditional varieties, play an important role in the sustainability and security of the global food system. Traditional varieties are an essential component of agricultural biodiversity, guaranteeing agricultural production adapted to the territory and ensuring the livelihood of a large proportion of people who depend on agriculture (1). Genetic diversity within crop species is wide. Germplasm grown under local environmental conditions can be optimized for small regional production areas that adjust to prevailing environmental and climatic patterns. However, in recent years there has been the phenomenon of genetic erosion within species, that is, "the loss of individual genes and the loss of particular combinations of genes, such as those manifested in locally adapted varieties" (2). This erosion has been supported for the contemporary plants breeding investigations, more focused on increasing the productivity of some crop species, than on enhancing cultivated genetic diversity (3). The work that small farmers have traditionally carried out in the conservation of genetic material adapted to local conditions (soil, climate, and consumption) must be highlighted, and in particular, the important role of organic farmers (4).

The health of people and the planet are at critical moments. There are synergies between intensivist global food systems and phenomena such as climate change, malnutrition, and obesity. Coexisting with scenarios of global loss of biodiversity, instability in the planet's natural systems, limits of the phosphorus and nitrogen cycles, lack of water resources, along with social and economic disturbances (5). The alternatives to these issues go through the development of a sustainable food system, including organically grown food. And consequently, the area devoted to the organic crop increases, ∼2% annually worldwide (6). In 2019, Spain was the third country in the world with the largest organic area, behind Australia and Argentina. Organic horticultural production, in Spain, is in the fifth position, behind cereal, olive tree, dried fruit, vineyard, and legume acreage. In line with this trend, an increasing proportion of tomato (*Solanum lycopersicum* L.) production is organically grown. The continuous growth in Spain of the agricultural area under organic production responds to the marked increase in organic consumption in European markets, and also, the growing demand in the domestic market. The main commercialization channels for autochthonous varieties are the local markets, better adapted to specific agro-climatic conditions, and especially recommended for organic agriculture. This phenomenon is observed with greater intensity in exquisite crops such as tomatoes (7, 8).

Tomato is an excellent source of nutrients and bioactive antioxidant compounds that are important for human health, including minerals, vitamins C and E, β-carotene, lycopene, flavonoids, organic acids, phenolics, and chlorophyll (9, 10). Some of the tomato components mentioned above have antioxidant properties (11), while others, such as sodium, potassium, magnesium, calcium, manganese, copper, zinc, and iodine, may reduce the risk of cardiovascular diseases (12) and its organic acids that may contribute to maintaining acid-base balance (13). The chemical composition of the tomato fruit depends on factors such as crop system, fruit maturity, environmental conditions (soil and climate), and the cultivation method in which the plants are grown (14–16). The results regarding the research on the effects of organic and conventional production on tomato quality are sometimes contradictory. In terms of quality, some studies report better taste, higher vitamin C contents, and higher levels of other quality-related compounds for organically grown tomatoes (17–19).

The identification of cultivars with high nutritive value represents a useful approach to selecting tomato cultivars with better quality and health-promoting properties. The diversity of tomatoes rapidly declined during the 20th century as a result of the industrialization of agriculture and the advance of plant breeding programs. Part of this diversity was collected and conserved in germplasm banks. The Spanish National Inventory contains 2,634 accessions of tomato conserved in different Spanish institutions. Several efforts have been made to characterize Spanish tomato materials (20, 21), there are even works on the characterization of tomato cultivars under organic farming conditions (22) and works that have characterized some varieties of tomato called "Cuelga" (23) or "for hanging" because the tomato trusses are hung from beams in the farmhouses. These types of tomatoes are grown and harvested during the natural cycle, in the open air during the warm months, and are commercialized and consumed in the winter. Historically, these various types have enabled the fresh consumption of tomatoes during the winter, without the need to force cultivation in greenhouse conditions. "Cuelga" tomatoes are a varietal type, which comprises a set of cultivars (integrated by inbreds) with great heterogeneity in fruit morphology. It is highly appreciated in the local cuisine and is the basis for sauces figuring in typical dishes. The characteristic traits are small, pallid fruits and long shelf life. In this context, this study mainly aims to characterize two Malacara tomato cultivars, differentiated by the red and yellow exocarp color, grown under organic farming conditions, through the characterization of physical parameters and nutritional composition. The literature shows the typification of other "Cuelga" tomatoes, but it is the first time that the nutritional composition of Malacara tomatoes has been characterized, identifying characteristics between the fruits of the two cultivars of different colors.

MATERIALS AND METHODS

Plant Material

Tomato crop was carried out during the summer in 2019 in the "La Verde" cooperative in Villamartín (Cadiz, Spain) (36° 52′ 0″ N, 5° 38′ 0″ W). The cooperative has been involved with organic agriculture certificates for 33 years. Its agricultural activity is mainly directed toward horticultural production. The cooperative has three hectares to produce nectarines, apples, pears, plums, citrus, and figs, and one hectare of olive grove, which is also needed to increase biodiversity. The rest of the area (ten hectares) is divided into 14 plots for the cultivation of horticulture species, which vary according to the season, following a precise crop rotation. The cooperative promotes re-seeding and seed exchange, understood as a model of *ex*

situ conservation in the country, involving the maintenance of varieties by their cultivation and closing cycles. At present, its seed bank contains over 250 varieties available in some 40 different species (24), including among the traditional varieties, the Malacara tomato seeds, which they grow for the local market. Although the local varieties have a high genotypic heterogeneity, the Malacara tomato has been in the "La Verde cooperative" for ∼25 years ago, as a result of seed exchange between local producers. It is possible that the lack of specialized markets has caused this tomato to spread little by other farmers. But the specific conditions of the cooperative (strong commitment to indigenous seeds, conservative tradition, local markets, and diversification) have made it possible to maintain these cultivars and introduce them annually on the market. Therefore, although the origin of the samples is from a single source, it is very robust to achieve the objectives.

The Malacara tomato was grown outdoors in the 2019 harvest, following organic farming methods, in clay-loam soil, fertilized with sheep manure in quantities of 2 kg of manure per m^2 and year. Plantlets were raised in seedling trays filled with organic compost and kept in an insect-free climate chamber until transplanted. No fertilization or phytosanitary treatments were applied before the transplant. Plants were cultivated in the summer cycle. The seedbed was carried out in February and the transplant to the field was carried out in April. A month later, the training of the plants is done. 1,000 tomato plants were grown, of different varieties, and 200 plants were from Malacara (100 for each cultivar and plot). The plants were spaced 1.2 m between rows and 0.45 m within the row. A total of 8 L of water per plant and week, in the pre-fruiting phase, were applied by a drip irrigation system, decreasing the frequency after fruiting. The average temperatures for the period were 15°C minimum and 30°C maximum. Weeding was done manually between plants, with three applications throughout the growing cycle, and mechanically between lines, with one application. Phytosanitary treatments consisted of the authorized use of sulfur powder as a preventive treatment. Harvesting of the tomato fruits began in June and continued until the end of October. In both cultivars, common practices of transplanting, weeding, training, pruning, and harvesting were similar.

Samples of tomatoes are shown in **Figure 1**. Tomato fruits were collected homogeneously, by hand, selecting fruits from the central plants of the plot. The fruits come from two harvests made commercially ripe, in early September. Of the collected fruits, ∼100 sample fruits of Malacara tomatoes by each color were supplied for analysis.

For composition determinations, the fruits were transversely cut in half, and the seeds were eliminated. One-half of the tomatoes were grouped and squeezed with a domestic extractor for the analysis of pH, titratable acidity, contents in soluble solids, reducing sugars (glucose, fructose), vitamin C, carotenoids, total polyphenols, and total antioxidant capacity analysis. The other tomato halves were homogenized, and one aliquot was dried for the proximate and mineral determination. Another fraction of fresh tomato was immediately freezing, in a vial and hermetically closed, for subsequent determination of volatile components. When using a headspace chromatography methodology, the volatile components are not significantly altered.

Morphological Parameters

The morphological parameters such as tomato dimensions (unit weight, smaller diameter, larger diameter, smaller height, larger height) and color parameters of exocarp, mesocarp, and endocarp were monitored on 60 tomatoes by cultivar. The fruit weights were measured with an analytical balance (CB-Junior, Cobos) with an accuracy of ± 0.01 g. The fruit's dimensions were measured using an electronic digital slide gauge (model CD-15 DC; Mitutoyo (UK) Ltd, Telford, UK) to within 0.01 mm accuracy. Color measurement in CieLab space (25) was carried out using a colorimeter (Konica Minolta CR-300, Photo Imaging Inc., Mahwah, NJ, USA). In each measurement the values of the three coordinates (L^*, a^*, b^*) are obtained which, combined with each other, gives rise to the color index. The Chroma (C) of each fruit is calculated using the formula: $C = \sqrt{a^2 + b^2}$

The Hue-Angle (H) of each fruit is calculated using the formula: $H = \tan^{-1}(\frac{b}{a})$

The color index (CI) of each fruit is calculated using the formula: $CI = \frac{1000^* a}{L^* b}$

The C shows the greater or lesser saturation toward that certain color. A high value of C is a highly saturated color. A value of zero for C indicates an achromatic stimulus. Hue (H) is the property of color associated with the dominant wavelength. With CI values close to 0, the yellow tones are evaluated, and values close to 20, the red tones are evaluated (26).

Nutritional Parameters

Proximate composition was carried out by official methods: moisture (AOAC 984.25), proteins (AOAC 984.13), fat (AOAC 983.23), fiber (AOAC 991.43), and ashes (AOAC 923.03). The carbohydrate (CH) content was calculated by difference. The energy was calculated by multiplying by 9 kcal the grams of fat and 4 kcal the grams of protein and carbohydrates each 100 g of tomato. The final results are expressed as g·100 g^{-1} of fresh weight (fw).

The mineral composition was determined by previous digestion of the samples to the method Association of Official Analytical Chemists (AOAC) 985.35. The samples were calcined in a Carbolite CWF 1100 muffle at 550°C, the ashes were dissolved and settled with concentrated HCl until a 2% HCl solution. The calibration curves were made by diluting the standards to the specific concentrations for each element. The analytical curves were obtained with a linear response for the selected concentration ranges. Mineral analysis was performed by atomic absorption spectroscopy, in a Thermo elemental AAseries spectrometer, software v.11.03, and hollow cathode lamps for each element, except for the phosphor, which was analyzed by colorimetry (27) using a UviLine 9400 (Schott Instruments) spectrometer. The determination of the soluble solids content (SSC) present in the tomato juice was carried out by refractometric techniques (27). The material used in this determination is in a hand-held refractometer with a range of 0–32 °Brix. The pH determination was made by direct potentiometric measurement of the homogenized tomato juice

FIGURE 1 | Red and yellow type of tomatoes cv. Malacara.

with pH & Ion-metro GLP 22 (CRISON). The determination of the total acidity (TA) consists of the potentiometric titration of the sample with an alkaline solution (0.5 *N* NaOH) up to pH = 8.1 of the acidity of the tomato juice (27). The results are expressed in grams of citric acid for 100 g of sample. The taste index of tomato (S), expressed in percentage, is based on the soluble solids content and total acidity content of fruit (28) and is determined by the expression: $S = TA + \frac{SSC}{20 * TA}$. The optimal value for flavor balance is considered when the taste index is higher than 1.

Contents in reducing sugars (fructose and glucose, F+G) were determined by titration with thiosulfate (27). The vitamin C (Vit C) concentration in the tomato juice was determined by potentiometric titration with chloramine-T, using an automatic titration equipment (702 SM Titrino, Metrohm, Herisau, Switzerland), the results were expressed as mg 100 g^{-1} fw of tomato. A UviLine 9400 (Schott Instruments) spectrometer was used to measure the absorbance for carotenoids, polyphenols, and antioxidants. The carotenoids, lycopene (Ly) and β-carotene (β-car), were determined by extraction in darkness with ethanol:hexane (4:3 v/v) and UV/V spectrophotometry following the protocol developed by Zscheille and Porter (29) with the modification of Russeaux et al. (30). For the total carotenoids (TC), the absorbance of the samples at 452 and 510 nm wavelengths were measured. Results were expressed as μg 100 g^{-1} fw. Total polyphenols (TP) were determined in an aliquot of the methanolic extract by a modification of the Folin–Ciocalteu assay, according to a previously published protocol (31), using gallic acid as the reference standard. The results are expressed in mg of gallic acid for 100 g of fruit fresh (mg EAG 100 g^{-1} fw). To measure the effect of the extract on the DPPH (2,4-dinitrophenylhydrazine) radical, the optimized method of Brand-Williams (32) adapted to tomatoes was used. This measure of the total antioxidant (AOT) capacity, it is carried employing methanol:HCl (99:1) as a dissolvent. The results are expressed in an activity equivalent to Trolox (μmol 100 g^{-1} of fresh fruit weight).

Volatile Composition

Headspace/solid-phase microextraction (HS/SPME) was used for the extractions of the volatile fraction according to the methodology of Moreno et al. (33). For the analysis, 2 g of fruit mesocarp per sample were put into a 20 mL sealed headspace vial, which was pre-incubated at 40°C for 30 min. After that, 40 min of extraction at 40°C was carried out, using an SPME fiber Divinylbenzene/Carboxen/Polydimethylsiloxane

(DVB/CAR/PDMS, 50/30 μm) (Supelco, Bellefonte, PA, USA) which was introduced into the vial headspace and adsorbed the volatiles. Then, the thermal desorption of the fiber was performed in the splitless mode at 250°C for 30 s in the gas chromatograph injection port. The analysis of volatiles was performed using a 6890 N Network Agilent gas chromatograph coupled by a Life-T-effluent (1:1) to a 5973 Inert Mass Selective detector (Agilent Technologies, Santa Clara, CA, USA). A silica capillary column (stationary phase: 5% phenyl, 95% dimethylpolysiloxane; 30 m × 0.25 mm × 0.25 μm) was used and helium was employed as carrier gas (1 mL·min^{-1}). MSD ChemStation D.02.00.275 (Agilent Technologies) was used to perform the chromatograms and mass spectra. For the identification of volatile compounds, a comparison of their mass spectra and GC retention time with reference commercial standards were carried out (RS. Sigma-Aldrich, Saint Louis, MO, USA), or tentatively by comparison of the mass spectrum (MS) with the NIST Mass Spectral library (MS Search 2.0) according to other bibliographic data or data from our own research library. For quantification, a total ion current chromatogram (TIC) was employed to integrate the peak area of each compound similarly to other previous works (34). Total volatiles and each individual volatile was studied on the basis of the GC-peak areas (traces values were not considered).

TABLE 1 | Morphological parameters for the two types of Malacara tomatoes (mean ± SD, $n = 30$ and p-value).

Parameter	Malacara Tomato		*P*-value
	Yellow	Red	
Fruit weight (g)	30.77 ± 10.18	53.23 ± 16.69	0.0000
Fruit width (mm)	38.35 ± 4.99	45.55 ± 4.84	0.0000
Fruit length (mm)	35.17 ± 3.65	42.04 ± 5.25	0.0000
L* exocarp	70.86 ± 2.05	63.57 ± 2.17	0.0000
a* exocarp	0.80 ± 0.50	15.11 ± 1.79	0.0000
b* exocarp	27.20 ± 2.36	15.68 ± 1.66	0.0000
C exocarp	27.21 ± 2.34	21.83 ± 1.95	0.0000
He exocarp	0.03 ± 0.02	0.77 ± 0.07	0.0000
CI exocarp	0.44 ± 0.32	15.32 ± 2.28	0.0000
L* mesocarp and endocarp	67.95 ± 2.65	59.01 ± 2.02	0.0000
a* mesocarp and endocarp	0.93 ± 0.64	14.98 ± 2.34	0.0000
b* mesocarp and endocarp	26.63 ± 3.08	16.87 ± 1.75	0.0000
C mesocarp and endocarp	26.65 ± 3.06	22.61 ± 2.44	0.0000
H mesocarp and endocarp	0.04 ± 0.03	0.72 ± 0.07	0.0000
CI mesocarp and endocarp	0.53 ± 0.41	15.13 ± 2.28	0.0000

Statistical Analysis

Every parameter analyzed was done in triplicate. Data obtained were processed using Statgraphics Plus version 5.1 software (StatPoint Technologies, Warrenton, VA, USA) which computed the means and standard errors. Differences between the effects of type, red (R) and yellow (Y) were tested using an ANOVA one-way analysis, and differences among groups were identified with the F-test and the Kruskal-Wallis test which compares medians instead of means at the 95.0% confidence level. The Kruskal-Wallis test tests the null hypothesis that the medians of parameters within each of the two types of Malacara tomato are the same. And it requires the fulfillment that the populations are normal, independent, and present homoscedasticity. Pearson's linear coefficients of correlation (r) between traits were calculated ($n = 6$) from regression analyzes between pairs of traits.

RESULTS

Morphological Parameters

Table 1 shows the results of the morphological parameters in Malacara tomato's fruits red and yellow. Statistically highly significant differences were found between all the morphological parameters (size and color) between all the two types of Malacara tomatoes studied. The red type was characterized for more variability for the morphological parameter than the yellow type. Red-type tomatoes are larger in size. On average the differences in weight are ∼22.5 g, the average differences in diameter are 7.2 mm, and the differences in height are 6.87 mm, in favor of red type Malacara tomatoes. The shape of the fruits is round, the width/length ratio is close to 1 (1.09 for yellow type and 1.03 for red type). For the color, in the L* it is seen that the exocarp of tomatoes present a measurement difference of 7.29, which means that the yellow Malacara tomatoes are significantly lighter than those of the red type. Also, the mesocarp and endocarp of the yellow-type tomatoes are more luminous, with differences of $\Delta L = 8.94$. The a* plane value for the exocarp, meso, and endocarp of tomatoes is significantly higher for Malacara red-type tomatoes, confirming visual appreciation. The significant differences between the values of plane b*, in the different parts of the tomato, also confirm the appreciation of the yellow color. The results show that the chroma, regardless of the part of the fruit, of the yellow Malacara tomatoes is significantly higher, that is yellow color is more marked than red. The tonality of the tomatoes is significantly higher, both in the exocarp, as well as in the meso and endocarp of the red Malacara tomatoes. The CI value close to 15 obtained for the peel and pulp in red Malacara tomato indicate a light shade of red or pink.

Nutritional Parameters

The nutritional fraction, proximate composition, and the content of each mineral studied, expressed in 100 g of the red and yellow, fresh Malacara tomatoes shown in **Table 2**. The total energy for each 100 g of fresh tomato is 46 kcal for yellow type and 36 kcal for red type. This greatest value is due to the high carbohydrates content (9.65 g 100 g^{-1} for yellow tomato and 7.16 g 100 g^{-1} for red tomato) and the lower moisture content of the yellow Malacara tomatoes since the calories from fat in the yellow tomato is 4.31% and in the case of red tomato 6.29%. Significant differences were found between the two types of tomatoes for, energy value, moisture, carbohydrates contain, and total fiber. No significant differences were found between the two types of tomatoes for protein and total fat. The most abundant mineral element in the Malacara tomato, regardless of color, is potassium, followed by sodium and magnesium for the yellow type, compared to phosphorus for red fruits, in third place. The most abundant trace element in the yellow tomato is iron,

TABLE 2 | Nutritional fraction and individual mineral content (100 g of fresh fruits) for the two types of Malacara tomatoes (mean ± SD, $n = 3$ and p-value).

Parameter	Malacara Tomato		*P*-value
	Yellow	Red	
Total energy (kcal)	45.96 ± 5.05	35.74 ± 1.80	0.0299
Moisture (g)	85.65 ± 1.15	89.26 ± 0.54	0.0081
Protein (g)	1.33 ± 0.18	1.20 ± 0.15	0.3951
Total fat (g)	0.22 ± 0.05	0.25 ± 0.03	0.3530
Fiber (g)	1.84 ± 0.13	1.03 ± 0.03	0.0005
Carbohydrates (g)	9.65 ± 0.98	7.16 ± 0.35	0.0144
Calcium (mg)	16.05 ± 0.49	13.01 ± 0.74	0.0041
Copper (mg)	0.108 ± 0.015	0.117 ± 0.008	0.5185
Iron (mg)	0.459 ± 0.027	0.339 ± 0.060	0.0349
Magnesium (mg)	31.41 ± 3.48	24.43 ± 1.48	0.0330
Phosphorus (mg)	27.04 ± 1.72	27.80 ± 2.49	0.6932
Potassium (mg)	833.59 ± 116.32	768.74 ± 23.13	0.3972
Sodium (mg)	55.68 ± 16.31	52.02 ± 13.73	0.7807
Zinc (mg)	0.351 ± 0.012	0.342 ± 0.030	0.6530

followed by zinc, and in the red tomato, zinc predominates over iron. Significant differences were found between the two types of tomatoes for calcium, iron, and magnesium content.

Table 3 shows the average content for the fruit traits evaluated related to tomato flavor and other bioactive characters, of the red and yellow Malacara tomatoes. The traits evaluated in the tomato Malacara demonstrate the importance of its consumption for aspects related to health and sensory attributes. The pH and the soluble solids content in fresh tomato are greatest value for yellow type (pH = 4.20 in yellow vs. pH = 4.14 in red Malacara tomato) and (6.20 °Brix in yellow vs. 5.87 °Brix in red Malacara tomato). The higher values of these parameters in the yellow tomato have, as a consequence, a higher value of the taste index. When considering the traits related to tomato flavor and other bioactive characters, no significant differences ($p > 0.05$) were found among the two types, except for lycopene, total carotenoids, and total antioxidant capacity (**Table 3**). The values of the parameters of bioactive components in the Malacara tomato are higher for the red type. For vitamin C, although the differences are not significant ($p = 0.1029$), the average contents are 12.4% higher (353.33 mg 100 g^{-1} fw in yellow and 403.33 mg 100 g^{-1} fw in red). Despite the existence of significant differences among means for the two types of Malacara tomato, for the flavor and other bioactive character traits, considerable variation was found within each of the types. In this respect, in most cases the ranges of variation overlap with the other value, the exception being the total antioxidant capacity, showing significant differences between yellow and red Malacara tomatoes, with slight increases for red tomatoes.

Volatile Composition

A total of 42 volatile compounds were found among the two types of Malacara tomato, grouped in 12 chemical families with quantitative and qualitative differences. **Table 4** shows the list of volatile compounds names, retention index (RI), identification method (RS: reference commercial standard, MS: comparison of the mass spectrum with NIST library and bibliographic data), aroma, and GC peak area total mean values. There are some volatile components that have only been found in the yellow tomato, specifically an aldehyde [(E)-2-hexenal)], three esters (methyl octanoate, isopentyl 3-methylbutanoate and methyl dihydrojasmonate), and two terpenes (carvone, α-thujene). Other components have only been detected in the red Malacara tomato, such as a monoterpene (perillene), four carotenoid components (β-cyclocitral, neral or β-citral, geranial or α-citral, and geranylacetone), and two polyphenolic derivatives (guaiacol and eugenol). In addition to the differences by compounds not detected between both types of tomato, statistically significant differences have been found for four monoterpenes (p-cymene, limonene, β-ocimene, and γ-terpinene), for the carotenoids 6-methyl-5-hepten- 2-one, for the phenol derivatives methyl salicylate, and the three nitrogen and sulfur compounds (2-Isobutylthiazole, pentane, 1-nitro and benzyl nitrile), in all cases, except for methyl salicylate, the concentrations are higher in yellow Malacara tomato.

In the aromatic profile of the yellow Malacara tomato, the major components (71.79% of its total volatile fraction) are the volatiles derived from nitrogen and sulfur compounds, mainly by 2-isobutylthiazole. Other volatile metabolites were monoterpenes (12.59%), aldehydes (7.24%), and 3.18% due to anethole (oxygenated benzene derivatives). The rest of the chemical families have a low percentage presence, without exceeding 0.67%. In the Malacara red tomato, the fraction of volatile components is more distributed, increasing the weights in some of them, vs. the derived from nitrogen and sulfur compounds. This remains the majority fraction with 44.49% of the total, followed by carotenoids (19.12%), monoterpenes (12.72%), aldehydes (7.86%), phenol derivatives (5.58%), furans (4.62%) due to the only presence of 2-pentylfuran, oxygenated benzene derivatives (3.30%) and 1.05% corresponding to the only detected alcohol (2-phenylethanol). The rest of the chemical families do not exceed 0.6%, being the group of terpenes with a single detected component (camphor) the one that is least represented in the aromatic fraction of the Malacara red tomato. In addition, for the red tomatoes group, the chemical family of esters is not detected in its volatile fraction.

Correlations Among Traits

Many significant correlations amongst traits were studied ($n = 6$). **Figure 2** represents the Pearson correlations between each pair of variables. These correlation coefficients range from −1 to +1, and they measure the strength of the linear relationship between the variables. Of the correlations detected, standing out are those shown between the most representative molecules involved with carbon (fiber, carbohydrate, soluble solids content) with the minerals [iron (Fe), magnesium (Mg), and calcium (Ca)], and with the more important volatile compounds, mainly of the derivatives of nitrogen and sulfur, terpenes, and (Z)-2-heptenal. The relationships between fiber and carbohydrates, and fiber vs. Fe, Mg, and Ca are statistically significant. Carbohydrates vs. Ca are also statistically significant,

TABLE 3 | Fruit traits related to tomato flavor and other bioactive characters for the two types of Malacara tomatoes (mean ± SD, $n = 3$ and p-value).

Parameter	Malacara Tomato		*P*-value
	Yellow	Red	
pH	4.20 ± 0.10	4.14 ± 0.110	0.5018
Soluble solids content (°Brix)	6.20 ± 0.20	5.87 ± 0.23	0.1318
Total sugar content (g 100 g^{-1} fw)	4.24 ± 0.57	4.25 ± 1.15	0.9865
Total acidity (g citric 100 g^{-1} fw)	0.43 ± 0.04	0.45 ± 0.06	0.7060
Taste index	1.153 ± 0.05	1.111 ± 0.05	0.3477
Lycopene (μg 100 g^{-1} fw)	34.92 ± 6.93	5512.22 ± 1046.29	0.0008
β-carotene (μg 100 g^{-1} fw)	533.85 ± 118.25	585.74 ± 125.58	0.6305
Total carotenoids (mg 100 g^{-1} fw)	0.44 ± 0.09	9.35 ± 1.81	0.0010
Vitamin C (mg 100 g^{-1} fw)	353.33 ± 7.02	403.33 ± 40.50	0.1029
Antioxidant capacity (AOT μmol ET 100 g^{-1} fw)	1238.87 ± 9.49	1284.86 ± 12.40	0.0070
Total phenolic content (mg EAG 100 g^{-1} fw)	55.60 ± 6.83	61.22 ± 17.91	0.6385

and those are related to carbohydrates and fiber and the indicated aromatic components. In addition, these hydrocarbon molecules have negative relationships with the antioxidant parameters analyzed (lycopene and carotenoids, vitamin C, and total antioxidant capacity), showing that these relationships have statistical significance (p-values below 0.05). In addition, these hydrocarbon molecules have negative relationships with the antioxidant parameters analyzed (lycopene and carotenoids, vitamin C, and total antioxidant capacity), showing that these relationships have statistical significance (p-values below 0.05). In general, the antioxidant parameters (lycopene and carotenoids, vitamin C, and total antioxidant capacity), show negative relationships against the volatile components of Malacara tomatoes, except for 6-methyl-5-hepten-2-one and methyl salicylate, with which positive relationships are found.

The typical aroma of tomato fruits depends on numerous volatile compounds and their synergic effects, the chemical nature, and odor threshold, which can be related to their composition as well crop conditions. In addition, Malacara is a long shelf life type of tomato, whose aromatic components can degrade over time to give rise to other components. Pearson correlations allow obtaining information on the most important relationships and the synergies or antagonisms of the aromatic components of the Malacara tomato and therefore of the possibilities of changes in the evolution of aromatic components in the Malacara tomato. Other significant correlations involved a positive correlation of 2-isobutylthiazole and (E)-2-pentenal, 2-isobutylthiazole and (Z)-2-heptenal, 2-isobutylthiazole and benzeneacetaldehyde, 2-isobutylthiazole and decanal, 2-isobutylthiazole and (E,E)-2,4-decadienal, 2-isobutylthiazole and 1-nitro pentane, 2-isobutylthiazole and benzyl nitrile, benzothiazole and p-cymene, benzothiazole and (Z)-2-heptenal, benzothiazole and decanal, benzothiazole and (E,E)-2,4-decadienal, benzothiazole and biphenyl, benzothiazole and estragole, benzothiazole and anethole, p-cymene and limonene, p-cymene and β-ocimene, p-cymene and estragole, p-cymene and anethole, limonene y β-ocimene, limonene and styrene, β-ocimene and (Z)-2-heptenal, β-ocimene and 1-nitro pentane, β-ocimene and benzyl nitrile, (E)-2-pentenal and (Z)-2-heptenal, (E)-2-pentenal and benzeneacetaldehyde, (E)-2-pentenal and decanal, (E)-2-pentenal and (E,E)-2,4-decadienal, (E)-2-pentenal and biphenyl, hexanal and anethole, (Z)-2-heptenal and benzeneacetaldehyde, (Z)-2-heptenal and decanal, (Z)-2-heptenal and (E,E)-2,4-decadienal, (Z)-2-heptenal and biphenyl, (Z)-2-heptenal y 1-nitro pentane, (Z)-2-heptenal and benzyl nitrile, benzeneacetaldehyde and decanal, benzeneacetaldehyde and (E,E)-2,4-decadienal, benzeneacetaldehyde and biphenyl, decanal and (E,E)-2,4-decadienal, decanal and biphenyl, (E,E)-2,4-decadienal and biphenyl, 6-methyl-5-hepten-2-one and methyl salicylate, biphenyl and estragole, biphenyl and anethole, estragole and anethole, pentane, 1-nitro and benzyl nitrile.

DISCUSSION

Tomatoes have a very varied carotenoid composition that is able to provide different tonalities from yellow to red. The Malacara tomato is a varietal type included in the group of "for hanging" tomatoes, which are the result of the natural selection carried out historically by farmers, in order to increase the conservation and extend the shelf life of the tomato and improve the quality and adaptation to local edaphoclimatic conditions (35). With this typology, two types differentiated by their color in ripe stage (yellow and red) are studied. Morphologically, Malacara is a tomato with velvety skin, and in terms of external appearance, it could be confused with plum fruit (**Figure 1**). The sizes found in Malacara tomatoes are within the range indicated by other authors (23, 36, 37). There are "for hanging" tomato lines, with fruit weight ranging from 21.7 to 168.4 g, although large fruits or more than 120 g have little acceptance among farmers and consumers (38), consequently, Malacara tomatoes, regardless of color, may have good commercial acceptance. The number of locules varies from 2 to 3 (**Figure 1**). This parameter is highly variable among the fruits of "for hanging" tomato lines (37). The spherical shape of the fruits is another attribute that increases the degree of acceptance of the two types of Malacara tomato. The characteristic color of tomato fruits is mainly due to the

TABLE 4 | List of volatile compounds names, retention index (RI), identification method (RS, reference commercial standard; MS, comparison of the mass spectrum with NIST library and bibliographic data), aroma, mean ± SD, $n = 3$ and p-value, for the two types of Malacara tomatoes.

Chemical family	Volatile compound	RI	IM	Aroma	Malacara Tomato		*P*-value
					Yellow	Red	
Alcohols	2-phenylethanol	1,136	RS	Floral, sweet, honey	489,694 ± 246,807	575,642 ± 238,376	0.6868
Aldehydes	(E)-2-pentenal	718	MS	Green, tomato, orange,	252,609 ± 297,412	46,941 ± 27,394.7	0.2989
	Hexanal	806	RS	Green, fresh, fatty, fruity	1.57E6 ± 1.29E6	1.41E6 ± 595,659	0.8562
	(E)-2-hexenal	814	MS	Green, sweet, bitter, fruity	764,938	Not detected	–
	(Z)-2-heptenal	913	MS	Sweet, green, slightly fatty	512,567 ± 90,361	361,269 ± 44,734.7	0.0601
	Benzaldehyde	982	MS	Bitter almond-like	490,981 ± 265,934	313,506 ± 46,386.9	0.4397
	(E,E)-2,4-heptadienal	921	MS	Sweet, creamy, fatty, citrus	255,776 ± 33,903.6	271,685 ± 38,853.4	0.6583
	2-phenylacetaldehyde	1,081	MS	Green, sweet, floral	648,893 ± 309,124	423,507 ± 89,267	0.2918
	Nonanal	1,104	RS	Aldehydic, waxy, rose	785,860 ± 310,322	804,128 ± 200,381	0.9359
	Decanal	1,204	RS	Aldehydic, sweet, waxy	372,449 ± 271,639	98,519.7 ± 18,316.5	0.1563
	(E,E)-2,4-decadienal	1,220	MS	Fatty, sweet, fresh	1.31E6 ± 864,940	595,208 ± 91,270.2	0.2257
Aliphatic and aromatic hydrocarbons	Styrene	883	MS	Almond	213,567 ± 60,656	104,270 ± 53,238.7	0.0789
	α-methylnaphthalene	1,345	MS	Green musty	123,585 ± 49,054.7	81,914.5 ± 27,784.3	0.3677
	Biphenyl	1,367	MS	Bergamot, cinnamon	143,821 ± 91,346.7	70,658 ± 43,686.8	0.2789
Esters	Methyl octanoate	1,083	MS	Green, fruity, waxy, citrus	155,334.33	Not detected	–
	Isopentyl 3-methylbutanoate	1,054	MS	Fruity, sweet, green	286,361.5	Not detected	–
	Methyl dihydrojasmonate	1,657	MS	Floral, citrus	177,300	Not detected	–
Furans	2-pentylfuran	1,040	RS	Fruity, green, earthy	2.31E6 ± 750,558	2.54E6 ± 740,288	0.7284
Ketones and methylketones	1-octen-3-one	943	MS	Earthy, fungal, green, oily	260,005 ± 26,349.7	248,907 ± 151,929	0.9068
	acetophenone	1,029	RS	Floral, sweet, pungent	58,117.3 ± 7,932.4	64,646 ± 41,419.5	0.7916
Monoterpenes	α-thujene	902	MS	Herbal	55,256.67	Not detected	–
	p-cymene	1,042	MS	Sweet, soft, fresh, lemon,	5.59E6 ± 813,109	3.06E6 ± 1.46E6	0.0591
	Limonene	1,018	RS	Citrus, herbal, terpenic	1.56E6 ± 182,424	726,484 ± 355,297	0.0220
	β-ocimene	958	RS	Floral, sweet, herbal, warm	517,027 ± 50,384	182,952 ± 77,229.3	0.0033
	γ-terpinene	998	MS	Citric, fatty, terpenic	4.38E6 ± 523,156	2.46E6 ± 250,811	0.0104
	Perillene	1,125	MS	Flowery, citrus-like	Not detected	570,774	–
Terpenes	Carvone	1,190	MS	Spearmint	70,377.33	Not detected	–
	Camphor	1,121	MS	Hot Turkish spices	130,320 ± 33,954.6	121,242 ± 93,096.3	0.9088
Carotenoids	6-methyl-5-hepten-2-one	938	MS	Lemon-grass	161,207 ± 29,513.3	1.01E7 ± 998,980	0.0001
	β-cyclocitral	1,204	MS	Tropical, herbal, sweet	Not detected	167,245.33	–
	Neral (β-citral)	1,174	MS	Lemon	Not detected	186,223.33	–
	Geranial (α-citral)	1,174	MS	Lemon	Not detected	816,193.33	–
	Geranylacetone	1,424	RS	Tropical, floral, fresh	not detected	418,298	–
Phenol derivatives	Guaiacol	1,090	MS	Phenolic, spicy, vanilla	Not detected	694755	-
	Methyl salicylate	1,281	RS	Minty, sweet, camphor	408,744 ± 212,970	2.18E6 ± 1.04E6	0.0438
	Eugenol	1,392	MS	Nutmeg, cinnamon	Not detected	195,951.33	–
Nitrogen and sulfur compounds	2-isobutylthiazole	1,067	MS	Woody, tomato-leaf notes	6.06E7 ± 1.99E7	2.22E7 ± 7.85E6	0.0359
	Benzothiazole	1,208	MS	Meaty, cooked, beefy	150,945 ± 59,548	88,841.7 ± 33,318.5	0.1901
	Pentane, 1-nitro	900	MS	Algae	7.83E6 ± 1.89E6	1.96E6 ± 503,511	0.0065
	Benzyl nitrile	1,138	MS	Bitter almonds, spicy, floral	433,907 ± 52,391.4	224,000 ± 58,611.4	0.0098
Oxygenated benzene derivatives	Estragole	1,172	MS	Anise	1.79E6 ± 717,612	1.01E6 ± 673,743	0.2430
	Anethole	1,190	MS	Anise	1.27E6 ± 591,754	804,140 ± 490,141	0.3488

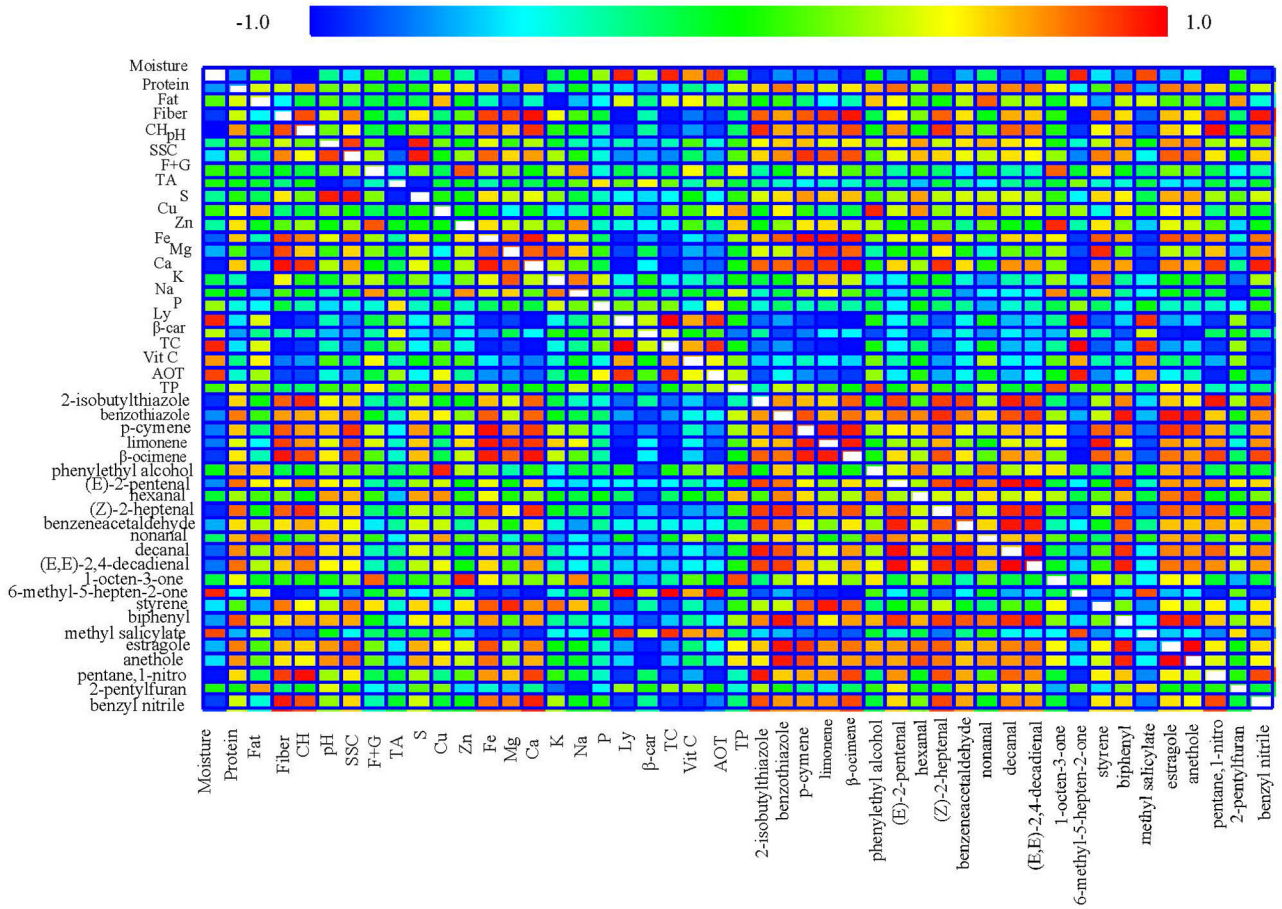


FIGURE 2 | Pearson linear correlations between the traits (nutritional and volatile) studied for the data obtained in the two Malacara tomatoes (yellow and red cv). A positive trend between each pair of variables (red color), a negative trend between each pair of variables (blue color), and no relationship between each pair of variables (green color). The strongest positive/negative relationships are displayed with the greatest intensity of color.

presence of carotenoids and their composition is influenced by several factors, including geographical origin, fruit maturity, and particularly, the variety (39). Color is one of the most remarkable features among the different tomato fruits and a key factor of external and internal quality and consumer acceptance (40). In fact, it has great importance in evaluating commercials. The color index shows significant differences in the case of both types of Malacara tomatoes. The largest number of "for hanging" tomato lines are slightly intense red fruit colors, there are also pink and orange fruits (41), but as far as in bibliography has been consulted, "for hanging" tomato lines have not been evaluated, of such intense yellow color, as those studied in the present work.

The nutritional composition of long shelf life tomatoes has been studied from different viewpoints. Patanè et al. (42) study the physicochemical components and the activities of enzymes related to the preservation of fruits, of two Sicilian landraces of long-shelf-life tomatoes, to contribute to the diversification in the agri-foods industry production. Conesa et al. (36) collect information on the morphological parameters, pH, soluble solids, and total acidity of Mediterranean landraces in order to highlight the importance of this genetic resource, to evaluate the shelf life of these tomatoes and the potential ability to adapt to changing conditions of the weather. Recently (43), the changes in quality and nutritional traits (dry matter, soluble sugar, organic acids, volatile compounds, and carotenoid contents) of one Italian long-shelf-life tomato landraces, over 120 days of natural storage, have been studied. Mainly to obtain data and to promote to traders and consumers during the winter, and early spring, when high-quality fresh tomatoes are not available. Regardless of these sources, the FAO database (InfoFoods) and the "Pera" tomato (44) were used to establish comparisons with the data obtained. Consequently, Malacara tomatoes have a lower moisture content and a higher energy value. In general, Malacara, mainly the yellow type, is a tomato that doubles the content of protein and fiber and multiplies the carbohydrate content by nine. The fat contents are similar to those shown in the bibliography (44). The quality of tomato concerning mineral contents may vary depending on interactions between cultivars, environmental factors such as light and temperature, the composition of the nutrient solution, crop management practices, and the interaction of all these

factors (45). One of the immediate consequences may be that with organic fertilization practices, higher concentrations of potassium accumulate in the edible parts (45), which could explain the high concentrations of potassium in the Malacara tomato, regardless of color.

Regarding fruit quality, soluble solid content in the two types of Malacara tomato studied ranged around 5.87 ± 0.23 °Brix (red tomato) to 6.20 ± 0.20 °Brix (yellow tomato), similar in average to values found by other authors (23, 37, 46) but slightly lower to the values found in Figàs et al. (47) in various landraces of tomato, including some long shelf life varieties. Similarly, when looking at reducing sugars, the mean values obtained for the two types of Malacara tomato are higher than those found by other authors (47) indicating the greater sweet taste of this tomato accession. The average titratable acidity in the two types was also similar to those found for other "for hanging" tomato lines (20, 37, 47, 48). Other authors (46), in Italian growing areas, have obtained means of titratable acidity higher than 1.0% in this type of long-lived tomatoes, possibly due to differences in the ripening stage at the time of harvesting. Regardless of color, in Malacara tomato, the taste index has been higher than 1, which is considered the optimal value for flavor balance (49) in salad tomato, and suggesting that this tomato has an excess of soluble solids. In most *alc* long shelf-life tomato varieties taste index has similar results (37).

The pH values, the soluble solids content, the titratable acidity, and the taste index, the vitamin C content, the antioxidant capacity, and the total phenolic content of Malacara tomatoes are within the range indicated in the literature for other "for hanging" tomato cultivars (23, 35). Compared with the FAO database (InfoFoods) for the "Pera" tomato (44), the yellow Malacara tomato studied contain, on average, around 2-fold more vitamin C and red Malacara tomato around 2.5-fold more vitamin C, and similar content in lycopene.

Lycopene is practically not detected in the yellow Malacara tomato, while the lycopene concentrations in the red Malacara tomato show twice the content of other "for hanging" tomato cultivars (35). Lycopene content and the total carotenoid content are aspects that are related to the pigments and the color that tomatoes develop. In general, ripe long shelf-life tomato fruits do not acquire the typical intense red coloration, with substantially low carotenoid levels. These observations may be related to the *alc* mutation, being able to influence the key regulators of carotenogenesis in the tomato fruits (41), this statement may be the cause of the yellow color in Malacara-type tomatoes.

The perception of flavor based on volatile compounds is very complex, as well as the biochemical chains that intervene in the development of aromas, because volatile compounds are secondary metabolites that, once synthesized, can undergo modifications to produce a new volatile or non-volatile compound. The important volatiles, which positively contribute to tomato aroma, are mainly derived from amino acids (phenolic and branched-chain compounds), fatty acids (includes the most abundant volatiles produced in the tomato fruit), and terpenoids (mono and sesquiterpenoids, and carotenoids). The presence of esters is extremely important to the aroma of fruit in many species. Esters have not been detected in the red Malacara tomato and a small fraction (0.64%) in the yellow tomatoes. Coinciding with these results, green-fruited wild tomato species accumulate considerably higher levels of esters compared with tomato red-fruited, due to the insertion of a retrotransposon in a position adjacent to the most enzymatically active tomato esterase, increasing gene expression. Enzymatic activity results in a dramatic reduction in the levels of many esters that are negatively correlated with human preference (50). In the matrix of Malacara tomatoes, 2-phenylethanol has been detected, which had been previously described as having a positive effect on tomato flavor, increasing floral aroma, and the perception of sweetness (51), this alcohol is 15% more in red tomatoes. Aldehydes, mainly fatty acid derivatives, constitute a class of compounds that include the most abundant volatiles produced in the tomato fruit: the C5 volatiles (E)-2-pentenal, C6 volatiles hexanal, (E)−2-hexenal, or benzaldehyde, C7 volatiles (Z) - 2-heptenal, (E, E)−2,4-heptadienal, and others with a higher number of carbon atoms, like decanal or (E, E)−2,4-decadienal. These compounds are classified as "green leaf" aromas due to their characteristic green, fresh scent of cut grass. In tomato fruits, the production of those compounds is increased at ripening, probably due to the loss of integrity of cellular membranes (52). The long shelf-life characteristic of the Malacara tomato prevents these degradative processes from taking place in the membranes and could be one of the causes of the lower concentration of aldehydes in the Malacara tomato compared with other varieties.

Based on their biosynthetic origin, the volatile compounds carotenoid derivatives are significant in the Malacara red tomato and only 6-methyl-5-hepten-2-one is found in the yellow type. This component is lycopene-derived and is not consistent (53) in its presence in yellow tomatoes. It is possible that the mutation of this tomato causes this concentration, providing an exceptional value for its color and the presence of this carotenoid component. Terpenoids are associated with fresh citrus-like flavors, with warm, peppery notes of the tomato stem, which supplement the aroma and attract consumer attention. Low concentrations of terpenes have been linked to breeding programs primarily focused on larger fruit yields and may have decreased the amount of defensive terpenoids produced in the vegetative part of the plant; therefore, terpene levels are relatively low in tomatoes (53). These compounds are related to numerous roles in plants (they are involved in membrane structure, growth, signaling, and defense mechanisms). The high values of terpenes (∼12% in both types of Malacara tomato) may be due to being a native variety. The phenolic volatiles of the Malacara tomato is practically negligible in the yellow type as opposed to the red type. These compounds are involved, positively or negatively, in the human capacity for tomato taste perception, and include a variety of compounds derived from the amino acid phenylalanine and its decarboxylation. Alternatively, it could be transformed into 1-nitro-2-phenylethane or benzyl nitrile by means of other unknown enzymes (54). These enzymatic mechanisms could be responsible for the high concentrations of nitrogen and sulfur compounds in the Malacara tomatoes in yellow and red type, giving rise to more spicy and ripe aromas, compared with floral and fresh aromas.

Although the vitamin C values have been high, our results indicate that in the Malacara tomato, the carotenoids may have a greater contribution to the total antioxidant activity, compared with what was found by other authors (47) where the greatest contribution to the antioxidant capacity comes from ascorbic acid. One of the characteristics of Malacara tomatoes is their low moisture content. The major water content of tomato fruits is negatively related to most of the parameters studied, except with the concentration of lycopene, total carotenoids, vitamin C content, total antioxidant capacity, and the volatile components 6-methyl-5-hepten-2-one and methyl salicylate. It has been shown that carbohydrates, fatty acids present in fat, and amino acids in proteins, represent the natural carbon reserves for the generation of volatile compounds (55). In general with Malacara tomato fruits, the volatile components detected increase when the concentration of the proximate fraction of the tomato is higher. The degradation of carotenoids in the Malacara tomato is responsible for the greater synthesis of the components of the volatile fraction, except in the case of 6-methyl-5-hepten-2-one and methyl salicylate, which are considered volatile derivatives of carotenoids.

Tomato is one of the most consumed and widely grown vegetable crops in the world, but the most commonly stated reason for consumer dissatisfaction with tomatoes is lack of flavor. Factors implicated in the tasteless tomatoes are complex as it comprises several components, including reducing sugars, free acids, minerals, amino acids, and volatile components (56). Factors that are mainly related to the production system, maturity stage at harvest, post-harvest treatment, storage period, and the genotype (57). The feature of the prolonged shelf life of Malacara tomatoes appears to involve attenuation of several metabolic processes associated with slower degradation of cell walls and the sustenance of firmness. In concordance with other "for hanging" tomato cultivars, this trait seems to be correlated with higher sucrose and reduced water loss (41). The high sucrose levels contribute to the Malacara tomato fruit's organoleptic qualities. For this reason, the Malacara tomato in either of its two ecotypes can help to break the trends sensorial of "tasteless tomatoes" and to re-availability of tasty tomatoes, and the results have practical implications for tomato growers, as well as the end consumer.

CONCLUSIONS

Local varieties create the potential to diversify global food production and better enable local adaptation to the diverse and changing environments humans inhabit, offering nutritional and organoleptic diversity. The Malacara tomato is an example of this. In its two versions color, Malacara tomato adjusts to the variability shown of "for hanging" tomato lines, with respect to morphological parameters, providing a yellow color characteristic with high value for commercialization and long shelf-life (12 moths proximally). Although the Malacara tomato is produced during the hot summer months, its long-life characteristic allows it to be consumed fresh, in the winter months, being a high source of bioactive components such as vitamin C, carotenoids, and polyphenols, and an important source mineral, especially potassium, without detracting from its aromatic characteristics.

One of the characteristics of Malacara tomatoes is their low moisture content. The major water content of tomato fruits is negatively related to most of the parameters studied, except with the concentration of lycopene, total carotenoids, vitamin C content, total antioxidant capacity, and the volatile components 6-methyl-5-hepten-2-one and methyl salicylate. In this sense, yellow tomatoes Malacara could be a greater reservoir of proteins, carbohydrates, fiber, and mineral components. While the red tomato Malacara shows a higher concentration of bioactive substances (vitamin C, carotenoids, and antioxidant capacity) and a higher and complex aromatic fraction.

This study provides extensive information on the typing of two Malacara tomato cultivars, differentiated by their color, grown in organic farming conditions that have never been described in the literature. The results incorporate an added value to the native horticultural heritage in the framework of local genetic resource conservation and can help in the selection of suitable tomato varieties, for a particular production system and the selection of local tomato varieties, of high organoleptic quality (rich in flavor and aromas, and attractive colors), high nutritional value and with characteristics to facilitate the improvement of the shelf life of tomato. Furthermore, consumers' dissatisfaction with taste concerning the tomato might be a reason for switching to specialty tomatoes, such as Malacara tomatoes in either of its two versions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MR planned the study and drafted the manuscript. MR and MG-M supervised the research. OC performed the morphological, agronomic, and chemical properties characterization. OC and MG-M performed the chemical composition characterization. OC, MG-M, and MR curated the data. OC and MR performed the statistical analyses. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors thank La Verde Cooperative, who kindly provided the materials used in this study on time and in sufficient quantity for his direct collaboration. OC thanks to the Erasmus+ programme that supports his traineeship.

REFERENCES

1. Johns T, Powell B, Maundu P, Eyzaguirre PB. Agricultural biodiversity as a link between traditional food systems and contemporary development, social integrity and ecological health. *J Sci Food Agric.* (2013) 93:3433–42. doi: 10.1002/jsfa.6351
2. FAO. The state of the world's biodiversity for food agriculture, Bélanger J, Pilling D, editors. *FAO Commission on Genetic Resources for Food and Agriculture Assessments*. Rome: Food and Agriculture Organization of the United Nations (2019) 572 p.
3. Brummer EC, Barber WT, Collier SM, Cox TS, Johnson R, Murray SC, et al. Plant breeding for harmony between agriculture and the environment. *Front Ecol Environ.* (2011) 9:561–8. doi: 10.1890/100225
4. Kliem L, Sievers-Glotzbach S. Seeds of resilience: the contribution of commons-based plant breeding and seed production to the social-ecological resilience of the agricultural sector. *Int J Agr Sustain.* (2021). doi: 10.1080/14735903.2021.1963598
5. Swinburn BA, Kraak VI, Allender S, Atkins VJ, Baker PI, Bogard JR, et al. The global syndemic of obesity, undernutrition, and climate change: the Lancet Commission report. *Lancet.* (2019) 393:791–846. doi: 10.1016/S0140-6736(19)30310-1
6. Willer H, Trávníček J, Meier C, Schlatter B, (editors.). *The World of Organic Agriculture. Statistics and Emerging Trends 2021*. Bonn: Research Institute of Organic Agriculture FiBL, Frick, and IFOAM – Organics International (2021) 337 p.
7. Moreno MM, Villena J, González-Mora S, Moreno C. Response of healthy local tomato (*Solanum lycopersicum* L.) populations to grafting in organic farming. *Sci Rep.* (2019) 9:4592. doi: 10.1038/s41598-019-41018-2
8. Pérez-Caselles C, Brugarolas M, Martínez-Carrasco L. Traditional varieties for local markets: a sustainable proposal for agricultural SMEs. *Sustainability.* (2020) 12:4517. doi: 10.3390/su12114517
9. Weisburger JH. Lycopene and tomato products in health promotion. *Exp Biol Med.* (2002) 227:924–7. doi: 10.1177/153537020222701014
10. Siddiqui MW, Ayala-Zavala JF, Dhua RS. Genotypic variation in tomatoes affecting processing and antioxidant attributes. *Crit Rev Food Sci Nutr.* (2015) 55:1819–35. doi: 10.1080/10408398.2012.710278
11. Navarro-González I, Periago MJ. El tomate, 'alimento saludable y/o funcional? *Rev Espanola de Nutr Hum y Diet.* (2016) 20:323–35. doi: 10.14306/renhyd.20.4.208
12. Mertz W. Trace minerals and atherosclerosis. *Federat Proce.* (1982) 41:2807–12.
13. Adedeji O, Taiwo KA, Akanbi CT, Ajani R. Physicochemical properties of four tomato cultivars grown in Nigeria. *J Food Process Preserv.* (2006) 30:79–86. doi: 10.1111/j.1745-4549.2005.00049.x
14. Caris-Veyrat C, Amiot MJ, Tyssandier V, Grasselly D, Buret M, Mikolajczak M. Borel P. Influence of organic versus conventional agricultural practice on the antioxidant microconstituent content of tomatoes and derived purees; consequences on antioxidant plasma status in humans. *J Agric Food Chem.* (2004) 52:6503–9. doi: 10.1021/jf0346861
15. Hernandez-Suarez M, Hernandez-Castillo FD, Gallegos-Morales G, Lira-Saldivar RH, Rodríguez-Herrera R, Aguilar CN. Biocontrol of soil fungi in tomato with microencapsulates containing *Bacillus subtilis*. *Am J Agric Biol Sci.* (2011) 6:189–95. doi: 10.3844/ajabssp.2011.189.195
16. Coyago-Cruz E, Corell M, Moriana A, Hernanz D, Benítez-González AM, Stinco CM, et al. Antioxidants (carotenoids and phenolics) profile of cherry tomatoes as influenced by deficit irrigation, ripening and cluster. *Food Chem.* (2018) 240:870–84. doi: 10.1016/j.foodchem.2017.08.028
17. Chassy AW, Bui L, Renaud EN, Van Horn M, Mitchell AE. Three-year comparison of the content of antioxidant microconstituents and several quality characteristics in organic and conventionally managed tomatoes and bell peppers. *J Agric Food Chem.* (2006) 54:8244–52. doi: 10.1021/jf060950p
18. Mitchell AE, Hong YJ, Koh E, Barrett DM, Bryant DE, Denison RF, et al. Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes. *J Agric Food Chem.* (2007) 55:6154–9. doi: 10.1021/jf070344
19. Vallverdú-Queralt A, Medina-Remón A, Casals-Ribes I, Lamuela-Raventos RM. Is there any difference between the phenolic content of organic and conventional tomato juices? *Food Chem.* (2012) 130:222–7. doi: 10.1016/j.foodchem.2011.07.017
20. Cebolla-Cornejo J, Roselló S, Nuez F. Phenotypic and genetic diversity of Spanish tomato landraces. *Sci Hortic.* (2013) 162:150–64. doi: 10.1016/j.scienta.2013.07.044
21. García-Martínez S, Corrado G, Ruiz JJ, Rao R. Diversity and structure of a sample of traditional Italian and Spanish tomato accessions. *Genet Resour Crop Evol.* (2013) 60:789–98. doi: 10.1007/s10722-012-9876-9
22. Cebrino FG, Ruiz ML, Yuste MCA, García MJB, Gómez DG. Characterization of traditional tomato varieties grown in organic conditions. *Span J Agric Res.* (2011) 9:444–52. doi: 10.5424/sjar/20110902-153-10
23. Casals J, Pascual L, Cañizares J, Cebolla-Cornejo J, Casañas F, Nuez F. Genetic basis of long shelf life and variability into Penjar tomato. *Genet Resour Crop Evol.* (2012) 59:219–29. doi: 10.1007/s10722-011-9677-6
24. Ramos García M, Soriano JJ, Gonzálvez V. Organic seeds and biodiversity in Spain. In: van Bueren EL, Raganathan R, Sorese N, editors. *Challenges and Opportunities for Organic Agriculture and the Seed Industry*. Bonn: International Federation of Organic Agriculture Movements. (2004). p. 68.
25. Weatherall IL, Coombs BD. Skin color measurements in terms of CIELAB color space values. *J Investig Dermatol.* (1992) 99:468–73. doi: 10.1111/1523-1747.ep12616156
26. DOGV. *Diari Oficial de la Comunitat Valenciana. Condiciones mínimas de calidad para la comercialización de frutos cítricos en fresco*. DOGV (2006) 5346:30321–8. Available Online at: https://dogv.gva.es/datos/2006/09/14/pdf/2006_10409.pdf
27. AOAC. *Official Method of Analysis of the Association of Official Analytical Chemists International*. Arlington: AOAC (2005).
28. Navez B, Letard M, Graselly D, Jost M. Les critéres de qualité de la tomate. *Infos-Ctifl.* (1999) 155:41–7.
29. Zscheille F, Porter JW. Analytical methods for carotenes of *Lycopersicon* species and strains. *Anal Chem.* (1947) 19:47–51. doi: 10.1021/ac60001a013
30. Rousseaux MC, Jones CM, Adams D, Chetelat R, Bennet A, Powell A. QTL analysis of fruit antioxidants in tomato using *Lycopersicon pennellii* introgression lines. *Theor Appl Genet.* (2005) 111:1396–408. doi: 10.1007/s00122-005-0071-7
31. Arnous A, Makris DP, Kefalas P. Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece. *J Food Compost Anal.* (2002) 15:655–65. doi: 10.1006/jfca.2002.1070
32. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft Technol.* (1995) 28:25–30. doi: 10.1016/S0023-6438(95)80008-5
33. Moreno E, Fita A, González-Mas MC, Rodríguez-Burruezo A. HS-SPME study of the volatile fraction of *Capsicum* accessions and hybrids in different parts of the fruit. *Sci Hortic.* (2012) 135:87–97. doi: 10.1016/j.scienta.2011.12.001
34. González-Mas MC, Rambla JL, Alamar MC, Gutiérrez A, Granell A. Comparative analysis of the volatile fraction of fruit juice from different Citrus species. *PLoS ONE.* (2011) 6:e22016. doi: 10.1371/journal.pone.0022016
35. Watada AE, Herner RC, Kader AA, Romani RJ, Staby GL. Terminology for the description of developmental stages of horticultural crops. *Hortscience.* (1984) 19:20–1.
36. Conesa MÀ, Fullana-Pericàs M, Granell A, Galmés J. Mediterranean long shelf-life landraces: an untapped genetic resource for tomato improvement. *Front Plant Sci.* (2020) 10:1651. doi: 10.3389/fpls.2019.01651
37. Figàs MR, Prohens J, Raigón MD, Fita A, García-Martínez MD, Casanova C. Soler S. Characterization of composition traits related to organoleptic and functional quality for the differentiation, selection and enhancement of local varieties of tomato from different cultivar groups. *Food Chem.* (2015) 187:517–24. doi: 10.1016/j.foodchem.2015.04.083
38. Sanz A, Martí M, Ariño J, Casals Missio J. El cultiu del tomàquet de Penjar. Un tipus varietal amb una gran variabilitat. In: El tomàquet de penjar. *Dossier Tècnic.* (2018) 94:9–14.
39. Ronen G, Carmel-Goren L, Zamir D, Hirschberg J. An alternative pathway to β-carotene formation in plant chromoplasts discovered by map-based cloning of beta and old-gold color mutations in tomato. *Proc Natl Acad Sci USA.* (2000) 97:11102–7. doi: 10.1073/pnas.190177497
40. Constantino LV, Rossetto LM, Benassi MT, Oliveira C, Zeffa DM, Koltun A. Gonçalves LSA. Physico-biochemical characterization of mini-tomatoes

and internal preference mapping based on consumer acceptance. *Sci Hortic.* (2021) 282:110034. doi: 10.1016/j.scienta.2021.110034
41. Kumar R, Tamboli V, Sharma R, Sreelakshmi Y. NAC-NOR mutations in tomato Penjar accessions attenuate multiple metabolic processes and prolong the fruit shelf life. *Food Chem.* (2018) 259:234–44. doi: 10.1016/j.foodchem.2018.03.135
42. Patanè C, Pellegrino A, Saita A, Siracusa L, Ruberto G, Barbagallo R. Mediterranean long storage tomato as a source of novel products for the agrifood industry: nutritional and technological traits. *LWT-Food Sci Technol.* (2017) 85:445–8. doi: 10.1016/j.lwt.2016.12.011
43. Parisi M, Lo Scalzo R, Migliori CA. postharvest quality evolution in long shelf-life "Vesuviano" tomato landrace. *Sustainability.* (2021) 13:11885. doi: 10.3390/su132111885
44. Charrondiere UR, Rittenschober D, Nowak V, Nicodemi C, Bruggeling P, Petracchi C. Fao/infoods e-learning course on food composition data. *Food Chem.* (2016) 193:6–11. doi: 10.1016/j.foodchem.2014.11.048
45. Martínez-Ballesta MC, Dominguez-Perles R, Moreno DA, Muries B, Alcaraz-López C, Bastías E, et al. Minerals in plant food: effect of agricultural practices and role in human health. a review. *Agron Sustain Dev.* (2010) 30:295–309. doi: 10.1051/agro/2009022
46. Fullana-Pericàs M, Conesa M, Douthe C, El Aou-ouad H, Ribas-Carbó M, Galmés J. Tomato landraces as a source to minimize yield losses and improve fruit quality under water deficit conditions. *Agric Water Manag.* (2019) 223:105722. doi: 10.1016/j.agwat.2019.105722
47. Figàs MR, Prohens J, Raigón MD, Pereira-Dias L, Casanova C, García-Martínez MD, et al. Insights into the adaptation to greenhouse cultivation of the traditional Mediterranean long shelf-life tomato carrying the *alc* mutation: a multi-trait comparison of landraces, selections, and hybrids in open field and greenhouse. *Front Plant Sci.* (2018) 9:1774. doi: 10.3389/fpls.2018.01774
48. Casals J, Marti R, Casañas F, Cebolla-Cornejo J. Sugar-and-acid profile of penjar tomatoes and its evolution during storage. *Sci Agric.* (2015) 72:314–21. doi: 10.1590/0103-9016-2014-0311
49. Rosa-Martínez E, Adalid AM, Alvarado LE, Burguet R, García-Martínez MD, Pereira-Dias L. Soler S. Variation for composition and quality in a collection of the resilient mediterranean 'de penjar' long shelf-life tomato under high and low n fertilization levels. *Front Plant Sci.* (2021) 12:441. doi: 10.3389/fpls.2021.633957
50. Goulet C, Mageroy MH, Lam NB, Floystad A, Tieman DM, Klee HJ. Role of an esterase in flavour volatile variation within the tomato clade. *Proc Natl Acad Sci USA.* (2012) 109:19009–14. doi: 10.1073/pnas.12165 15109
51. Baldwin EA, Goodner K, Plotto A. Interactions of volatiles, sugars, and acids on perception of tomato aroma and flavor descriptors. *J Food Sci.* (2008) 73:S294–307. doi: 10.1111/j.1750-3841.2008.00825.x
52. Klee HJ, Giovannoni JJ. Genetics and control of tomato fruit ripening and quality attributes. *Annu Rev Genet.* (2011) 45:41–59. doi: 10.1146/annurev-genet-110410-132507
53. Oluk AC, Ata A, Ünlü M, Yazici E, Karaşahin Z, Eroglu EÇ, Canan I. Biochemical characterisation and sensory evaluation of differently coloured and shaped tomato cultivars. *Not Bot Horti Agrobot Cluj Napoca.* (2019) 47:599–607. doi: 10.15835/nbha47311382
54. Rambla JL, Tikunov YM, Monforte AJ, Bovy AG, Granell A. The expanded tomato fruit volatile landscape. *J Exp Bot.* (2014) 65:4613–23. doi: 10.1093/jxb/eru128
55. Schwab W, Davidovich-Rikanati R, Lewinsohn E. Biosynthesis of plant-derived flavor compounds. *Plant J.* (2008) 54:712–32. doi: 10.1111/j.1365-313X.2008.03446.x
56. Fernqvist F, Hunter E. Who's to blame for tasteless tomatoes? the effect of tomato chilling on consumers' taste perceptions. *Europ J Hort Sci.* (2012) 77:193–8.
57. Zhao X, Chambers E IV, Matta Z, Loughin TM, Carey EE. Consumer sensory analysis of organically and conventionally grown vegetables. *J Food Sci.* (2007) 72:87–91. doi: 10.1111/j.1750-3841.2007.00277.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Raigón, García-Martínez and Chiriac. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 31 January 2022
doi: 10.3389/fnut.2022.833723

Response of Tomato Fruit Quality Depends on Period of LED Supplementary Light

Shuya Wang[1], *Ning Jin*[1], *Li Jin*[1], *Xuemei Xiao*[1], *Linli Hu*[1], *Zeci Liu*[1], *Yue Wu*[1], *Yandong Xie*[1], *Wen Zhu*[1], *Jian Lyu*[1,2]* *and Jihua Yu*[1,2]*

[1] College of Horticulture, Gansu Agricultural University, Lanzhou, China, [2] State Key Laboratory of Aridland Crop Science, Gansu Agricultural University, Lanzhou, China

Light is an important environmental factor that regulates the activity of metabolism-related biochemical pathways during tomato maturation. Using LED to improve lighting conditions during the process of tomato growth and development is a feasible and efficient method to improve the quality of tomato fruit. In this study, red and blue LEDs were used to supplement light on "MicroTom" tomato plants for different periods of time in the morning and evening, and the differences between the primary and secondary metabolites and other nutrient metabolites in the tomato fruit were analyzed using liquid chromatography and liquid chromatography mass spectrometry and other methods. Supplementing light in the morning promoted the accumulation of vitamin C, organic acids, amino acids, carotenoids, phenolic acids, and other health-promoting substances in the tomato fruits. Supplementing light in the evening significantly increased the content of sugars, flavonoids, and aromatic substances in tomato fruits, whereas the promoting effect of LED on the accumulation of amino acids and carotenoids was lower in the evening than in the morning. Both morning and evening light supplementation reduced the mineral content of fruit. In conclusion, morning light supplementation improved the nutritional quality of tomato fruits, while evening light supplementation improved their flavor.

Keywords: tomato, LED, period, flavor quality, nutrition quality

OPEN ACCESS

Edited by:
José Pinela, Polytechnic Institute of Bragança (IPB), Portugal

Reviewed by:
Ivana Tomaz, University of Zagreb, Croatia
Shixiang Yao, Southwest University, China
Miguel Simón, Polytechnic University of Valencia, Spain

***Correspondence:**
Jian Lyu
lvjiangs@126.com
Jihua Yu
yujihuagg@163.com

Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *12 December 2021*
Accepted: *07 January 2022*
Published: *31 January 2022*

Citation:
Wang S, Jin N, Jin L, Xiao X, Hu L, Liu Z, Wu Y, Xie Y, Zhu W, Lyu J and Yu J (2022) Response of Tomato Fruit Quality Depends on Period of LED Supplementary Light. Front. Nutr. 9:833723. doi: 10.3389/fnut.2022.833723

INTRODUCTION

Tomatoes are one of the most widely cultivated vegetables in the world, and are deeply loved for their unique flavor and rich nutritional quality. Tomatoes contain soluble sugars, organic acids, polyphenols, carotenoids, amino acids and other nutrients (1). Eating tomatoes can prevent cancer and other diseases and have great health benefits (2, 3). The ever-increasing population has led to the pursuit of high yields of tomatoes. In order to increase yields, breeders have to lower the requirements for tomato quality and discard some good quality traits and varieties (4, 5). In addition, factors such as climate change, intensification of environmental pollution and improper use of fertilizers have led to the deterioration of tomato quality (6, 7). In recent years, with the improvement of people's requirements for food quality, it is generally believed that tomatoes have lost their "childhood taste," and the goal of increasing the quality of tomato fruits has attracted more and more attention.

Previous studies have found that there are many ways to improve tomato fruit quality. Appropriate fertilization and irrigation methods can promote the nutritional quality of tomato fruits (8–10). Root application or foliar spraying of certain concentrations of exogenous substances such as methyl jasmonate, quercetin, selenium, and potassium can increase the contents of sugars, carotenoids, and other nutrients in tomato fruits (1, 11–13). Small molecular gases such as nitric oxide and hydrogen sulfide also significantly improved the nutritional and sensory quality of tomato fruits (14, 15).

Light is an important environmental factor that regulates the activities of metabolite-related biochemical pathways during tomato ripening and plays an important role in determining tomato quality (16). Both the leaves and fruits of tomato contain photoreceptors, including phytochrome and cryptochrome (17, 18), and appropriate illumination can promote the absorption of light and enhance the photosynthetic capacity of tomato (19). Moreover, light can contribute to promoting the absorption and transport of water and nutrients (20), thereby influencing metabolic processes and metabolite production (21). Light-emitting diodes (LEDs) are an efficient environmentally friendly source of artificial light, the supplementary illumination provided by which can promote the growth and development of tomatoes and other vegetables (22, 23), and the findings of recent studies have revealed that such supplementary light can play an important role in improving tomato fruit quality. LEDs with different spectra and supplementary LED illumination of differing durations can have different effects on tomato fruit metabolites and contribute to enhancing the amounts of key compounds related to tomato fruit quality characteristics (16, 24). For example, compared with white light, a combination of red and blue (R:B, 3:1) LED illumination has been shown to increase the contents of soluble solids, glucose, fructose, and sucrose in tomato fruits by promoting the accumulation of proteins related to glucose metabolic pathways (25), whereas compared with lighting using high pressure sodium lamps, LEDs have been found to significantly increase the content of lycopene in tomato fruits (26). Similarly, a combination of red and blue LEDs has been demonstrated to promote lycopene biosynthesis and the accumulation of carbohydrates by increasing the contents of melatonin in tomato fruits (27), whereas either blue or a combination of red and blue LEDs was found to increase the contents of potassium and beta carotene in fruits by promoting the absorption and transport of potassium by tomato roots (28). Furthermore, it has been observed that blue light LEDs can significantly increase the contents of soluble solids, lycopene, and phenolic compounds in post-harvest tomato fruits (29), whereas high-intensity blue light LEDs can stimulate the antioxidant system in tomato fruit, thereby enhancing the ascorbic acid content (30). Continuous illumination with red light LEDs has also been found to significantly promote increases in the contents of lycopene, beta carotene, phenolic acid, and flavonoids in post-harvest tomato fruits (31), whereas significant increases in the contents of free amino acids in post-harvest tomato fruits have been detected in response to continuous illumination with blue light LEDs (32). In addition, the provision of red light LED lighting can improve the content of Mg, Ca, Cu, and other mineral elements in tomato fruits (19), and the sensory qualities of aroma and texture in fruits can be significantly enhanced in response to an increase far-red irradiation (33). In our previous studies, we have also found that exposing tomato plants to red-blue light LEDs can improve the soluble sugar and soluble protein contents of fruits (34).

Collectively, the findings of these studies indicate that appropriate LED lighting can contribute to enhancing the quality of tomato fruit. However, although previous studies in this regard have tended to focus on the quality and intensity of LED light, as well as the duration of supplementary illumination, it is also well-established that the metabolic processes of plants are regulated by circadian rhythms and biological clocks (35, 36). Consequently, it might be predicted that the effects of supplementary LED illumination on tomato quality would be dependent on an appropriate timing of light supplementation. It is thus considered necessary to examine the temporal effects supplementary LED lighting on the quality of tomato fruit. At present, however, the responses of tomato fruit to the provision of supplementary light at different times during the day have yet to be sufficiently well-characterized. Accordingly, in this study, we examined the effects supplementary light treatments with red and blue LEDs provided at different periods of time on tomato, with a specific focus on differences between primary and secondary metabolites and other nutritional metabolites, analyzed based on liquid chromatography and liquid chromatography mass spectrometry and other approaches. The principle objectives of study were to clarify the effects of the timing of LED supplementation on tomato fruit quality, provide new insights for enhancing the quality of tomato fruit quality, and establish a new theoretical basis for supplementary LED lighting technology for tomatoes.

MATERIALS AND METHODS

Plant Materials and Growing Conditions

The tomato variety used in this experiment was "Micro Tom." After soaking in warm water, tomato seeds were placed in a dark artificial climate box at 28°C to promote germination. After 36 h, the uniformly germinated seeds were sown on the seedling substrate, and then put into the artificial climate box where the seedlings were raised. The conditions of the artificial climate chamber were 12 h of illumination and 12 h of darkness. When illuminating, the temperature was 26°C, the humidity was 70%, and the light intensity was 450 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Under dark conditions, the temperature was 18°C and the humidity was 50%. When the seedlings had two leaves and one heart, they were moved into the greenhouse for normal management.

Experimental Design

The experiment was carried out in a plant light supplement cultivation frame completely isolated from natural light in the greenhouse. Two kinds of LED light sources were installed on the cultivation rack: one was a full-spectrum LED plant growth light (T8-0.9M-28W-220V, Xiamen rural Hui Photoelectric Technology Co., Ltd.), which provided 10 h of daylight between 8 A.M. and 18 P.M., with light intensity 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The other was a red and blue (7R2B) LED plant growth

light (HY-85CM-27 × 3W-RB, Shenzhen Houyi Energy Saving Tech Co., Ltd.), which was the supplementary light source in this experiment, with light intensity 51 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The experiment consisted of three treatments: treatment without supplementary light (CK), red and blue light supplementation for 3 h before 8 o'clock in the morning (T1), and red and blue light supplementation for 3 h after 18:00 in the evening (T2) (**Figure 1**). Fifty tomato plants with the same flowering time were selected for each treatment when the first flower was just blooming, and transferred to the plant light cultivation rack where the treatment commenced. When the tomato fruit matured, fruit of the same size and maturity were selected to determine the quality, and all the index analyses were set up with 3 biological repeats in each treatment.

Sugar Components

Tomato fruit (5 g) were homogenized, transferred to a 25 mL volumetric flask with ultrapure water to make the volume constant, and then sonicated in a water bath at 30°C for 60 min, filtered into a 50 mL centrifuge tube at 4°C, centrifuge for 10 min at 10,000 $r \cdot min^{-1}$. Supernatant (2 mL) was extracted and filtered through a 0.22 μm aqueous filter, and the filtrate was used for liquid chromatography. The measuring instrument was a high performance liquid chromatograph (HPLC) with a differential refractive index display (Agilent 1100 Series, Agilent Technologies, USA), the chromatographic column was an LC-NH2 amino column (250 × 4.6 mm, Phenomenex, USA), the mobile phase was V(acetonitrile): V(water) = 75: 25, isocratic elution, flow rate 1.0 $mL \cdot min^{-1}$, column temperature 30°C, and the injection volume was 20 μL.

Organic Acid Components

A fresh sample of tomato fruit (0.5 g) was transferred to a 25 mL volumetric flask with ultrapure water to make the volume constant, shaken well, transferred to a 50 mL centrifuge tube, centrifuged at 4°C, 10,000 $r \cdot min^{-1}$ for 10 min; 2 mL of supernatant was extracted and filtered through a 0.22 μm water system Filter by filter, and the filtrate was used for liquid chromatography (HPLC) determination. The measuring instrument was a high performance liquid chromatograph with a UV detector (Agilent 1260 Infinity II, Agilent Technologies, USA), the chromatographic column was X-Peonyx AQ-C18 (250 × 4.6 mm, FeiniGen Instrument, China), the detection wavelength was 210 nm, and the mobile phase was 0.2 $mmol \cdot L^{-1}$ dihydrogen phosphate sodium, isocratic elution, flow rate 1.2 $mL \cdot min^{-1}$, column temperature 30°C, injection volume was 5 μL.

Amino Acid Components

Amino acid components were determined according to the method of Ma et al. (37) with slight modifications. The tomatoes were heated in a constant temperature drying oven at 105°C for 15 min, and then dried at 80°C to a constant weight. After grinding, 0.5 g was weighed into a 50 mL Erlenmeyer flask, 25 mL of 0.1% hydrochloric acid solution was added, then ultrasonically extracted for 15 min. The extract was transferred into a 50 mL centrifuge tube, centrifuged at 4°C, 10,000 $r \cdot min^{-1}$ for 10 min, the supernatant was filtered through a 0.22 μm aqueous filter, and the filtrate supplied for HPLC-MS (LC-MS) determination. The measuring instrument was a triple quadrupole LC/MS system (Agilent 1290 Infinity, Agilent 6460 Triple Quad, Agilent Technologies, USA), the chromatographic column was Poroshell 120 HILIC-Z (100 × 2.1 mm, Agilent Technologies, USA), and the mobile phase A was 20 mM ammonium formate (pH = 3): water = 1: 9, the mobile phase B was 20 mM ammonium formate (pH = 3): acetonitrile = 1:9, gradient elution (0 min, 100% mobile phase B; 11.5 min, 30% mobile phase A and 70% mobile phase B; 12 min, 100% mobile phase B), the flow rate was 0.5 $mL \cdot min^{-1}$, the column temperature 25°C, the injection volume 1 μL. The mass spectrometry ionization mode was the ESI positive ion mode, the drying gas temperature was 330°C, the gas flow rate 13.0 $L \cdot min^{-1}$, and the capillary voltage 1,500 V. The mass spectrometry detection parameters of each amino acid, such as parent ion and product ion, are detailed in **Supplementary Table 1**.

Carotenoid Components

Carotenoid components were determined according to Kang et al. 's method (38) with slight modification. The tomato fruits were freeze-dried at −30°C for 72 h in a freeze dryer (Alpha 1-2 LDplus, Martin Christ, Germany). The freeze-dried tomatoes were ground into powder, weighed (0.5 g) into a 50 mL centrifuge tube, with 30 mL of petroleum ether and acetone mixture (2:1, v/v), then to a water bath for ultrasonic extraction. For carotene, the extract was collected in a brown bottle, and 30 mL of a mixture of petroleum ether and acetone was added until all the color was removed. The combined filtrate was transferred to a separatory funnel, washed twice with 250 mL of distilled water, the water phase was drained, and a small amount of anhydrous sodium sulfate was added to the upper extract to remove the water phase. The extract was filtered into a round-bottomed flask with a sand core funnel under vacuum and placed in a rotary evaporator at 40°C until dry. The dried extract was then dissolved with 25 mL of an acetonitrile: dichloromethane: methanol (55:20:25) mixture, and filtered through a 0.22 μm oil filter. The filtrate was used for liquid chromatography determination. The extraction process was protected from light. The measuring instruments were high performance liquid chromatograph (Alliance Waters e2695, Waters, USA) and ultraviolet detector, the chromatographic column was HPLC C18 (250 × 4.6 mm, Waters, USA), the detection wavelengths were 286 nm, 450 nm, 470 nm and 665 nm, and the mobile phase was V(acetonitrile): V(two Chloromethyl): V(methanol) = 55: 20: 25, isocratic elution, the flow rate 1.2 $mL \cdot min^{-1}$, the column temperature 30°C; the injection volume 10 μL.

Phenolic Acids and Flavonoids

To determine the phenolic acids and flavonoids, 0.1 g of freeze-dried fruit powder was weighed and placed in a 5 mL centrifuge tube with 2 mL of methanol, and placed at 4°C for 1 h, shaking it 3 times during the hour. The tube was centrifuged at 8,000 $r \cdot min^{-1}$ at 4°C for 10 min, then 2 mL of supernatant was extracted, filtered through a 0.22 μm oil-based filter, and the filtrate was

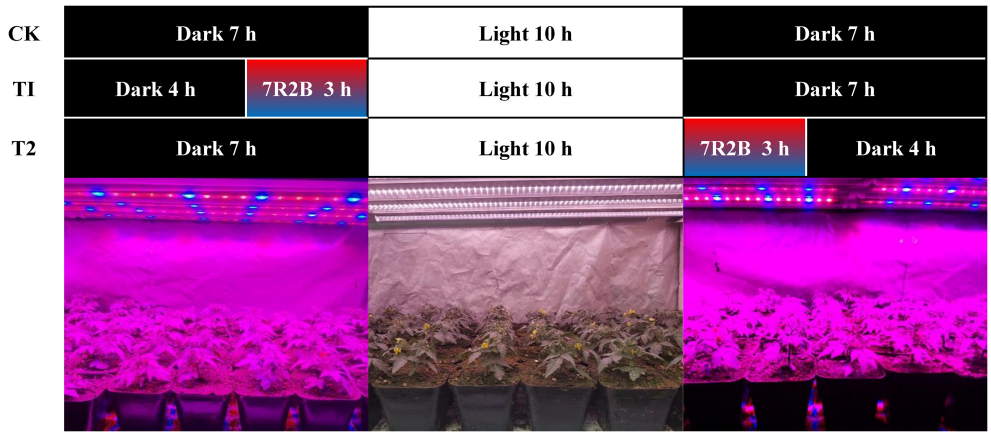


FIGURE 1 | Schematic diagram of the illumination time of each treatment. CK, no light supplementation control; T1, light supplementation for 3 h in the morning; T2, light supplementation for 3 h in the evening.

used for liquid chromatography measurement. The measuring instruments were high performance liquid chromatograph (Alliance Waters e2695, Waters, USA) and ultraviolet detector, the chromatographic column was HPLC C18 (250 × 4.6 mm, Waters, USA), the detection wavelength 240 nm, 280 nm and 322 nm, mobile phase A was methanol, mobile phase B was 1% acetic acid, gradient elution (**Supplementary Table 2**), flow rate 1.1 mL·min^{-1}, column temperature 30°C, injection volume 10 μL.

Mineral Element Content

To determine the mineral element content, 0.5 g of dried and ground tomato fruit was placed in a 150 mL erlenmeyer flask, and digested using the H_2SO_4-H_2O_2 digestion method. The digestion solution was washed into a 50 mL volumetric flask without damage, with ultrapure water to make the volume constant, and shaken well. The extract was used for the determination of mineral elements phosphorus (P) and potassium (K). 0.5 g of dried and ground tomato fruit was put into a porcelain crucible and ashing was carried out by dry ashing method. The ash was dissolved with 5 mL 6 mol·L^{-1} HCL, filtered and filled with water in a constant volume of 50 mL volumetric flask. The extract was used for the determination of mineral elements such as calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn) and sodium (Na). Phosphorus was determined using the Mo-Sb colorimetric method. The K, Ca, Mg, Cu, Fe, Mn, Zn, and Na contents were determined by an atomic absorption spectrometer (ZEEnit700P, Analytik Jena AG, Germany).

Volatile Compounds

To determine the contents of volatile compounds a sample of fresh tomato fruit (0.5 g), was added to 1.5 g anhydrous sodium sulfate, ground quickly and fully, and then pourrf into a headspace bottle. After closing the cover, the headspace bottle was placed on a magnetic stirrer, heated and stirred at 70°C for 10 min to balance the internal headspace gas, and then the electronic nose (PEN3, Airsense, Germany) was used to detect the volatile substances in the gas at the upper part of the headspace bottle. The detection conditions of the electronic nose were as follows: the flushing time was 60 s, the sensor zeroing time 5 s, the pre sampling time 5 s, the injection flow rate 400 ml·min^{-1}, and the measurement time 120 s. Please refer to **Supplementary Table 3** for the material type and performance description of the sensor.

Statistical Analysis

There were replicates for each treatment and the results are presented as mean ± standard deviation (SD). One-way analysis of variance was performed, and Duncan's honesty significant difference test was used to assess the differences between two groups, using SPSS ver. 21 (SPSS, Inc., Chicago, IL, USA). Heat map production and hierarchical cluster analysis (HCA) were carried out in Origin 2021 (Origin, Inc., San Francisco, California, USA). HCA was based on the calculation of Pearson correlation. $P < 0.05$ were deemed statistically significant.

RESULTS

Sugar Contents

Sugars are important plant constituents that contribute to the flavor of tomato fruit and play key roles in determining fruit quality (39). We first determined and analyzed the contents of the main soluble sugars (fructose, glucose, and sucrose) in tomato fruits. Compared with the control group, 3 h of evening light supplementation significantly increased the contents of fructose (**Figure 2A**) and glucose (**Figure 2B**) in the tomato fruits. Compared with the control group 3 h of morning light supplementation made no significant difference in the contents of fructose and glucose in the tomato fruits. Light supplementation in the evening significantly increased the sucrose content in tomato fruit, and conversely, light supplementation in the morning decreased the sucrose content (**Figure 2C**). Consistently, we found that the total sugar contents of tomato fruit were significantly increased by 3 h of evening light supplementation, although there was no significant difference between treatment and control fruits when supplementary light was provided in the morning (**Figure 2D**).

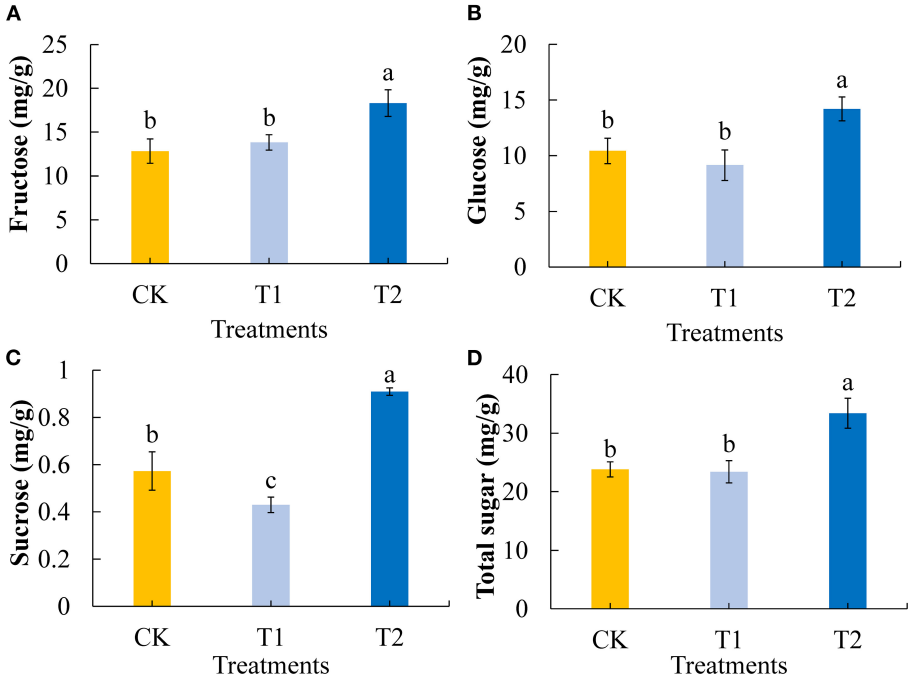


FIGURE 2 | Fructose **(A)**, glucose **(B)**, sucrose **(C)**, and total sugar **(D)** contents in tomato fruits under different LED supplementary light periods. The data are expressed as average values ± SD ($n = 3$). $^{a-c}$Indicate significant differences between treatments ($P < 0.05$, Duncan's multiple range test). The bars indicate standard errors. CK, no light supplementation control; T1, light supplementation for 3 h in the morning; T2, light supplementation for 3 h in the evening.

Organic Acid Components

Among the organic acids present in tomato fruits, we selected five to evaluate the differences in organic acid contents in tomato fruits in response to supplementary light at different times of the day. Compared with the control, 3 h of light supplementation in the morning and evening significantly promoted the accumulation of tartaric acid in tomato fruits (**Figure 3A**) but had no effect on the accumulation of malic acid (**Figure 3B**). Light supplementation for 3 h in the morning significantly promoted the accumulation of ascorbic acid (**Figure 3C**) and oxalic acid (**Figure 3D**) in the tomato fruits, while light supplementation for 3 h in the evening had no significant effect on the contents of ascorbic acid and oxalic acid. In contrast, exposing plants to supplementary light for 3 h in the evening promoted a significantly reduction in the accumulation of citric acid, whereas we detected no significant differences in the citric acid contents of treatment and control fruit when light supplementation was provided in the morning (**Figure 3E**). Light supplementation for 3 h in the morning significantly promoted the accumulation of total organic acids (**Figure 3F**) in tomato fruits, while light supplementation for 3 h in the evening had no significant effect on the accumulation of total organic acids.

Amino Acid Contents

Amino acids are the basic substances that constitute proteins, maintain the normal metabolism of plants, and provide the material basis for life activities. In the present study, we quantitatively analyzed 21 amino acids in tomato fruits using liquid chromatography-mass spectrometry. We found that compared with the control treatment, with the exception of serine, for which there was no significant effect, supplementary lighting for 3 h in the morning significantly increased the contents of the assessed amino acids. Supplementing light for 3 h at night significantly increased the content of phenylalanine, leucine, isoleucine, tryptophan, methionine, valine, proline, tyrosine, alanine, glycine, glutamate, arginine, glutamine, and lysine in tomato fruits, significantly reduced the content of aspartic acid, and had no significant effect on the accumulation of cystine, histidine, serine, cysteine, asparagine, and threonine. Compared with the treatment of supplementing light for 3 h in the evening, supplementing light for 3 h in the morning significantly increased the accumulation of threonine, phenylalanine, leucine, isoleucine, asparagine, methionine, valine, proline, tyrosine, cysteine, alanine, histidine, aspartic acid, arginine, and cystine in tomato fruits (**Figure 4A**; see also **Supplementary Table 4**). Moreover, providing light supplementation for 3 h in both the morning and evening promoted significant increases in the accumulation of total amino acids in tomato fruits, with amounts in the fruit of plants exposed morning supplementation being significantly higher than those in plants receiving evening supplementation (**Figure 4B**).

Carotenoid Components

Carotenoids are important natural pigments in plants. We determined four common carotenoids in tomato fruits. We found that providing supplementary light for 3 h in the evening promoted a significant increase in the fruit contents of

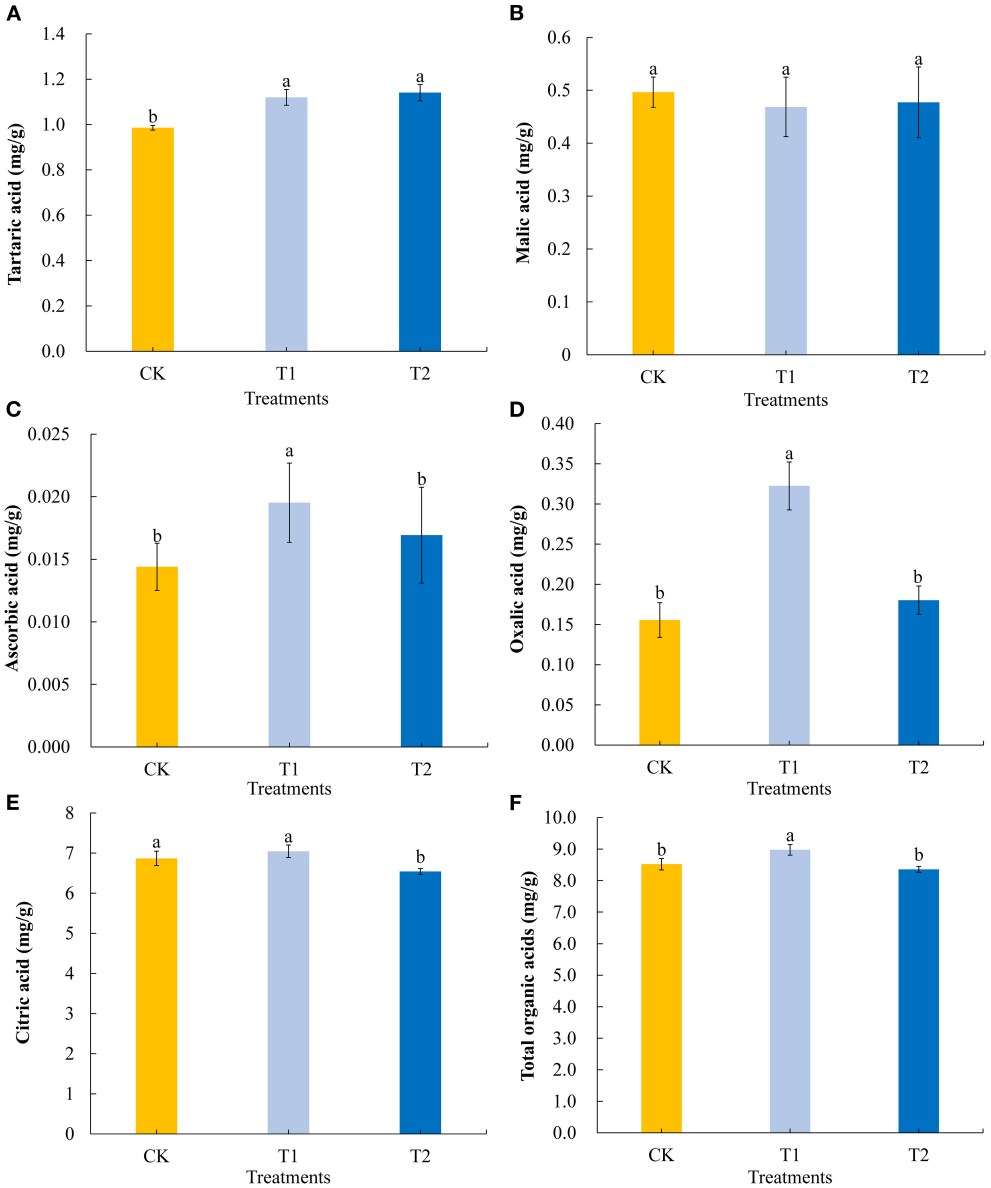


FIGURE 3 | Tartaric acid **(A)**, malic acid **(B)**, ascorbic acid **(C)**, oxalic acid **(D)**, citric acid **(E)**, and total organic acids **(F)** contents in tomato fruits under different LED supplementary light periods. The data are expressed as average values ± SD ($n = 3$). $^{a-c}$Indicate significant differences between treatments ($P < 0.05$, Duncan's multiple range test). The bars indicate standard errors. CK, no light supplementation control; T1, light supplementation for 3 h in the morning; T2, light supplementation for 3 h in the evening.

phytoene (**Figure 5A**), and that 3 h of both morning and evening light supplementation significantly increased the contents of beta carotene, with morning supplementation promoting a significantly higher accumulation of this carotene (**Figure 5B**). Both morning and evening light supplementation for 3 h had no significant effect on the lutein content in tomato fruits (**Figure 5C**), Both morning and evening light supplementation for 3 h significantly promoted the accumulation of lycopene (**Figure 5D**). Similarly, 3 h of both morning and evening light supplementation were found to promote significant increases in the accumulation of total carotenoids in tomato fruits, with morning supplementation having a more pronounced effect in this regard (**Figure 5E**).

Phenolic Acids and Flavonoids

Phenolic acids and flavonoids are natural antioxidants in plants, which can prevent diseases in the human body. We quantitatively analyzed 12 phenolic acids and 4 flavonoids in the tomato fruits. Compared with the control, supplementing light for 3 h in the morning significantly increased the contents of six phenolic acids and one flavonoid in the tomato fruit, including *p*-hydroxybenzoic acid, caffeic acid, cynarin, cinnamic acid,

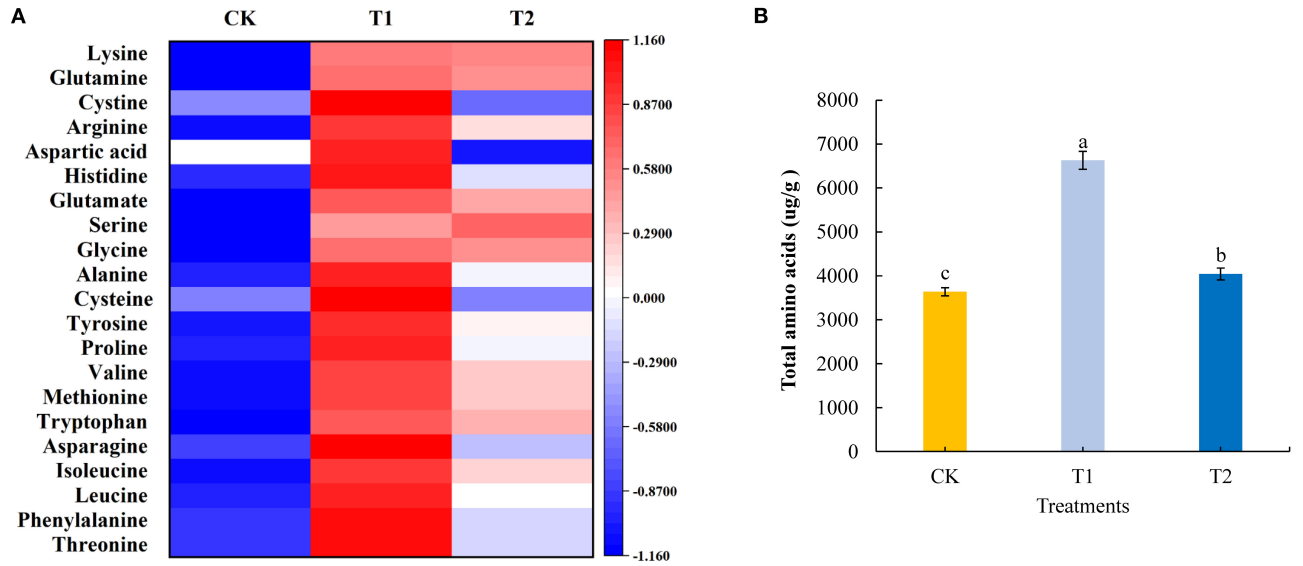


FIGURE 4 | Amino acid component **(A)**, total amino acids **(B)** contents in tomato fruits under different LED supplementary light periods. The data are expressed as average values ± SD ($n = 3$). $^{a-c}$Indicate significant differences between treatments ($P < 0.05$, Duncan's multiple range test). The bars indicate standard errors. CK, no light supplementation control; T1, light supplementation for 3 h in the morning; T2, light supplementation for 3 h in the evening.

benzoic acid, ferulic acid, and quercetin, and the contents of three phenolic acids and one flavonoid: gentisic acid, 4-coumaric acid, gallic acid, and rutin decreased significantly. Supplementing light for 3 h in the morning had no significant effect on the content of three phenolic acids and two flavonoids: protocatechuic acid, chlorogenic acid, sinapic acid, kaempferol, and naringenin. Supplementing light for 3 h at night significantly increased the contents of four phenolic acids and two flavonoids: *p*-hydroxybenzoic acid, sinapic acid, cinnamic acid, ferulic acid, quercetin, and rutin in tomato fruits, while the contents of four phenolic acids and one flavonoid, caffeic acid, gentisic acid, 4-coumaric acid, gallic acid, and naringenin, decreased significantly. Supplementing light for 3 h at night had no significant effect on the contents of four phenolic acids and one flavonoid: protocatechuic acid, chlorogenic acid, cynarin, benzoic acid, and kaempferol (**Figure 6A**, see also **Supplementary Table 5**). Moreover, compared with the control plants, supplementary lighting for 3 h in the morning was found to promote significant accumulations of total phenolic acids in tomato fruits, whereas we detected significant reductions in total flavonoid contents. Contrastingly, there were significant increases in the contents of total flavonoids in tomato fruits in response to 3 h of light supplementation in the evening, although this treatment had no significant effects on the accumulation of total phenolic acids (**Figures 6B,C**).

Mineral Contents

Table 1 shows the contents of minerals in tomato fruits under different light supplementation periods. Compared with the control, supplementing light for 3 h in the morning significantly increased the accumulation of Ca in tomato fruit, but significantly decreased the contents of P, Cu, Fe and Zn in tomato fruit, and had no significant effect on the contents of K, Mg, Na and Mn. Supplementing light for 3 h in the evening significantly increased the accumulation of Ca in tomato fruit but decreased the content of P and Zn, and there was no significant difference in the content of K, Mg, Na, Cu, Fe, and Mn.

Differences in the Aroma Characteristics of Tomato Fruits

In order to study the effect of supplementary light period on the aroma characteristics of tomato, we used electronic nose (E-nose) to analyze the volatile compounds of tomato fruit. The radar map presented in **Figure 7A** shows the difference in the response values of the 10 sensors of the E-nose to volatile compounds in tomato fruits exposed to the different treatments. We found that the W5S, W1C, W2W, W3C, and W3S sensors, which are specifically responsive to nitrogen oxides, aromatic benzene compounds, aromatic organic sulfide substances, aromatic ammonia compounds, and long-chain alkanes, respectively, were more sensitive to the volatile compounds in tomato fruits. We also carried out hierarchical cluster analysis (**Figure 7B**) on the aroma characteristics of tomato fruits exposed to different supplementary light treatments. We accordingly found that the three treatments clustered into two categories, with the control treatment without supplementary lighting comprising one cluster, and the 3 h morning and evening supplementary light treatments comprising the other. This result further indicated that the supplementary light treatment changed the aroma components of tomato fruits. Supplementary light for 3 h in the morning increased the contents of ammonia aromatics and long chain alkanes in the tomato fruits, while light supplementation for 3 h in the evening increased the contents of ammonia

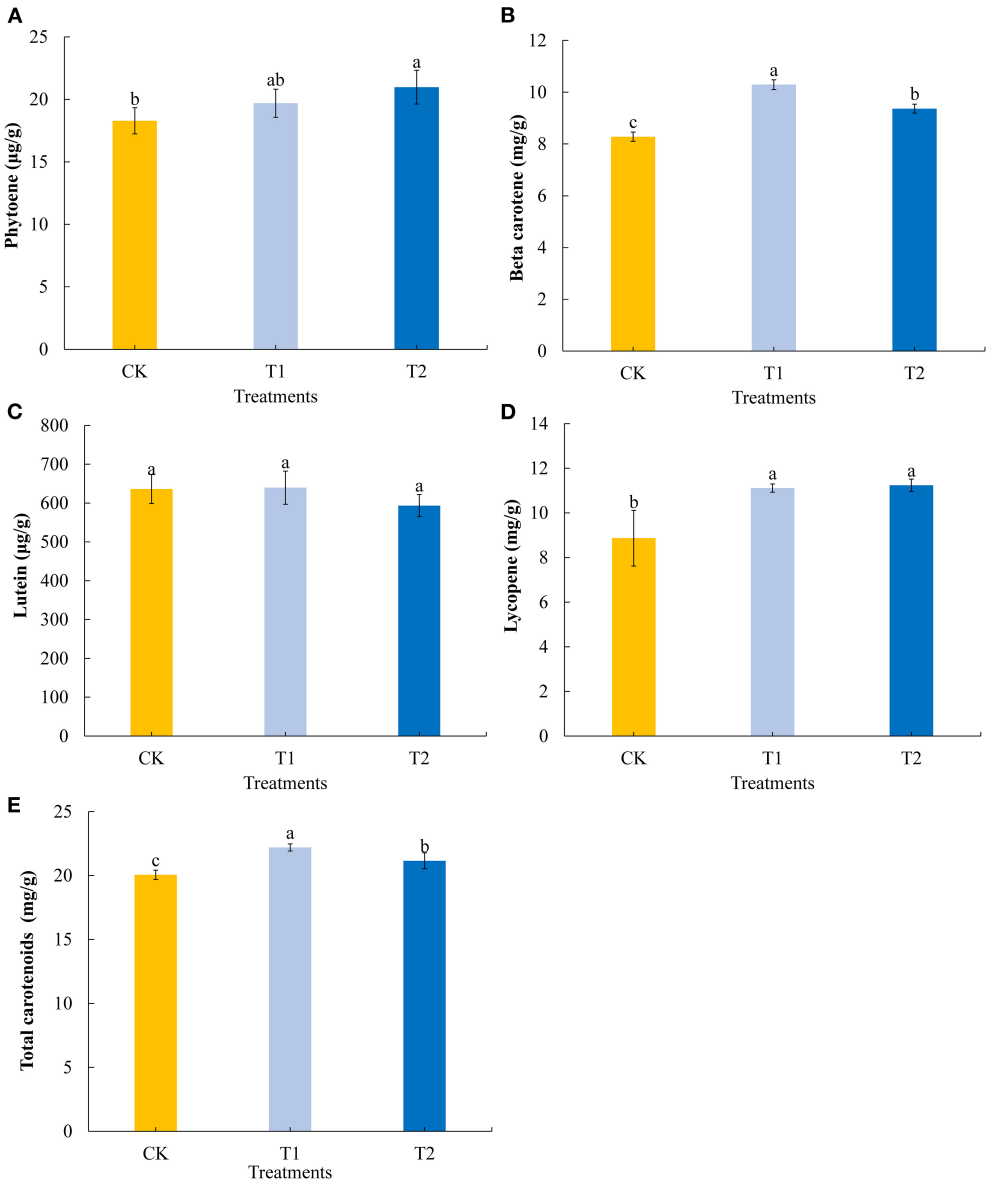


FIGURE 5 | Phytoene **(A)**, beta carotene **(B)**, lutein **(C)**, lycopene **(D)**, and total carotenoids **(E)** contents in tomato fruits under different LED supplementary light periods. The data are expressed as average values ± SD ($n = 3$). $^{a-c}$Indicate significant differences between treatments ($P < 0.05$, Duncan's multiple range test). The bars indicate standard errors were indicated by bars. CK, no light supplementation control; T1, light supplementation for 3 h in the morning; T2, light supplementation for 3 h in the evening.

aromatics, long chain alkanes, and benzene aromatics in the tomato fruits (**Supplementary Table 6**).

DISCUSSION

With the rapid development of LED light supplement technology, its use to improve the quality of vegetables and fruits has attracted research attention (40–42). We used red and blue (7R2B) LEDs to supplement the light of the flowering tomatoes in the morning and evening and analyzed the differences in the quality of tomatoes with different treatments using metabolomics-related technology. Sugars and organic acids in tomato fruit are the key components that affect tomato quality and people's favorite degree of flavor, so appropriately increasing the contents of sugar and organic acid in tomato fruit can improve the quality of tomatoes (43). Our study found that light supplementation for 3 h in the evening promoted the accumulation of fructose, glucose, sucrose, and total sugar in tomato fruit, while light supplementation for 3 h in the morning had no significant effect on the contents of fructose, glucose, and total sugar. However, light supplementation for 3 h in the morning significantly reduced the content of sucrose

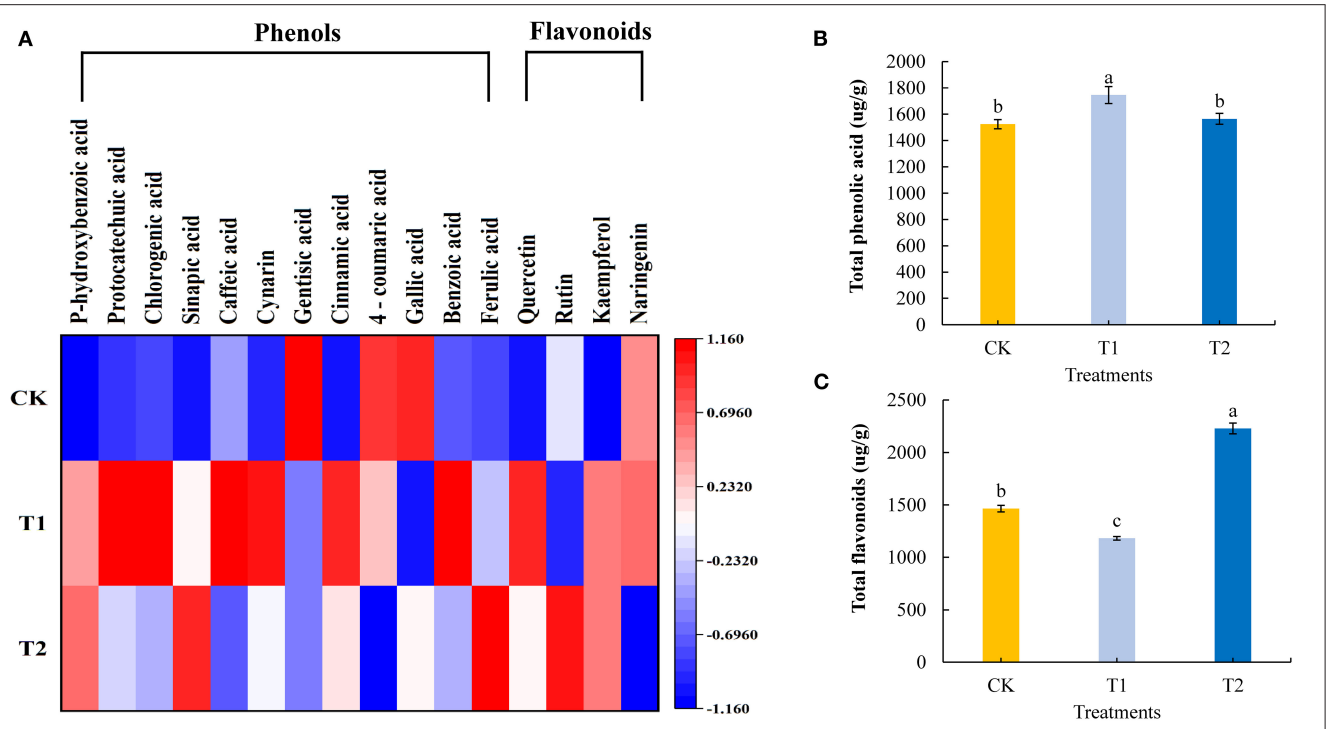


FIGURE 6 | Phenolic acid component and flavonoids component **(A)**, total phenolic acid **(B)**, and total flavonoids **(C)** contents in tomato fruits under different LED supplementary light periods. The data are expressed as average values ± SD ($n = 3$). $^{a-c}$Indicate significant differences between treatments ($P < 0.05$, Duncan's multiple range test). The bars indicate standard errors. CK, no light supplementation control; T1, light supplementation for 3 h in the morning; T2, light supplementation for 3 h in the evening.

TABLE 1 | Mineral contents in tomato fruits under different LED supplementary light periods (mg/g).

Treatments	P	K	Ca	Mg	Na	Cu	Fe	Mn	Zn
CK	8.24 ± 0.30^{a}	55.13 ± 5.45^{a}	1.31 ± 0.04^{b}	1.47 ± 0.09^{a}	0.089 ± 0.013^{a}	0.334 ± 0.076^{a}	0.159 ± 0.022^{a}	0.0246 ± 0.003^{a}	0.0856 ± 0.009^{a}
T1	7.52 ± 0.33^{b}	50.25 ± 2.88^{a}	1.53 ± 0.03^{a}	1.60 ± 0.04^{a}	0.067 ± 0.002^{a}	0.199 ± 0.014^{b}	0.121 ± 0.005^{b}	0.0252 ± 0.004^{a}	0.0433 ± 0.008^{b}
T2	7.31 ± 0.17^{b}	51.05 ± 3.62^{a}	1.60 ± 0.05^{a}	1.49 ± 0.08^{a}	0.072 ± 0.006^{a}	0.236 ± 0.076^{ab}	0.148 ± 0.016^{ab}	0.0256 ± 0.004^{a}	0.0567 ± 0.009^{b}

The data are expressed as average values ($n = 3$). Different lowercase letters indicate significant differences between treatments ($P < 0.05$) according to Duncan's multiple range test. CK, no light supplementation control; T1, light supplementation for 3 h in the morning; T2, light supplementation for 3 h in the evening.

in the fruit, because there was no significant change in the content of total sugar in the fruit (**Figure 2**). This may be because the supplementary light in the morning promoted the decomposition of sucrose in tomato fruit to some extent and accelerated the conversion of sucrose to fructose and glucose (44). The study of organic acid in tomato fruit showed that supplementing light for 3 h in the morning increased the contents of tartaric acid, ascorbic acid, oxalic acid, and total organic acid in tomato fruit, but had no significant effect on the accumulation of malic acid and citric acid. Supplementing light for 3 h at night increased the content of tartaric acid and decreased the content of citric acid in tomato fruit but had no significant effect on the contents of malic acid, ascorbic acid, oxalic acid, and total organic acid (**Figure 3**). The sour taste of tomato is mainly attributed to citric acid and malic acid, and citric acid accounts for the highest proportion of the organic acids in tomato fruit (45). Thus, these results showed that supplementing light for 3 h in the evening significantly reduced the sour taste of tomato fruit, while supplementing light for 3 h in the morning increased the sour taste of tomato fruit. To sum up, supplementing light for 3 h in the evening can increase the content of soluble sugar in tomato fruit and reduce the content of organic acid, improve the flavor quality of tomato fruit, and may enhance the favorite degree of consumers.

Amino acids are the basic substances that make up proteins, which can maintain the normal metabolism of life and provide material basis for life activities. Amino acids have rich tastes such as sweetness, sourness, bitterness and saltiness, and are important taste substances in tomato fruits (46). Our study found that supplementing light for 3 h in the morning could significantly increase the contents of most amino acids and total amino acids in tomato fruits, while supplementing light for 3 h in the evening could also increase the contents of some amino acids and total amino acids in tomato fruits. However, the type and degree of

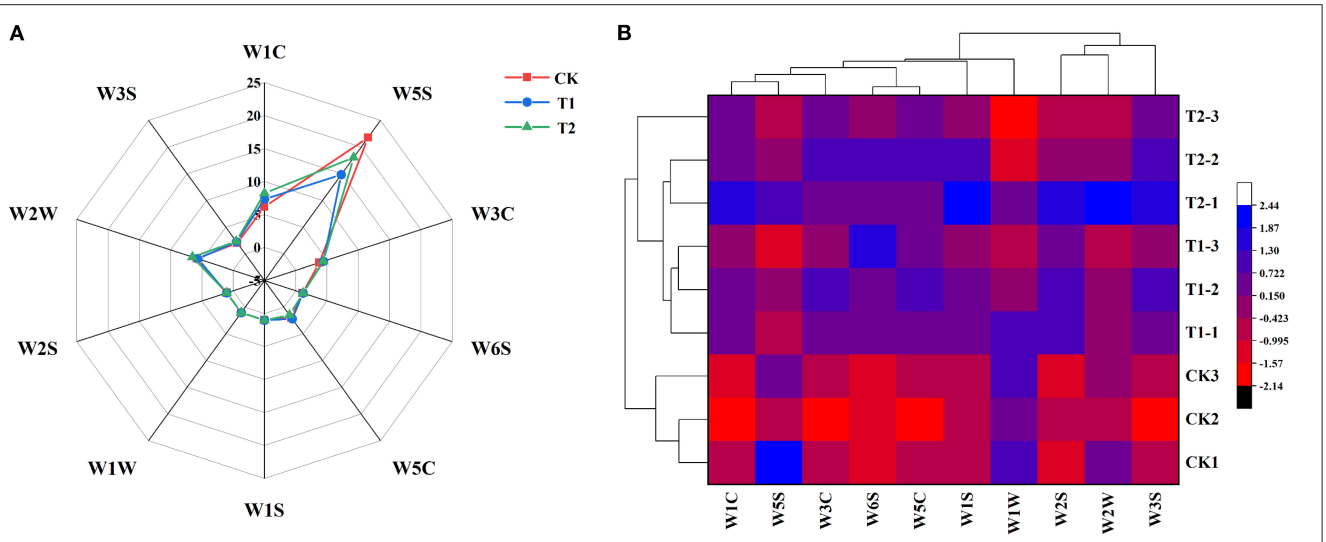


FIGURE 7 | Aroma characteristic response values **(A)** and HCA **(B)** of tomato fruits under different LED supplementary light periods. The data are expressed as average values ($n = 3$). CK, no light supplementation control; T1, light supplementation for 3 h in the morning; T2, light supplementation for 3 h in the evening.

improvements were significantly weaker than those following light supplement for 3 h in the morning (**Figure 4**). These results showed that supplementing light for 3 h in the morning and 3 h in the evening promoted the accumulation of amino acids in tomato fruits, but the accumulation effect of 3 h in the morning was better, which was similar to the effect of blue light LED irradiation on the content of amino acids in tomato fruits (32).

Carotenoid is the main pigment of tomato fruit and the precursor of odor and flavor synthesis (47). Carotenoid is also a substance that promotes human health and has a good effect on reducing coronary heart disease and lung cancer (48). Our study found that light supplementation for 3 h in the morning promoted the accumulation of beta carotene, lycopene, and total carotenoids in tomato fruits. Light supplementation for 3 h in the evening promoted the accumulation of octahydrolycopene, beta carotene, lycopene, and total carotenoids in tomato fruits, but the increase in the contents of beta carotene and total carotenoids in tomato fruits after light supplementation for 3 h in the evening was weaker than that in the morning. However, the two supplementary light treatments had no significant effect on the accumulation of lutein in fruit (**Figure 5**). This is similar to the results of Dannehl et al. (26) and Panjai et al. (49); they treated tomatoes with full spectrum LED and red-light LED, respectively, and found that lycopene and beta carotene in tomato fruit increased significantly after the treatments with LEDs.

Phenolic acids and flavonoids are important substances that regulate the sensory quality of vegetables and fruits (50). They have attracted wide attention because of their physiological effects such as antioxidation, anti-inflammation, and anti-allergy; these are important substances that can promote human health (51, 52). Among the 12 phenolic acids and 4 flavonoids we determined, supplementing light for 3 h in the morning increased the accumulation of 6 phenolic acids and total phenolic acids in the tomato fruits but decreased the accumulation of total flavonoids. Supplementing light for 3 h at night increased the accumulation of total flavonoids in tomato fruit but had no significant effect on the accumulation of total phenolic acid (**Figure 6**). These results showed that supplementing light for 3 h in the morning and 3 h at night had different effects on the accumulation of phenolic acids and flavonoids in tomato fruits, which was similar to results from previous studies. Previous studies found that supplementing light with white light and blue light LED could significantly increased the accumulation of phenols in okra (53), and red-blue light and red-blue-green light LED treatment significantly increased the accumulation of total phenols and flavonoids in tea callus (54).

We also studied the mineral content in tomato fruit. Minerals not only regulate the growth and development of plants, but also play an important role in human health and growth (55, 56). Our study showed that light supplementation for 3 h in the morning and 3 h in the evening promoted the accumulation of Ca in tomato fruits, but light supplementation for 3 h in the morning decreased the contents of P, Cu, Fe, and Zn in tomato fruits, and light supplementation for 3 h in the evening decreased the contents of K, Mg, Na, Cu, Fe, and Mn (**Table 1**). Some studies have found that red-blue light LED can promote the ripening of tomato fruit by increasing the content of melatonin in tomato fruit (27). In the process of tomato fruit ripening, some mineral content in tomato fruit will decrease to a certain extent (57). In this study, the change of mineral content in tomato fruit may be due to the fact that LED light supplementation promoted the ripening of tomato fruit, which led to the decrease of mineral content in advance of that in fruit without light supplement. Therefore, the mineral content in tomato fruit decreased in different degrees under different light supplement treatments.

Volatile aroma, along with sugars and organic acids, is an important factor affecting tomato fruit flavor, and aroma characteristics are important indicators to distinguish

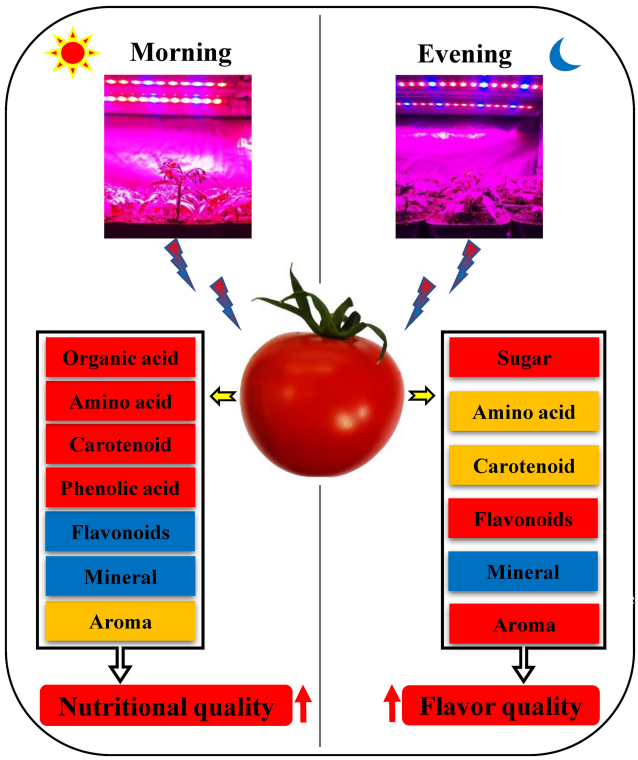


FIGURE 8 | Effects of LED supplementation on fruit quality of tomato in the morning and evening. Red represents an increase in the substance content, orange also represents an increase in the substance content, but the increase is weaker than the red box, and blue represents a decrease in the substance content.

different vegetables and fruits (58). Our study showed that light supplementation for 3 h in the morning and evening significantly increased the contents of ammonia aromatics and long-chain alkanes in tomato fruits, and light supplementation for 3 h in the evening also increased the content of benzene aromatics in tomato fruits. The aroma characteristics of tomato fruits of 3 h in the morning and 3 h in the evening were similar and were clustered into the same category, while the aroma characteristics of tomato fruits without supplemental light treatment were clustered into a separate category (**Figure 7**). This indicated that the aroma characteristics of tomato fruits were changed by supplementing light for 3 h in the morning and evening, and the benzene aromatic substances in tomato fruits were increased by supplementing light for 3 h in the evening. The latter makes the aroma of tomato fruit more rich (59), which may be more treasured by consumers.

CONCLUSION

The use of red and blue (7R2B) LEDs to supplement the light of the flowering tomato plants in the morning and evening had different effects on the flavor and nutritional quality of the tomato fruit. Supplementing light for 3 h in the morning significantly increased VC, total organic acids, amino acids, carotenoids, phenolic acids, ammonia aromatics, and long-chain alkanes in the fruit aroma of tomato fruit, and reduced the contents of flavonoids and some minerals, without significant effects on the sugar content of the fruit. Supplementing light for 3 h in the evening significantly increased the contents of sugars, amino acids, carotenoids, flavonoids in tomato fruit, and ammonia aromatic substances, long-chain alkanes, and benzene aromatic substances in the fruit aroma, and decreased the content of minerals, but had no significant effect on the contents of total organic acids and phenolic acids in tomato fruits. However, compared with 3 h of light supplementation in the evening, 3 h of light supplementation in the morning promoted the accumulation of health-promoting substances such as VC, amino acids and carotenoids in tomato fruits. In conclusion, supplementing light for 3 h in the morning improved the nutritional quality of tomato fruit, while supplementing light for 3 h in the evening improved the flavor quality (**Figure 8**).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

JL, JY, and SW contributed to conception and design of the study. SW, YX, and WZ participated in the experiment. NJ, LJ, and LH organized the database. SW, NJ, XX, and ZL performed the statistical analysis. SW wrote the first draft of the manuscript. JL, JY, and YW reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This research was funded by the Education science and technology innovation project of Gansu Province (GSSYLXM-02), the Special project of central government guiding local science and technology development (ZCYD-2021-07), Gansu people's livelihood science and technology project (20CX9NA099), the Fuxi Young Talents Fund of Gansu Agricultural University (GAUfx-04Y03), the Special project of national modern agricultural industrial system (CARS-23-C-07), and the Gansu excellent postgraduates Innovation Star project (2021CXZX-374).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.833723/full#supplementary-material

REFERENCES

1. Liu HR, Meng FL, Miao HY, Chen SS, Yin TT, Hu SS, et al. Effects of postharvest methyl jasmonate treatment on main health-promoting components and volatile organic compounds in cherry tomato fruits. *Food Chem.* (2018) 263:194–200. doi: 10.1016/j.foodchem.2018.04.124
2. Lenucci MS, Cadinu D, Taurino M, Piro G, Dalessandro G. Antioxidant composition in cherry and high-pigment tomato cultivars. *J Agr Food Chem.* (2006) 54:2606–13. doi: 10.1021/jf052920c
3. Maharaj R, Arul J, Nadeau P. UV-C irradiation effects on levels of enzymic and non-enzymic phytochemicals in tomato. *Innov Food Sci Emerg.* (2014) 21:99–106. doi: 10.1016/j.ifset.2013.10.001
4. Giovannoni J. Tomato multiomics reveals consequences of crop domestication and improvement. *Cell.* (2018) 172:6–8. doi: 10.1016/j.cell.2017.12.036
5. Bertin N, Génard M. Tomato quality as influenced by preharvest factors. *Sci Hortic.* (2018) 233:264–76. doi: 10.1016/j.scienta.2018.01.056
6. Kechasov D, Verheul MJ, Paponov M, Panosyan A, Paponov IA. Organic waste-based fertilizer in hydroponics increases tomato fruit size but reduces fruit quality. *Front Plant Sci.* (2021) 12:680030. doi: 10.3389/fpls.2021.680030
7. Ruiz-Nieves JM, Ayala-Garay OJ, Serra V, Dumont D, Vercambre G, Génard M, et al. The effects of diurnal temperature rise on tomato fruit quality. Can the management of the greenhouse climate mitigate such effects? *Sci Hortic.* (2021) 278:109836. doi: 10.1016/j.scienta.2020.109836
8. Sellitto VM, Golubkina NA, Pietrantonio L, Cozzolino E, Cuciniello A, Cenvinzo V, et al. Tomato yield, quality, mineral composition and antioxidants as affected by beneficial microorganisms under soil salinity induced by balanced nutrient solutions. *Agriculture.* (2019) 9:5:110. doi: 10.3390/agriculture9050110
9. Paskovic I, Soldo B, Ban SG, Radic T, Lukic M, Urlic B, et al. Fruit quality and volatile compound composition of processing tomato as affected by fertilisation practices and arbuscular mycorrhizal fungi application. *Food Chem.* (2021) 359:129961. doi: 10.1016/j.foodchem.2021.129961
10. Yang LJ, Qu H, Zhang YL, Li FS. Effects of partial root-zone irrigation on physiology, fruit yield and quality and water use efficiency of tomato under different calcium levels. *Agric Water Manag.* (2012) 104:89–94. doi: 10.1016/j.agwat.2011.12.001
11. Stakhova LN, Ladygin VG, Stakhov LF. The effect of quercetin on the accumulation of carbohydrates and amino acids in tomato fruits. *Russ J Plant Physl.* (2001) 48:160–4. doi: 10.1023/A:1009087614221
12. Narváez-Ortiz WA, Becvort-Azcurra AA, Fuentes-Lara LO, Benavides-Mendoza A, Valenzuela-García JR, González-Fuentes JA. Mineral composition and antioxidant status of tomato with application of selenium. *Agronomy.* (2018) 8:9:185. doi: 10.3390/agronomy8090185
13. Weinert CH, Sonntag F, Egert B, Pawelzik E, Kulling SE, Smit I. The effect of potassium fertilization on the metabolite profile of tomato fruit (*Solanum lycopersicum* L.). *Plant Physiol Biochem.* (2021) 159:89–99. doi: 10.1016/j.plaphy.2020.12.010
14. Zuccarelli R, Rodriguez-Ruiz M, Lopes-Oliveira PJ, Pascoal GB, Andrade SCS, Furlan CM, et al. Multifaceted roles of nitric oxide in tomato fruit ripening: NO-induced metabolic rewiring and consequences for fruit quality traits. *J Exp Bot.* (2021) 72:941–58. doi: 10.1093/jxb/eraa526
15. Zhong TY, Yao GF, Wang SS, Li TT, Sun KK, Tang J, et al. Hydrogen sulfide maintains good nutrition and delays postharvest senescence in postharvest tomato fruits by regulating antioxidative metabolism. *J Plant Growth Regul.* (2021) 40:2548–2559. doi: 10.1007/s00344-021-10377-4
16. Ntagkas N, Vos RCH, Woltering EJ, Nicole CCS, Labrie C, Marcelis LFM. Modulation of the tomato fruit metabolome by LED light. *Metabolites.* (2020) 10:6:266. doi: 10.3390/metabo10060266
17. Alba R, Cordonnier-Pratt MM, Pratt LH. Fruit-localized phytochromes regulate lycopene accumulation independently of ethylene production in tomato. *Plant Physiol.* (2000) 123:1:363–70. doi: 10.1104/pp.123.1.363
18. Giliberto L, Perrotta G, Pallara P, Weller JL, Fraser PD, Bramley PM, et al. Manipulation of the blue light photoreceptor cryptochrome 2 in tomato affects vegetative development, flowering time, and fruit antioxidant content. *Plant Physiol.* (2005) 137:1:199–208. doi: 10.1104/pp.104.051987
19. Tang YK, Mao, RX, Guo, SS. Effects of LED spectra on growth, gas exchange, antioxidant activity and nutritional quality of vegetable species. *Life Sci in Space Res.* (2020) 26:77–84. doi: 10.1016/j.lssr.2020.05.002
20. Li QM, Liu YP, Tian SB, Liang ZW, Li SH, Li YM, et al. Effect of supplemental lighting on water transport, photosynthetic carbon gain and water use efficiency in greenhouse tomato. *Sci Hortic.* (2019) 256:108630. doi: 10.1016/j.scienta.2019.108630
21. Kim YX, Son S, Lee S, Jung E, Lee Y, Sung J, et al. Combined effects of nutrients× water× light on metabolite composition in tomato fruits (*Solanum lycopersicum* L.). *Plants.* (2021) 7:1437. doi: 10.3390/plants10071437
22. Paponov M, Kechasov D, Lacek J, Verheul MJ, Paponov IA. Supplemental light-emitting diode inter-lighting increases tomato fruit growth through enhanced photosynthetic light use efficiency and modulated root activity. *Front Plant Sci.* (2019) 10:1656. doi: 10.3389/fpls.2019.01656
23. Liu XY, Chen Z, Jahan MS, Wen YX, Yao XY, Ding HF, et al. RNA-Seq analysis reveals the growth and photosynthetic responses of rapeseed (*Brassica napus* L.) under red and blue LEDs with supplemental yellow, green, or white light. *Hortic Res.* (2020) 7:206. doi: 10.1038/s41438-020-00429-3
24. Gil HJ, Kim YX, Sung J, Jung ES, Singh D, Lee Y, et al. Metabolomic insights of the tomato fruits (*Solanum lycopersicum* L.) cultivated under different supplemental led lighting and mineral nutrient conditions. *Hortic Environ Biote.* (2020) 61:2:415–27. doi: 10.1007/s13580-019-00215-8
25. Dong F, Wang CZ, Sun XD, Bao ZL, Dong C, Sun CH, et al. Sugar metabolic changes in protein expression associated with different light quality combinations in tomato fruit. *Plant Growth Regul.* (2019) 88:267–82. doi: 10.1007/s10725-019-00506-1
26. Dannehl D, Schwend T, Veit D, Schmidt U. Increase of yield, lycopene, and lutein content in tomatoes grown under continuous PAR spectrum LED lighting. *Front Plant Sci.* (2021) 12:611236. doi: 10.3389/fpls.2021.611236
27. Li Y, Liu C, Shi QH, Yang FJ, Wei M. Mixed red and blue light promotes ripening and improves quality of tomato fruit by influencing melatonin content. *Environ Exp Bot.* (2021) 185:104407. doi: 10.1016/j.envexpbot.2021.104407
28. Wang W, Liu DX, Qin M, Xie ZB, Chen RY, Zhang YT. Effects of supplemental lighting on potassium transport and fruit coloring of tomatoes grown in hydroponics. *Int J Mol Sci.* (2021) 22:2687. doi: 10.3390/ijms22052687
29. Kong DH, Zhao WT, Ma Y, Liang H, Zhao XY. Effects of light-emitting diode illumination on the quality of fresh-cut cherry tomatoes during refrigerated storage. *Int J Food Sci Tech.* (2020) 56:2041–52. doi: 10.1111/ijfs.14836
30. Zushi K, Suehara C, Shirai M. Effect of light intensity and wavelengths on ascorbic acid content and the antioxidant system in tomato fruit grown *in vitro*. *Sci Hortic.* (2020) 274:109673. doi: 10.1016/j.scienta.2020.109673
31. Panjai L, Noga G, Hunsche M, Fiebig A. Optimal red light irradiation time to increase health-promoting compounds in tomato fruit postharvest. *Sci Hortic.* (2019) 251:189–96. doi: 10.1016/j.scienta.2019.03.019
32. Dhakal R, Baek KH. Metabolic alternation in the accumulation of free amino acids and γ-aminobutyric acid in postharvest mature green tomatoes following irradiation with blue light. *Hortic Environ Biote.* (2014) 55:36–41. doi: 10.1007/s13580-014-0125-3
33. Kim HJ, Yang T, Choi S, Wang YJ, Lin MY, Liceaga AM. Supplemental intracanopy far-red radiation to red LED light improves fruit quality attributes of greenhouse tomatoes. *Sci Hortic.* (2020) 261:108985. doi: 10.1016/j.scienta.2019.108985
34. Wang SY, Lv J, Yu JH, Jin N, Jin L, Liu XQ, et al. Effects of different light supplement duration on growth, yield and quality of tomato cultivated in solar-greenhouse. *Chinese vegetables.* (2018) 10:35–9. (In Chinese).
35. Müller NA, Wijnen CL, Srinivasan A, Ryngajllo M, Ofner I, Lin T, et al. Domestication selected for deceleration of the circadian clock in cultivated tomato. *Nat Genet.* (2016) 48:89–93. doi: 10.1038/ng.3447
36. Boxall SF, Kadu N, Dever LV, Knerova J, Waller JL, Gould PJD, et al. Kalanchoe PPC1 is essential for crassulacean acid metabolism and the regulation of core circadian clock and guard cell signaling genes. *Plant Cell.* (2020) 32:4:1136–60. doi: 10.1105/tpc.19.00481
37. Ma R, Bao FW, Feng WN, Xie M, Zhang YF, Chen WH. Determination of free amino acids in tobacco by liquid chromatography tandem mass spectrometry. *China Measure Test.* (2013) 39:34–7. (In Chinese).

38. Kang BS, Zhao WE, Hou YB, Tian P. Expression of carotenogenic genes during the development and ripening of watermelon fruit. *Sci Hortic.* (2010) 124:368–75. doi: 10.1016/j.scienta.2010.01.027
39. Gao YM, Tian P, Li J, Cao Y, Xu WR, Li JS. Transcriptional changes during tomato ripening and influence of brackish water irrigation on fruit transcriptome and sugar content. *Plant Physiol Bioch.* (2019) 145:21–33. doi: 10.1016/j.plaphy.2019.10.025
40. Zhou CB, Shao MJ, Liu WK, Li BS, Wang Q, Liu JY, et al. Regulation of ascorbate accumulation and metabolism in lettuce by end-of-production high light irradiation provided by red and blue LEDs. *Environ Exp Bot.* (2021) 189:104567. doi: 10.1016/j.envexpbot.2021.104567
41. Molmann JAB, Hansen E, Johansen TJ. Effects of supplemental LED light quality and reduced growth temperature on swede (*Brassica napus* L. *ssp.* rapifera Metzg.) root vegetable development and contents of glucosinolates and sugars. *J Sci Food Agric.* (2021) 101:2422–7. doi: 10.1002/jsfa.10866
42. Díaz-Galián MV, Torres M, Sanchez-Pagán JD, Navarro PJ, Weiss J, Egea-Cortines M. Enhancement of strawberry production and fruit quality by blue and red LED lights in research and commercial greenhouses. *S Afr J Bot.* (2021) 140:269–75. doi: 10.1016/j.sajb.2020.05.004
43. Zhao JT, Xu Y, Ding Q, Huang XL, Zhang YT, Zou ZR, et al. Association mapping of main tomato fruit sugars and organic acids. *Front Plant Sci.* (2016) 7:1286. doi: 10.3389/fpls.2016.01286
44. Duran-Soria S, Pott DM, Osorio S, Vallarino JG. Sugar signaling during fruit ripening. *Front Plant Sci.* (2020) 11:564917. doi: 10.3389/fpls.2020.564917
45. Bastías A, López-Climent M, Valcárcel M, Rosello S, Gómez-Cadenas A, Casaretto JA. Modulation of organic acids and sugar content in tomato fruits by an abscisic acid-regulated transcription factor. *Physiol Plantarum.* (2011) 141:215–26. doi: 10.1111/j.1399-3054.2010.01435.x
46. Kato H, Rhue MR, Nishimura T. Role of free amino acids and peptides in food taste. *Am Chem Soc.* (1989) 13:158–74. doi: 10.1021/bk-1989-0388.ch013
47. Quinet M, Angosto T, Yuste-Lisbona FJ, Blanchard-Gros R, Bigot S, Martinez JP, et al. Tomato fruit development and metabolism. *Front Plant Sci.* (2019) 10:1554. doi: 10.3389/fpls.2019.01554
48. Bruno A, Durante M, Marrese PP, Migoni D, Laus MN, Pace E, et al. Shades of red: Comparative study on supercritical CO_2 extraction of lycopene-rich oleoresins from gac, tomato and watermelon fruits and effect of the α-cyclodextrin clathrated extracts on cultured lung adenocarcinoma cells' viability. *J Food Compos Anal.* (2018) 65:23–32. doi: 10.1016/j.jfca.2017.08.007
49. Panjai L, Röhlen-Schmittgen S, Ellenberger J, Noga G, Hunsche M, Fiebig A. Effect of postharvest irradiation with red light on epidermal color and carotenoid concentration in different parts of tomatoes. *J Food Meas Charact.* (2021) 15:1737–46. doi: 10.1007/s11694-020-00770-0
50. Cory H, Passarelli S, Szeto J, Tamez M, Mattei J. The role of polyphenols in human health and food systems: A mini-review. *Front Nutr.* (2018) 5:87. doi: 10.3389/fnut.2018.00087
51. Shahidi F, Yeo JD. Bioactivities of phenolics by focusing on suppression of chronic diseases: a review. *Int J Mol Sci.* (2018) 19:1573. doi: 10.3390/ijms19061573
52. Tohge T, Souza LPD, Fernie AR. Current understanding of the pathways of flavonoid biosynthesis in model and crop plants. *J Exp Bot.* (2017) 68:4013–28. doi: 10.1093/jxb/erx177
53. Wilawan N, Ngamwonglumlert L, Devahastin S, Chiewchan N. Changes in enzyme activities and amino acids and their relations with phenolic compounds contents in okra treated by LED lights of different colors. *Food and Bioprocess Tech.* (2019) 12:1945–54. doi: 10.1007/s11947-019-02359-y
54. Jang EB, Ho TT, Park SY. Effect of light quality and tissue origin on phenolic compound accumulation and antioxidant activity in Camellia japonica calli. *In Vitro Cell Dev.* (2020) 56:567–77. doi: 10.1007/s11627-020-10121-9
55. Singh S, Parihar P, Singh R, Singh VP, Prasad SM. Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. *Front Plant Sci.* (2016) 6:1143. doi: 10.3389/fpls.2015.01143
56. Martínez-Ballesta MC, Dominguez-Perles R, Moreno DA, Muries B, Alcaraz-López C, Bastías E, et al. Minerals in plant food: effect of agricultural practices and role in human health. A review. *Agron Sustain Dev.* (2010) 30:295–309. doi: 10.1051/agro/2009022
57. Costa F, Baeta MDL, Saraiva D, Verissimo MT, Ramos F. Evolution of mineral contents in tomato fruits during the ripening process after harvest. *Food Anal Method.* (2010) 4:410–5. doi: 10.1007/s12161-010-9179-8
58. Baldwin EA, Goodner K, Plotto A. Interaction of volatiles, sugars, and acids on perception of tomato aroma and flavor descriptors. *J Food Sci.* (2008) 73:S294–307. doi: 10.1111/j.1750-3841.2008.00825.x
59. Martina M, Tikunov Y, Portis E, Bovy AG. The genetic basis of tomato aroma. *Genes.* (2021) 12:2:226. doi: 10.3390/genes12020226

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Wang, Jin, Jin, Xiao, Hu, Liu, Wu, Xie, Zhu, Lyu and Yu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 08 March 2022
doi: 10.3389/fnut.2022.849163

Bioaccessibility and Antioxidant Capacity of Bioactive Compounds From Various Typologies of Canned Tomatoes

Luana Izzo [1†], Luigi Castaldo [1†], Sonia Lombardi [1], Anna Gaspari [1], Michela Grosso [2,3*] and Alberto Ritieni [1,4]*

[1] Laboratory of Food Chemistry, Department of Pharmacy, School of Medicine, University of Naples Federico II, Naples, Italy, [2] Laboratory of Biochemistry and Molecular Biology, Department of Molecular Medicine and Medical Biotechnology, School of Medicine, University of Naples Federico II, Naples, Italy, [3] CEINGE-Biotecnologie Avanzate, Naples, Italy, [4] Health Education and Sustainable Development, University of Naples Federico II, Naples, Italy

OPEN ACCESS

Edited by:
José Pinela, Polytechnic Institute of Bragança (IPB), Portugal

Reviewed by:
Senem Kamiloglu, Uludag University, Turkey
Chafik Hdider, Institut National de la Recherche Agronomique de Tunisie (INRAT), Tunisia

***Correspondence:**
Luana Izzo
luana.izzo@unina.it
Michela Grosso
michela.grosso@unina.it

†These authors have contributed equally to this work and share first authorship

Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *05 January 2022*
Accepted: *07 February 2022*
Published: *08 March 2022*

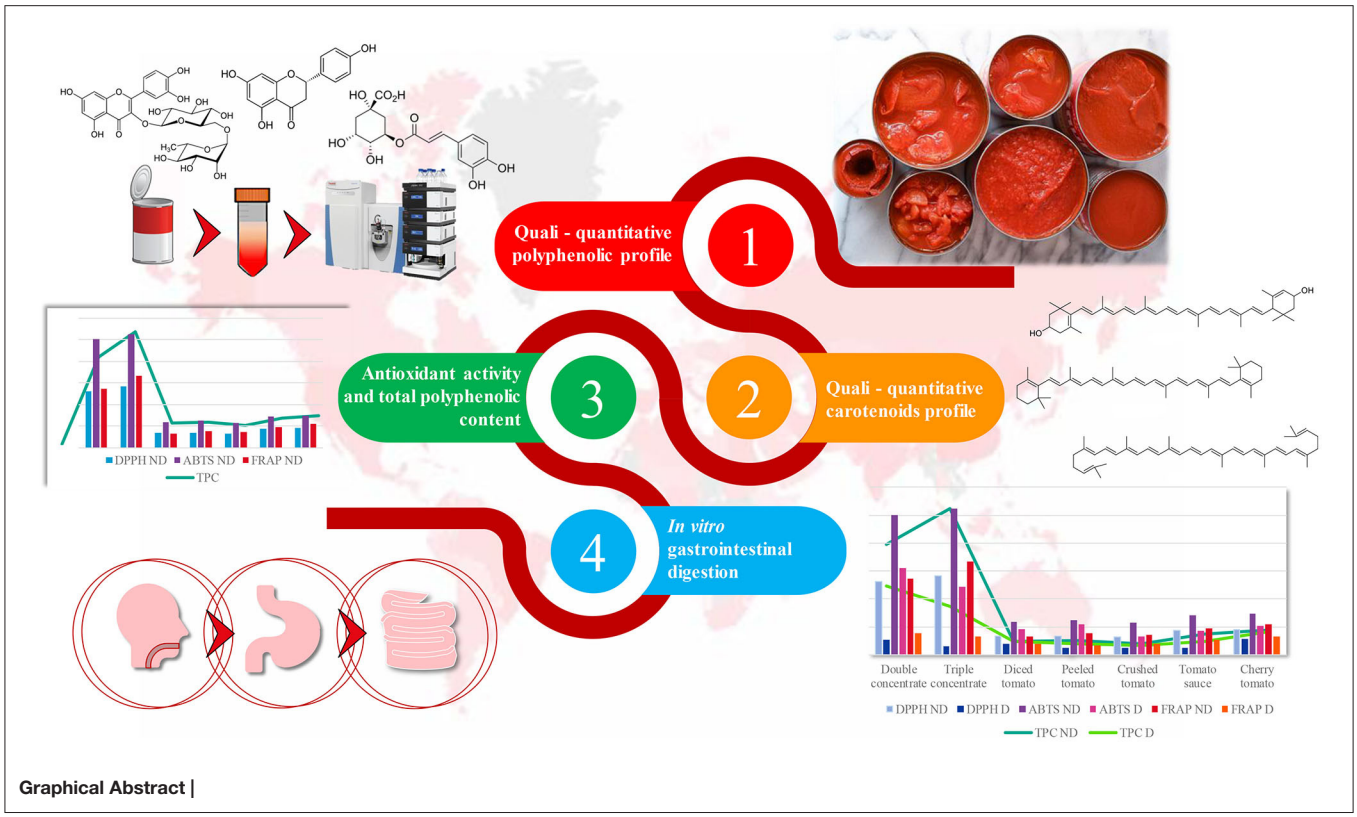
Citation:
Izzo L, Castaldo L, Lombardi S, Gaspari A, Grosso M and Ritieni A (2022) Bioaccessibility and Antioxidant Capacity of Bioactive Compounds From Various Typologies of Canned Tomatoes. Front. Nutr. 9:849163. doi: 10.3389/fnut.2022.849163

Tomato (*Solanum lycopersicum* L.) is one of the most consumed vegetables in the world; it contains high amounts of antioxidant phytochemicals and essential nutrients. Although it is commonly consumed fresh, more than 80% of its consumption derives from processed products. Since limited information on changes in the bioaccessibility of bioactive compounds during gastrointestinal digestion was reported, this current study aimed to monitor the antioxidant activity, total polyphenolic and carotenoid content, and bioaccessibility during *in vitro* gastrointestinal digestion of different typologies ($n = 7$) of canned tomatoes. A comprehensive evaluation of the polyphenolic profile of digested and not digested samples was ascertained by ultra-high-performance liquid chromatography combined with high-resolution Orbitrap mass spectrometry. The results highlighted a considerable content of rutin (1.191–9.516 mg/100 g), naringenin (0.359–1.452 mg/100 g), chlorogenic acid (1.857–11.236 mg/100 g), and lycopene (50.894–222.061 mg/kg) in the analyzed matrices. After *in vitro* gastrointestinal digestion, large variability, losses and low recovery were recorded. An appreciable percentage of rutin (30.7%), naringenin (29.6%), chlorogenic acid (25.8%), and lycopene (varied between 9.3 and 20%) remained bioaccessible after the *in vitro* gastrointestinal digestion. Our study could be a valid support to evaluate which content of bioactive compounds could be really bioaccessible to exercise beneficial effects on human health.

Keywords: canned tomatoes, *in vitro* gastrointestinal digestion, bioaccessibility, antioxidant activity, polyphenol, carotenoid

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most produced vegetables in the world, with an annual production of 186, 821 million tons. According to latest data reported by the Food and Agriculture Organization (FAO), Italy ranks 4th in world production of tomato and its products, with an annual production of 6,248 million tons, 27.4% of overall European amount in the year 2020. Although it is commonly consumed as fresh, more than 80% of its consumption derives from processed products (1, 2). The Italian consumption of preserved whole tomatoes or in pieces corresponds to 34 g per capita/day (3).



Graphical Abstract |

Consumption of fresh or processed tomato plays an important role in nutrition because of well-established health benefits. Tomato is known as a reliable source of biologically active compounds and essential nutrients owing to the array of phenolics and carotenoids it contains (4, 5).

Polyphenols are considered one of the most numerous and widely distributed groups of natural products synthesized by plants (6). There is a broad collection of natural products with similar structural properties that include various subgroups of phenolic compounds, essentially divided into flavonoid and non-flavonoid compounds. Flavonoids contain two benzene rings connected by a 3-carbon linking chain from the nearby pyran ring, whereas phenolic acids, non-flavonoid polyphenolic compounds, are substances composed of a phenolic ring and an organic carboxylic acid function (C6-C1 skeleton) (7, 8). Polyphenols have an important impact on reduction in the risk of chronic degenerative diseases and prevention of cardiovascular heart disease, inflammatory effects, and gastrointestinal disorders (9–11).

Non-nutritive phytochemicals such as lycopene, β-carotene, and lutein belonging to the carotenoid class are also present in significant amounts in tomato (12). Carotenoids constitute a polyene chain that is sometimes terminated by rings and may have additional oxygen atoms attached. Carotenoids are responsible for pigmentation of fruits and vegetables, and play an important role in human health because of their powerful antioxidant potential. In particular, they are associated with anti-inflammation, anti-aging, and anticancer, and they have anti-ulcer capacity as well as other chemoprotective capabilities (13).

As widely reported in literature, tomato contains considerable amounts of phenolic acids and flavonoids, such as rutin, naringenin, chlorogenic acid, and carotenoids. Tomato and its derived products represent major sources of lycopene, which is particularly abundant in ripe tomatoes with a concentration ranging between 30 and more than 200 mg per kg of fresh product (14–16).

To confer beneficial effects on health, bioactive compounds need to be bioaccessible before they are bioavailable to reach target tissues after gastrointestinal (GI) digestion (17). Bioaccessibility is defined as the fraction of nutrients released from the food matrix during GI digestion that is available for absorption (18). Several factors, such as food conservation, cooking, combinations of macronutrients, gastric pH, processing and preservation methods, and lytic enzymes, are able to influence the bioaccessibility of bioactive compounds, which, although they are present in the matrix as such, may not be absorbed (19).

Until now, several analytical approaches are being reported for polyphenol determination in tomato (20–23), such as liquid chromatography coupled to mass spectrometry (LC-MS). Recently, ultra-HPLC combined with high-resolution mass spectrometry has represented an optimal choice for appropriate identification and characterization of compounds with reduction in run time and amelioration in peak shape and accuracy (24–26).

Hence, this current study aimed to investigate the antioxidant activity and total polyphenol content of different typologies ($n = 7$) of commercially canned tomatoes, and evaluate their bioaccessibility during an *in vitro* GI digestion. In addition, qualitative-quantitative profiling of polyphenolic ($n = 43$) and carotenoid ($n = 3$) compounds was performed on extracts and after *in vitro* GI digestion by ultra-high-performance liquid chromatography coupled to high-resolution Orbitrap mass spectrometry and HPLC-diode-array UV/VIS detector analysis, respectively. To the best of the authors' knowledge, this is the first study that investigated these aspects of Italian canned tomatoes.

MATERIALS AND METHODS

Chemicals and Reagents

Water for chromatography (LC-MS grade) (< 18 MΩ/cm resistivity) used for the experiments was acquired from Merck SpA (Milan, Italy). The acetonitrile (Acn), methanol (MeOH), formic acid (FA), acetic acid (AcOH), ethanol (EtOH), hydrochloric acid (HCl), chloroform, and n-hexane of HPLC grade used for analyses were purchased from CARLO ERBA Reagents (Milan, Italy).

All the salts: anhydrous magnesium sulfate ($MgSO_4$), sodium chloride (NaCl), potassium thiocyanate (KSCN), sodium bisphosphate (NaH_2PO_4) potassium persulfate ($K_2S_2O_8$), potassium hydroxide (KOH), sodium hydroxide (NaOH), butylhydroxytoluene (BHT), calcium chloride ($CaCl_2$), sodium bicarbonate (Na_2CO_3), diamine salt of 2,2'-azino-bis (3-ethylbenzothiazolin-6-sulfonic) acid (ABTS), ferrous chloride, 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, Trolox 6-hydroxy−2,5,7,8-tetramethylcroman-2-carboxylic acid; and enzymes: α-amylase (≥ 5 units/mg), pepsin from porcine gastric mucosa (≥ 250 units/mg), pancreatin from porcine pancreas (8 × USP), and bile salts were purchased from Sigma Aldrich (Milan, Italy). Hydrophilic polytetrafluoroethylene (PTFE) syringe filters (15 mm; 0.2 μm) were acquired from Phenomenex (Castel Maggiore, Italy).

Sampling

Three batches of seven different typologies of canned tomatoes ($n = 7$), which included double and triple tomato concentrates, diced tomatoes, peeled tomatoes, crushed tomatoes, tomato sauce, and cherry tomatoes were analyzed. Double and triple concentrate, sauce, crushed are produced from Parma round tomato variety. Peeled tomatoes are produced from the long tomato variety. Diced tomatoes are produced from the "Datterino" tomato variety. Cherry tomatoes are produced from cherry tomato variety. All the typologies were produced with 100% Italian tomatoes and were acquired from various shops located in Campania region, Italy. After arrival in the laboratory, all the samples were properly stored at room temperature in original packaging, and the analyses were carried out before the expiration date. Prior to the analyses, the canned tomato samples were homogenized with an Ultra Turrax® instrument (T 25 digital ULTRA-TURRAX®) to obtain a homogeneous sample from all parts.

TABLE 1 | Levels of moisture content found in the assayed canned tomato samples.

Sample	Moisture content	
	g/100 g	±SD
Double concentrate	74.7[a]	0.5
Triple concentrate	63.8[b]	0.4
Diced tomato	89.4[c]	0.5
Peeled tomato	90.4[c]	0.7
Crushed tomato	89.8[c]	0.8
Tomato sauce	88.8[c]	0.8
Cherry tomato	89.6[c]	0.7

[a−c]*Different letters show significant difference ($p < 0.05$) among the different typologies of canned tomatoes.*

Moisture Content

Determination of moisture content of the canned tomato samples was performed according to the protocol reported in (4). In short, 5 g of the samples was dried at 70 °C using a laboratory oven for 6 h. Moisture content was determined by weighing the samples after drying. Data were expressed as g/100 g of samples.

Extraction of Polyphenol Compounds From Canned Tomatoes

Extraction of polyphenols was carried out following the procedure reported in (23), with some modifications. Following this method, 5 mL of 80% ethanol acidified with 0.1% formic acid was added to 2 g of the samples. The mixture was mixed for 1 min and subsequently sonicated for 5 min. A freezer pack was placed in a water bath to prevent degradation of bioactive compounds. Afterward, the samples were centrifuged (X3R Heraeus Multifuge, Thermo Fisher Scientific) for 5 min, at 2,800 × g at 4°C. The supernatant was recovered and kept, and the pellet was re-extracted. The pooled supernatant (about 10 mL) was dried using a nitrogen evaporator (Laborata 4000; Heidolph Instruments Italia Srl, Milan, Italy) and then reconstituted with 2 mL of water acidified to 0.1% formic acid. Finally, the extract was filtered with 0.22 μm nylon filters and was ready for successive analyses.

UHPLC Q-Orbitrap HRMS

Polyphenol determination was performed as previously described in (27). The analysis was performed using a UHPLC system (Dionex UltiMate 3000; Thermo Fisher Scientific, Waltham, MA, United States) equipped with a degassing system, a quaternary UHPLC pump, and an autosampler device. Chromatography separation was accomplished with a thermostated (25°C) Kinetex F5 (50 mm × 2.1 mm, 1.7 μm) column (Phenomenex, Castel Maggiore, Italy). Mobile phases consisted of H_2O containing 0.1% formic acid (A) and MeOH containing 0.1% formic acid (FA) (B). Chromatographic separation was carried out under the following conditions: 0–0.5 min, 0% B; 0.5–1 min, 0–40% B; 1–2 min, 40–80% B; 2–5 min, 80–100% B; 5–9 min, 100% B; 9–11 min, 100–0% B; 11–13 min, 0% for column re-equilibration. Flow rate was set at 500

TABLE 2 | Chromatographic and spectrometric parameters: retention time (RT), chemical formula, theoretical and measured masses (*m/z*), accuracy, and sensibility for phenolic acids and flavonoids ($n = 25$) in the investigated canned tomato samples.

Analyte	RT (min)	Chemical formula	$[M-H]^-$ theoretical mass (*m/z*)	$[M-H]^-$ found mass (*m/z*)	MS/MS fragment ions (*m/z*)	Accuracy (Δ ppm)	LOD (mg/kg)	LOQ (mg/kg)
Protocatechuic acid	2.41	$C_7H_6O_4$	153.01930	153.01857	109.02840	−4.77064	0.026	0.078
Epicatechin	2.98	$C_{15}H_{14}O_7$	289.07176	289.07202	221.94647–203.09201–161.04478	0.89943	0.013	0.039
Caffeic acid	3.05	$C_9H_8O_4$	179.03498	179.03455	134.99960	−2.40177	0.013	0.039
Vanillic acid	3.07	$C_8H_8O_4$	167.03490	167.03428	151.03905–123.04387	−3.71180	0.026	0.078
Chlorogenic acid	3.11	$C_{16}H_{18}O_9$	353.08780	353.08798	191.05594–84.98998	0.50979	0.013	0.039
Catechin	3.18	$C_{15}H_{14}O_6$	289.07175	289.07205	247.02241–205.10712–151.03923–125.02335	1.03780	0.026	0.078
Daidzein	3.29	$C_{15}H_9O_4$	253.05063	253.04977	209.96429–225.00984	−3.39853	0.052	0.156
p-coumaric acid	3.31	$C_9H_8O_3$	163.04001	163.03937	119.04917	−3.92542	0.026	0.078
Ferulic acid	3.38	$C_{10}H_{10}O_4$	193.05063	193.05016	178.02666–149.06009-−134.99963	−2.43459	0.013	0.039
Syringic acid	3.39	$C_9H_{10}O_5$	197.04555	197.04503	182.02153–166.99791	−2.63898	0.026	0.078
Genistin	3.40	$C_{15}H_{10}O_5$	269.04554	269.04562	241.14435–213.14908–151.03935	0.29735	0.013	0.039
Isoquercetin	3.51	$C_{21}H_{20}O_{12}$	463.08820	463.08853	431.09848–187.09698–174.95542	0.71261	0.013	0.039
Rutin	3.55	$C_{27}H_{30}O_{16}$	609.14611	609.14673	300.99911–271.05026–255.12390	1.01782	0.013	0.039
Naringin	3.56	$C_{27}H_{32}O_{14}$	579.17193	579.17212	515.11951–477.10406–463.08841–359.07724	0.32805	0.026	0.078
Quercetin 3-glucoside	3.59	$C_{21}H_{20}O_{12}$	463.08820	463.08817	447.09344–359.07730	−0.06478	0.026	0.078
Vitexin	3.58	$C_{21}H_{20}O_{10}$	431.09837	431.09824	317.03000–174.95531	−0.30156	0.026	0.078
Diosmin	3.60	$C_{28}H_{32}O_{15}$	607.16684	607.16711	593.15240–463.08835–447.09323–317.03027	0.44469	0.013	0.039
Ellagic acid	3.61	$C_{14}H_6O_8$	300.99899	300.99911	245.91669-229.93712-185.01208-117.00336	0.39867	0.013	0.039
Isorhamnetin 3-rutinoside	3.62	$C_{28}H_{32}O_{16}$	623.16117	623.16223	507.10849–447.09338–317.03012–253.05043	1.70100	0.026	0.078
Kaempferol 3-glucoside	3.63	$C_{21}H_{20}O_{11}$	447.09328	447.09332	300.99915-273.07690-227.07104	0.08947	0.026	0.078
Myricetin	3.64	$C_{15}H_{10}O_8$	317.03029	317.03040	300.99899–253.05046–128.95857	0.34697	0.013	0.039
Quercetin	3.75	$C_{15}H_{10}O_7$	301.03538	301.03508	174.95551	−0.99656	0.013	0.039
Naringenin	3.80	$C_{15}H_{12}O_5$	271.06120	271.06110	235.92595–151.03917	−0.36892	0.013	0.039
Kaempferol	3.86	$C_{15}H_{10}O_6$	285.04046	285.04086	93.00679	1.40331	0.013	0.039
Apigenin	3.93	$C_{15}H_{10}O_5$	269.04555	269.04550	248.96060–174.95537–91.00249	−0.18584	0.013	0.039

TABLE 3 | Quantitative analysis of bioactive compounds in the investigated canned tomato extracts ($n = 7$) performed by UHPLC-Q-Orbitrap HRMS analysis.

Analyte	Double concentrate		Triple concentrate		Diced tomato		Peeled tomato		Crushed tomato		Tomato sauce		Cherry tomato	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Protocatechuic acid	0.001^{a}	0.000	0.002^{b}	0.000	0.002^{b}	0.000	0.002^{b}	0.000	0.002^{b}	0.000	0.003^{c}	0.000	0.001^{a}	0.000
Chlorogenic acid	6.597^{a}	0.967	7.112^{a}	0.756	3.730^{b}	0.143	1.857^{c}	0.283	3.432^{d}	0.119	5.048^{e}	0.494	11.236^{f}	0.567
Caffeic acid	0.745^{a}	0.050	0.894^{b}	0.016	0.189^{c}	0.003	0.205^{c,d}	0.023	0.204^{c,d}	0.002	0.292^{d,e}	0.097	0.355^{e}	0.011
p-coumaric acid	0.060^{a}	0.001	0.080^{b}	0.002	0.045^{c}	0.001	0.036^{d}	0.001	0.048^{c}	0.002	0.056^{e}	0.002	0.060^{a,e}	0.002
Ferulic acid	0.450^{a}	0.080	0.470^{a}	0.089	0.408^{a}	0.065	0.256^{b}	0.050	0.350^{a}	0.041	0.411^{a}	0.036	0.430^{a}	0.048
Genistin	0.015^{a}	0.003	0.015^{a}	0.002	0.080^{b}	0.003	0.050^{c}	0.003	0.080^{b}	0.002	0.080^{b}	0.001	0.080^{b}	0.000
Naringin	0.080^{a}	0.001	0.100^{b}	0.003	0.096^{b}	0.001	0.092^{c}	0.001	0.087^{d}	0.001	0.090^{c}	0.001	0.080^{a}	0.001
Quercetin 3-glucoside	0.002^{a}	0.000	0.002^{a}	0.000	0.000^{b}	0.000	0.001^{c}	0.000	0.001^{c}	0.000	0.001^{c}	0.000	0.001^{c}	0.000
Kaempferol 3-glucoside	0.003^{a}	0.000	0.004^{b}	0.000	0.001^{c}	0.000	0.001^{c}	0.000	0.001^{c}	0.000	0.001^{c}	0.000	0.002^{d}	0.000
Rutin	7.905^{a}	0.321	9.516^{b}	0.167	2.248^{c}	0.058	1.191^{d}	0.003	1.441^{e}	0.051	3.481^{f}	0.202	3.722^{f}	0.059
Vitexin	0.123^{a}	0.015	0.156^{a}	0.018	0.050^{b}	0.001	0.056^{c}	0.002	0.059^{c}	0.001	0.065^{d}	0.001	0.089^{e}	0.013
Isorhamnetin 3-rutinoside	0.037^{a}	0.002	0.045^{b}	0.001	0.009^{c}	0.000	0.010^{c}	0.001	0.010^{c,d}	0.000	0.015^{d,e}	0.005	0.018^{e}	0.001
Myricetin	0.016^{a}	0.001	0.019^{b}	0.001	*<loq*	-	*<loq*	-	*<loq*	-	*<loq*	-	0.016^{a}	0.000
Naringenin	1.202^{a,d}	0.123	1.452^{a}	0.163	0.426^{b}	0.002	0.359^{c}	0.050	0.530^{c}	0.140	0.590^{c}	0.097	1.103^{d}	0.056
Kaempferol	0.005^{a,b}	0.001	0.007^{a}	0.001	*<loq*	-	*<loq*	-	*<loq*	-	*<loq*	-	0.003^{b}	0.001
Quercetin	0.013^{a}	0.001	0.018^{b}	0.002	0.010^{c}	0.000	0.010^{c}	0.000	0.009^{c}	0.000	0.009^{c}	0.000	0.013^{a}	0.001
Apigenin	0.010^{a}	0.000	0.013^{b}	0.001	0.009^{a}	0.000	0.006^{c}	0.000	0.006^{c}	0.000	0.008^{d}	0.000	0.009^{a}	0.000

Results are expressed in mg/100 g of fresh weight and reported as mean ± SD from three independent experiments.
$^{a-f}$Different letters show a significant difference ($p < 0.05$) among the different typologies of canned tomatoes.

TABLE 4 | Retrospective data analysis, identification, and semi-quantitative analysis of 18 no-target polyphenols in the different types of analyzed canned tomato samples ($n = 7$).

Analyte	Double concentrate		Triple concentrate		Diced tomato		Peeled tomato		Crushed tomato		Tomato sauce		Cherry tomato	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Protocatechuic acid O-hexoside	0.032^{a}	0.001	0.042^{b}	0.002	0.020^{c,d}	0.002	0.017^{c}	0.001	0.023^{d}	0.001	0.021^{d}	0.001	0.017^{c}	0.001
Vanillic acid hexoside	0.063^{a}	0.000	0.067^{a}	0.010	0.021^{b}	0.000	0.014^{c}	0.000	0.018^{d}	0.000	0.026^{e}	0.000	0.047^{f}	0.000
Coumaric acid hexoside	0.304^{a}	0.015	0.332^{b}	0.005	0.040^{c}	0.002	0.018^{d}	0.000	0.079^{e}	0.001	0.422^{f}	0.131	0.526^{f}	0.030
Caffeic acid hexoside	0.580^{a}	0.009	0.548^{b}	0.005	0.206^{c}	0.004	0.101^{d}	0.006	0.178^{e}	0.009	0.326^{f}	0.077	0.680^{g}	0.008
Cryptochlorogenic acid	0.695^{a,f}	0.151	1.076^{b}	0.047	0.554^{a}	0.008	0.094^{c}	0.003	0.192^{d}	0.010	0.528^{e}	0.013	0.822^{f}	0.090
Rutinhexoside	0.027^{a}	0.002	0.019^{b}	0.000	0.009^{c}	0.000	0.003^{d}	0.001	0.010^{e}	0.000	0.051^{f}	0.001	0.026^{a}	0.005
Cumaroylquinic acid	0.072^{a}	0.000	0.025^{b}	0.000	0.042^{c}	0.000	0.021^{d}	0.001	0.042^{c}	0.000	0.041^{c}	0.002	0.034^{e}	0.001
Feruloylquinic acid	0.008^{a}	0.000	0.012^{b}	0.000	0.006^{c}	0.000	0.008^{a}	0.000	0.006^{c}	0.000	0.006^{c}	0.000	0.008^{a}	0.000
Naringenin C hexoside	0.436^{a}	0.005	0.562^{b}	0.005	0.104^{c}	0.006	0.120^{d}	0.005	0.094^{c}	0.004	0.201^{e}	0.005	0.200^{e}	0.004
Eriodicthiol	0.180^{a}	0.005	0.210^{b}	0.006	0.083^{c}	0.002	0.053^{d}	0.003	0.039^{e}	0.004	0.048^{f}	0.004	0.095^{g}	0.004
Tricaffeoilquinic acid	0.031^{a}	0.001	0.035^{b}	0.002	0.025^{c}	0.004	0.023^{c}	0.003	0.024^{c}	0.002	0.026^{c}	0.001	0.027^{c}	0.003
Caffeic Acid dihexoside*	0.580^{a}	0.009	0.614^{b}	0.005	0.206^{c}	0.004	0.101 ^{d}	0.006	0.178^{e}	0.009	0.326^{f}	0.077	0.680^{g}	0.008
Naringenin C diglycoside*	0.432^{a}	0.015	0.583^{b}	0.016	0.160^{c}	0.021	0.046^{d}	0.002	0.061^{e}	0.008	0.105^{f}	0.002	0.137^{c}	0.007
Eriodicthiol O hexoside *	0.109^{a}	0.002	0.156^{b}	0.000	0.019^{c}	0.000	0.022^{d}	0.001	0.013^{e}	0.000	0.019^{c}	0.001	0.035^{f}	0.003
Quercetin O dihexoside *	0.034^{a}	0.001	0.046^{b}	0.001	0.009^{c}	0.000	0.039^{d}	0.001	0.015^{e}	0.001	0.017^{e}	0.001	0.026^{f}	0.000
Rutin O pentoside *	1.009^{a}	0.006	1.271^{b}	0.047	0.147^{c}	0.011	0.106^{d}	0.003	0.201^{e}	0.004	0.398^{f}	0.035	0.411^{g}	0.021
Floretindiglycoside *	0.662^{a}	0.031	0.755^{b}	0.030	0.233^{c}	0.041	0.067^{d}	0.004	0.306^{f}	0.036	0.363^{f}	0.080	0.596^{g}	0.025
Kaempferol 3-O-rutinoside *	0.079^{a}	0.004	0.155^{b}	0.001	0.060^{c}	0.002	0.025^{d}	0.000	0.027^{e}	0.000	0.077^{a}	0.006	0.094^{f}	0.003

Results are expressed in mg/100 g of fresh weight and reported as mean ± SD from three independent experiments.
$^{a-g}$Different letters show a significant difference ($p < 0.05$) among the different typologies of canned tomatoes.
**Semi-quantification with rutin.*

μL/min and injection volume at 5 μL. Detection was performed using a Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, United States) operated in positive and negative modes. Full ion MS and all ion fragmentation (AIF) scan events were set. The following settings were fixed in full MS scan mode: scan range 80–1,000 *m/z*, resolution power of

TABLE 5 | Quantitative analysis of bioactive compounds in the investigated canned tomato samples ($n = 7$) in the intestinal stage performed by UHPLC-Q-Orbitrap HRMS analysis.

Analyte	Double concentrate		Triple concentrate		Diced tomato		Peeled tomato		Crushed tomato		Tomato sauce		Cherry tomato	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
							Intestinal phase							
Protocatechuic acid	0.000^{a}	0.000	0.001^{b}	0.000	-	-	-	-	0.001^{b}	0.000	0.001^{b}	0.000	0.000^{a}	0.000
Chlorogenic acid	1.100^{a}	0.120	2.341^{b}	0.126	0.963^{c}	0.024	0.280^{d}	0.047	1.123^{a}	0.020	1.452^{e}	0.082	3.256^{f}	0.210
Caffeic acid	0.169^{a}	0.104	0.287^{a}	0.131	0.051^{b,d}	0.001	0.045^{b,d}	0.005	0.058^{c,d}	0.000	0.063^{d,e}	0.022	0.120^{e}	0.056
p-coumaric acid	0.008^{a}	0.000	0.026^{b}	0.006	0.011^{c}	0.000	0.007^{d}	0.000	0.009^{e}	0.000	0.018^{f}	0.000	0.027^{b,f}	0.011
Ferulic acid	0.059^{a}	0.018	0.145^{b}	0.020	0.097^{c}	0.014	0.049^{a}	0.014	0.114^{c}	0.009	0.142^{b}	0.008	0.186^{d}	0.012
Genistin	0.003^{a}	0.001	0.005^{b}	0.000	0.018^{c}	0.001	0.001^{a}	0.001	0.025^{d}	0.000	0.028^{d}	0.003	0.038^{e}	0.008
Naringin	0.021 ^{a}	0.009	0.032^{b}	0.001	0.023^{a}	0.000	0.023^{a}	0.000	0.024^{a}	0.000	0.034^{b}	0.004	0.032^{b}	0.009
Quercetin 3-glucoside	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kaempferol 3-glucoside	0.001^{a}	0.000	0.001^{a}	0.000	-	-	-	-	-	-	-	-	-	-
Rutin	1.658^{a}	0.071	3.154^{b}	0.037	0.543^{c}	0.013	0.256^{d}	0.013	0.426^{e}	0.011	1.568^{a}	0.045	1.520^{a}	0.130
Vitexin	0.024^{a}	0.002	0.048^{b}	0.004	0.013^{c}	0.000	0.001^{d}	0.001	0.017^{e}	0.000	0.018^{e}	0.002	0.041^{b}	0.009
Isorhamnetin 3-rutinoside	0.008^{a}	0.001	0.008^{a}	0.000	0.002^{b}	0.000	0.002^{b}	0.000	0.002^{b}	0.000	0.001^{b}	0.001	0.008^{a}	0.002
Myricetin	0.003^{a}	0.000	0.006^{b}	0.000	-	-	-	-	-	-	-	-	0.004^{c}	0.000
Naringenin	0.404^{a}	0.024	0.478^{b}	0.032	0.098^{c}	0.000	0.068^{d}	0.000	0.168^{e}	0.000	0.224^{f}	0.023	0.321^{a,f}	0.140
Kaempferol	0.001^{a}	0.000	0.002^{a}	0.000	-	-	-	-	-	-	-	-	0.001^{a}	0.000
Quercetin	0.002^{a}	0.000	0.005^{b}	0.000	0.003^{c}	0.000	0.002^{a}	0.011	0.002^{a}	0.000	0.003^{a,c}	0.001	0.004^{c}	0.000
Apigenin	0.002^{a}	0.000	0.004^{b}	0.000	0.002^{a}	0.000	0.001^{a}	0.000	0.002^{a}	0.000	0.002^{a}	0.000	0.003^{b}	0.000

The results are expressed in mg/100 g of fresh weight and reported as mean ± SD from three independent experiments.
$^{a–f}$Different letters show a significant difference ($p < 0.05$) among the different typologies of canned tomatoes.

70,000 full width at half maximum, microscan 1, automatic gain control target 1×10^6, maximum injection time 200 ms, sheath gas flow rate 18, auxiliary gas 3, sweep gas flow rate 0, spray voltage 3.5 KV, capillary temperature 320°C; S-lens RF level 60, and auxiliary gas heater temperature 350°C. In the AIF scan mode, the resolution power was set as: scan range 80–1,000 *m/z*, resolution power of 17,500 full width at half maximum, microscan 1, automatic gain control target 1×10^5, maximum injection time 200 ms, sheath gas flow rate 18, auxiliary gas 3, sweep gas flow rate 0, spray voltage 3.5 kV, capillary temperature 320°C, S-lens RF level 60, and auxiliary gas heater temperature 350°C. Collision energy (CE) was set at 15, 30, and 45 eV to achieve a representative product ion spectrum. Mass tolerance was fixed at 5 ppm for identification and confirmation of target molecular ions and at 1 ppm for retrospective analysis of data; scan time = 0.10 s and retention time to 30 s. Data processing was performed using the Quan/Qual Browser Xcalibur software 3.1.66.19 (Xcalibur; Thermo Fisher Scientific, Waltham, MA, United States).

Carotenoid Extraction

Extraction of carotenoids was performed following the protocol in (28), with slight modifications. The procedure involves extraction of 1 g of samples, with 6 mL of 0.1% BHT ethanol. After 1 min of vortex, the samples were incubated in a water bath for 5 min at 85°C. Then, 120 μL of an 80% aqueous KOH solution was added, vortexed for 1 min, and re-incubated for saponification for 10 min. Then, the samples were cooled for 5 min in a freezer at −80°C. Addition of 3 mL of hexane and 3 mL of water is followed by centrifugation (X3R Heraeus Multifuge; Thermo Fisher Scientific) for 5 min at 2,800 × g. After centrifugation, the hexane phase was collected. The extraction procedure was repeated two more times, and the supernatants were combined, dried with nitrogen, resuspended in 1 mL of chloroform, and filtered with 0.2-μm nylon filters before analysis.

HPLC-Diode-Array UV/VIS Detector (DAD) Analysis

Carotenoid analysis was performed on the Jasco HPLC Model 2000 Plus Series (Jasco, Cremella, Italy) equipped with a pump (PU-2080), a UV-Vis detector (UV-2075 Plus; Jasco), and an autosampler (AS-2055 Plus; Jasco,). Chromatography separation was carried out using a Gemini C18 (250 mm × 4.6 mm, 100 A, 5 μm) column (Phenomenex, Castel Maggiore, Italy). Mobile phases consisted of acetonitrile as solvent system A and *n*-hexane, ethanol, and dichloromethane (1:1:1) as mobile phase B. Chromatographic separation was carried out under the following conditions: initial 18% B, increased to 24% B in 8 min, to 42% B in 4 min, and to 61% B in 6 min. The gradient was reduced to 18% B in 4 min and another 5 min for column re-equilibration at 18%. Total run time was 27 min, and flow rate was 1 mL/min. The absorbance of lutein, β-carotene, and lycopene was measured at 450 nm.

In vitro GI Digestion

In vitro gastrointestinal digestion was performed following the protocol reported in (29), with slight modifications. The *in vitro* GI digestion includes three steps: the oral, gastric, and intestinal

TABLE 6 | Retrospective data analysis, identification, and semi-quantitative analysis of 18 no-target polyphenols in the different types of analyzed samples ($n = 7$) in the intestinal stage performed by UHPLC-Q-Orbitrap HRMS analysis.

Analyte	Double concentrate		Triple concentrate		Diced tomato		Peeled tomato		Crushed tomatoes		Tomato sauce		Cherry tomato	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
							Intestinal phase							
Protocatechuic acid O-hexoside	0.015^{a}	0.001	0.025^{b}	0.001	0.006^{c}	0.000	0.007^{c}	0.000	0.008^{d}	0.000	0.008^{d}	0.001	0.013^{a}	0.001
Vanillic acid hexoside	0.015^{a}	0.001	0.017^{b}	0.000	0.002^{c}	0.000	0.016^{b}	0.001	0.008^{d,e}	0.001	0.007^{d}	0.000	0.009^{e}	0.000
Coumaric acid hexoside	0.160^{a}	0.001	0.202^{b}	0.008	0.025^{c}	0.001	0.012^{d}	0.001	0.008^{e}	0.005	0.085^{f}	0.009	0.120^{g}	0.000
Caffeic acid hexoside	0.330^{a}	0.002	0.354^{b}	0.000	0.134^{c}	0.000	0.089^{d}	0.000	0.132^{e}	0.000	0.156^{f}	0.000	0.320^{g}	0.000
Cryptochlorogenic acid	0.192^{a}	0.005	0.272^{b}	0.020	0.065^{c}	0.003	0.044^{d}	0.000	0.085^{e}	0.004	0.125^{a,b,e,f}	0.132	0.210^{f}	0.039
Rutinhexoside	0.012^{a}	0.000	0.015^{b}	0.000	0.006^{c}	0.000	0.001^{d}	0.000	0.002^{d}	0.000	0.032^{e}	0.000	0.018^{f}	0.000
Cumaroylquinic acid	0.015^{a}	0.001	0.021^{b,e}	0.001	0.021^{b,d,e}	0.002	0.008^{c}	0.001	0.024^{d}	0.001	0.020^{a,b,c,d,e}	0.011	0.019^{e}	0.002
Feruloylquinic acid	0.003^{a,b}	0.002	0.002^{a}	0.000	0.003^{a,b}	0.000	0.001^{a}	0.000	0.002^{a}	0.000	0.001^{a}	0.000	0.003^{b}	0.000
Naringenin C hexoside	0.206^{a}	0.004	0.285^{b}	0.002	0.074^{c}	0.001	0.085^{d}	0.001	0.042^{e}	0.002	0.065^{c}	0.009	0.101^{f}	0.007
Eriodicthiol	0.058^{a}	0.000	0.069^{b}	0.001	0.036^{c}	0.001	0.049^{d}	0.001	0.012^{e}	0.000	0.041^{c,d}	0.009	0.047^{d}	0.005
Tricaffeoilquinic acid	0.021^{a}	0.003	0.021^{a}	0.002	0.012^{b}	0.001	0.020^{a}	0.003	0.014^{c}	0.003	0.014^{c}	0.001	0.013^{c}	0.001
Caffeic Acid dihexoside *	0.320^{a}	0.001	0.360^{b}	0.002	0.080^{c}	0.002	0.098^{d}	0.003	0.150^{e}	0.003	0.215^{f}	0.003	0.250^{g}	0.001
Naringenin C diglycoside *	0.321^{a}	0.000	0.496^{b}	0.000	0.029^{c}	0.001	0.035^{d}	0.000	0.052^{e}	0.001	0.062^{f}	0.001	0.089^{g}	0.001
Eriodicthiol O hexoside *	0.089^{a}	0.003	0.130^{b}	0.003	0.001^{c}	0.001	0.009^{d}	0.001	0.008^{d}	0.001	0.019^{e}	0.059	0.028^{f}	0.022
Quercetin O dihexoside *	0.021 ^{a}	0.001	0.041^{b}	0.001	0.001^{c}	0.000	0.024^{d}	0.001	0.009^{e}	0.001	0.009^{e}	0.001	0.011^{e}	0.001
Rutin O pentoside *	0.725^{a}	0.000	0.812^{b}	0.002	0.086^{c}	0.000	0.081^{d}	0.000	0.160^{e}	0.001	0.142^{f}	0.012	0.236^{g}	0.000
Floretindiglycoside *	0.321^{a}	0.002	0.486^{b}	0.000	0.213^{c}	0.000	0.052^{d}	0.001	0.280^{e}	0.000	0.241^{f}	0.001	0.286^{g}	0.005
Kaempferol 3-O-rutinoside *	0.063^{a}	0.001	0.080^{b}	0.001	0.056^{c}	0.001	0.012^{d}	0.001	0.005^{e}	0.000	0.060^{a}	0.006	0.075^{f}	0.003

The results are expressed in mg/100 g of fresh weight and reported as mean ± SD from three independent experiments.

$^{a-g}$*Different letters show a significant difference ($p < 0.05$) among the different typologies of canned tomatoes.*

**Semi-quantification. with rutin.*

phases. To simulate the oral phase, 5 g of the samples were combined with 3.5 mL of simulated salivary fluid (SSF), 500 μL of a-amylase solution (1,500 U/mL in SSF), 25 μL of 0.3 M calcium chloride dihydrate, and 0.975 mL of distilled water. Then, the pH was adjusted to 7 using HCl 1 M, and the mixture was incubated at 37°C for 2 min at 150 rpm in a water bath orbital shaker (GFL-1086; Biosigma S.p.A., Venice, Italy).

The oral stage continues with the gastric phase, in which 7.5 mL of simulated gastric fluid (SGF), 1.6 mL of a pepsin solution (25,000 U/mL in SGF), 5 μL of 0.3 M calcium chloride dihydrate, and 0.695 mL of distilled water were added. In this step, the pH was adjusted to 3 with HCl 6 M, and the mixture was incubated at 37°C for 2 h at 150 rpm in the water bath orbital shaker (GFL-1086; Biosigma S.p.A., Venice, Italy).

Finally, in the intestinal phase, 11 mL of simulated intestinal fluid (SIF), 5 mL of pancreatin solution (800 U/mL), 2.5 mL of bile salts (160 mM), 40 μL of 0.3 M calcium chloride dihydrate, and 1,310 μL of distilled water were added. It is suggested to verify the pH value and adjust it to 7 using NaOH 6 M. The mixture was incubated at 37°C for 2 h at 150 rpm in the water bath orbital shaker (GFL-1086; Biosigma S.p.A., Venice, Italy).

At the end of the intestinal phase, the mixture was centrifuged using X3R Heraeus Multifuge; (Thermo Fisher Scientific) for 5 min at 2,800 × g. The supernatants were collected and freeze-dried, resuspended in methanol, and centrifuged for min at 5,000 rpm; the supernatants without salts were used for further experiments.

The preparation of SSF, SGF, and SIF is schematized in a Table of our previously published scientific study (30).

Total Phenolic Content

Total phenolic content (TPC) was measured on the diluted extract following the method reported by (31). In short, 0.125 mL of polyphenolic extract was added to 0.5 mL of deonized water and 0.125 mL of the Folin–Ciocalteu reagent. After 6 min of incubation, 1.25 mL of 7.5% sodium carbonate solution and 1 mL of deionized water were added to the mixture. After 90 min of incubation under dark conditions and at room temperature, the absorbance was recorded at 760 nm. Autozero was carried out with distilled water. All the experiments were conducted in triplicate. The results were expressed in mg gallic acid equivalent (GAE)/100 g of fresh weight.

Antioxidant Activity

Antioxidant activity was measured by three different assays: the DPPH, ABTS, and FRAP tests. All the experiments were conducted in triplicate. The results were expressed in mmol Trolox/kg of fresh weight.

DPPH Assay

Radical-scavenging activity was determined using the method suggested by (56), with slight modifications. Briefly, 2 mg of DPPH salt were diluted with methanol until to reach an absorbance value at 515 nm of 0.9 (±0.02). Once the working solution was obtained, to 1 mL of the DPPH•+ solution, we

TABLE 7 | Total phenolic content of the investigated samples measured in digested and non-digested samples.

Samples	Not digested		Digested		%
	Mean	±DS	Mean	±DS	
Double concentrate	126.976[a]	2.626	85.638[a]	0.637	67.4
Triple concentrate	162.597[b]	0.783	65.587[b]	0.627	40.3
Diced tomatoes	30.888[c]	0.822	30.383[c]	0.303	98.4
Peeled tomatoes	31.750[c]	0.673	28.487[d]	0.511	89.7
Crushed tomatoes	27.895[d]	0.256	26.317[e]	0.491	94.3
Tomato sauce	37.614[e]	0.139	30.318[c]	0.042	80.6
Cherry tomatoes	41.692[f]	0.28	40.306[f]	0.216	96.7

Data are displayed as mean of mg GAE/100 g of the samples and standard deviation (SD). [a–f] *Different letters show a significant difference ($p < 0.05$) among the different typologies of canned tomatoes.*

added 200 μL of the diluted sample. Absorbance was measured after waiting for 10 min. Autozero was carried out with methanol.

ABTS Assay

Free radical-scavenging activity was determined with the method previously reported by (32). In short, 9.6 mg of ABTS salt was dissolved in 2.5 mL of deionized water. To this mixture, we added 44 μL of potassium persulfate. The solution was maintained under dark conditions at 4°C for 16 h prior to use. Then, the solution was diluted in ethanol until an absorbance value at 734 nm of 0.7 (±0.02). Once the working solution was obtained, to 1 mL of the $ABTS^{\bullet+}$ solution, we added 100 μL of the diluted sample. The absorbance was rapidly measured after waiting for 2.5 min. Autozero was carried out with ethanol.

FRAP Assay

Ferric reducing antioxidant power (FRAP) was measured based on the protocol reported in (33), with slight changes. A FRAP solution was prepared by mixing a TPTZ solution (10 mM, in HCl 40 mM), a ferric chloride solution (20 mM, in water), and an acetate buffer (0.3 M; pH 3.6) with a ratio of 1:1:10 (*v/v/v*). In short, 150 μL of diluted samples were added to 2,850 mL of the FRAP solution. The absorbance was recorded after 4 min at 593 nm. Autozero was carried out with methanol.

Statistical Analysis

The results were expressed as average ± standard deviation (SD) evaluated on three independent replication. Tukey's test was performed to evaluate differences among the different typologies of tested samples. Tukey's test at a level of $p < 0.05$ was considered significant. Statistical analysis was performed using the software Info-Stat version 2008 (https://www.infostat.com.ar/index.php?mod=page&id=15).

RESULTS

Moisture Content

The moisture content obtained by gravimetric analysis is summarized in **Table 1**. The moisture content found in different typologies of the tomato samples ranged from 63.8 to 90.4 g/100 g of sample.

Quantification and Retrospective Analysis of Polyphenol Compounds in Canned Tomatoes by UHPLC-Q-Exactive HRMS

Bioactive compounds of canned tomato extracts were profiled by UHPLC-Q-Orbitrap HRMS. A total of 25 different polyphenolic compounds such as flavonoids and phenolic acids were investigated by combining MS and MS/MS spectra (**Table 2**). Analysis of phenolic acids and flavonoids was performed in ESI, producing the deprotonated molecular ion [M-H]. Identification was carried out by comparison to their relative reference pure standards. Quantitative –determination was performed through calibration curves at nine concentration levels (5–0.019 μg/kg).

Results of the quantitative analysis are shown in **Table 3**. Predominant compounds found in all the studied typologies of canned tomatoes were represented by rutin, naringenin, and chlorogenic acid. In particular, chlorogenic acid was in the range of 1.857–11.236 mg/100 g, rutin ranged between 1.191 and 9.516 mg/100 g, and naringenin ranged from 0.359 to 1.452 mg/100 g. As far as phenolic acids are concerned, chlorogenic acid was the compound quantified with highest concentration in double- and triple-concentrated canned tomatoes, followed by the cherry tomato typology, with an average value of 4.852 mg/100 g. With regard to flavonoids, rutin and naringenin were found to have an average value of 4.215 and 0.808 mg/100 g, respectively. With regard to concentration of the other investigated polyphenols, great variability was recorded among the typologies, and the amount determined was significantly lower ($p < 0.05$).

Retrospective analysis allowed for the identification and semi-quantification of 18 further polyphenolic compounds (**Table 4**). For the quantitative analysis of compounds, for which a reference standard was not available, a representative standard of the same group was selected (rutin and quercetin 3-glucoside). The most representative compounds were represented by caffeic acid hexoside (range 0.101–0.68 mg/100 g; average 0.384 mg/100 g), cryptochlorogenic acid (range 0.094–1.076 mg/100 g; average 0.566 mg/100 g), caffeic acid diesoside (range 0.101–0.68 mg/100 g; average 0.384 mg/100 g), rutin O-pentoside (range 0.106–1.271 mg/100 g; average 0.506 mg/100 g), and floretin diglycoside (range 0.067–0.755 mg/100 g; average 0.426 mg/100 g).

Bioaccessibility of Polyphenol Compounds in Canned Tomatoes by UHPLC-Q-Exactive HRMS

Polyphenolic profile was followed during *in vitro* GI digestion by UHPLC Q-Orbitrap HRMS. The results are reported in **Table 5**. In particular, the analysis was conducted in the intestinal stage. Among the various analyzed compounds, there were large variability and losses, and low recovery amount was obtained (7.9–69.7%). Rutin was recovered at a percentage of 30.7% (range: 21–45.1%), naringenin at a parcentage of 29.6% (range 18.9–38%), and chlorogenic acid at a percentage of 25.8% (range 15.1–32.9%). Quercetin 3-glucoside and Kaempferol 3-glucoside were not bioaccessible during the GI tract digestion. For protocatechuic acids, Kaempferol and myricetin were reported to have low recovery in the analyzed intestinal stage.

TABLE 8 | Antioxidant activity of digested and non-digested canned tomato samples (n = 7).

Samples	DPPH					ABTS					FRAP				
	Not digested		Digested		%	Not digested		Digested		%	Not digested		Digested		%
	Mean	±DS	Mean	±SD		Mean	±SD	Mean	±SD		Mean	±SD	Mean	±SD	
Double concentrate	1.308^{a}	0.046	0.268^{a}	0.023	20.5	2.508^{a}	0.076	1.552^{a}	0.053	61.9	1.374^{a}	0.063	0.387^{a}	0.021	27.6
Triple concentrate	1.413^{b}	0.035	0.149^{b}	0.016	10.5	2.618^{a}	0.086	1.212^{b}	0.048	46.3	1.676^{b}	0.063	0.335^{a}	0.033	19.5
Diced tomatoes	0.334^{c}	0.027	0.197^{c}	0.026	59.0	0.595^{b}	0.042	0.453^{c}	0.036	76.2	0.338^{c}	0.048	0.204^{b}	0.031	58.6
Peeled tomatoes	0.339^{c}	0.034	0.121^{b}	0.015	35.7	0.621^{b,c}	0.061	0.538^{d}	0.049	86.5	0.383^{c}	0.061	0.176^{b}	0.027	45.9
Crushed tomatoes	0.319^{c}	0.029	0.117^{b}	0.017	36.7	0.571^{b}	0.057	0.320^{e}	0.026	55.9	0.355^{c}	0.025	0.214^{b}	0.025	55.6
Tomato sauce	0.429^{d}	0.016	0.123^{b}	0.013	28.7	0.713^{c,d}	0.059	0.430^{c}	0.036	60.3	0.477^{d}	0.039	0.276^{c}	0.023	56.5
Cherry tomatoes	0.447^{d}	0.023	0.286^{a}	0.021	64.0	0.733^{d}	0.049	0.522^{d}	0.039	71.2	0.545^{d}	0.044	0.329^{c}	0.030	59.6

The results are expressed in mmol Trolox/Kg ±DS.

$^{a-e}$Different letters show a significant difference ($p < 0.05$) among the different typologies of canned tomatoes.

TABLE 9 | Correlation between total phenolic content (TPC) and data obtained by the DPPH, ABTS, and FRAP tests.

Assay	Not digested samples R^2	Digested samples R^2
DPPH	0.964	0.525
ABTS	0.953	0.942
FRAP	0.973	0.858

Assessment of bioaccessibility was also performed during GI digestion for semi-quantified compounds. The results reported in **Table 6** show that coumaric acid hexoside, caffeic acid hexoside, cryptochlorogenic acid, naringenin C hexoside, rutin O-pentoside, and floretindiglycoside were the most abundant compounds. After GI digestion, rutin hexoside was recovered at a percentage of 53.6%, rutin O-pentosideat a percentage of 63.3% and caffeic acid hexoside at a percentage of 42.2%. Among the other investigated typologies, cherry tomatoes showed comparable concentrations to those of double and triple concentrates for most of the studied analytes.

Total Phenolic Content and *in vitro* Bioaccessibility

Total phenolic content was determined using the Folin-Ciocalteu assay, and the bioaccessibility of tomato polyphenols was assessed using an *in vitro* digestion protocol in order to provide valuable insights into their bioaccessibility. Therefore, the TPC content of non-digested samples were compared with that of digested ones. As shown in **Table 7**, the TPC content in the digested and non-digested samples was quantified to be in the range of 26.317 to 85.638 and 27.895 to 162.597 mg GAE/100 g, respectively. Moreover, the data highlighted that all the digested samples showed significantly lower TPC values ($p < 0.05$) than the digested ones, except for diced tomatoes. The percentage of decrease in TPC following GI digestion ranges from 1.6 (diced tomatoes) to 59.7% (triple concentrate), as indicated in **Table 7**. Furthermore, the data revealed that five out of the seven samples showed a decrease in the bioaccessibility of polyphenols of <20%, whereas the two remaining samples showed a decrease in polyphenol bioaccessibility between about one-thirds (double concentrate) and two-thirds (triple concentrate) compared with the initial TPC values.

Antioxidant Capacity and *in vitro* Bioaccessibility

The antioxidant capacity of the assayed samples recorded in the initial samples and following GI digestion was evaluated and compared. Three spectrophotometric methods, namely, DPPH, ABTS, and FRAP, were used to monitor variations in antioxidant capacity. The findings are summarized in **Table 8**. The data highlighted that the digestion process affected the active compounds present in the assayed samples, resulting in decreased antioxidant activity. In fact, compared to the initial values, the samples subjected to simulated GI digestion ended up with a significantly ($p < 0.05$) lower antioxidant activity. In particular, the results of antioxidant capacity revealed lowered values ranging from 36.1 to 89.5% (DPPH test), 13.5 to 53.7% (ABTS test), and 40.4 to 80.5% (FRAP test). Furthermore, the triple concentrate sample was found as the sample that showed highest decrease in antioxidant activity measured in all the three tests performed in this study.

Furthermore, strong positive correlations between TPC content and antioxidant capacity measured by DPPH, ABTS, and FRAP were observed for the initial values and following the GI digestion process, except for the DPPH test of the digested samples ($R^2 = 0.525$), as shown in **Table 9**.

Carotenoids Content and Their *in vitro* Bioaccessibility

The carotenoid profile of the most representative compounds such as lutein, β-carotene, and lycopene was quantified using an HPLC method. Calibration curves with real standards at 12 concentration levels were employed (regression coefficient > 0.99) for quantitative determination of the assayed compounds. **Table 10** shows the results (mean value and SD) obtained from the initial samples and following simulated GI digestion. In addition, the percentage of bioaccessibility (non-digested

TABLE 10 | Intestinal bioaccessibility of carotenoids evaluated by the HPLC-DAD method in the digested and non-digested canned tomato samples ($n = 7$).

Samples	Lutein					β-carotene					Lycopene				
	Not digested		Digested		%	Not digested		Digested		%	Not digested		Digested		%
	Mean	±DS	Mean	±DS		Mean	±DS	Mean	±DS		Mean	±DS	Mean	±DS	
Double concentrate	2.851[a]	0.142	0.301[a]	0.052	10.5	40.622[a]	2.133	7.602[a]	0.423	18.7	222.061[a]	19.123	28.325[a]	1.093	12.8
Triple concentrate	4.018[b]	0.243	0.360[a]	0.068	9.0	52.404[b]	3.138	7.264[a]	0.418	13.9	385.643[b]	23.248	35.740[b]	2.138	9.3
Diced tomatoes	0.892[c]	0.073	0.110[b,c]	0.031	12.3	12.696[c]	0.893	2.522[b]	0.323	19.9	69.395[c]	11.183	13.829[c]	1.286	19.9
Peeled tomatoes	0.654[d]	0.064	0.100[b]	0.011	15.3	9.313[d]	0.544	2.133[b]	0.124	22.9	50.894[d]	9.662	7.421[d]	0.863	14.6
Crushed tomatoes	0.779[c,d,f]	0.071	0.131[c]	0.014	16.8	11.273[c]	0.521	3.110[c,d]	0.215	27.6	60.147[c,d]	9.119	10.005[e]	0.529	16.6
Tomato sauce	1.073[e]	0.084	0.178[d]	0.012	16.6	15.238[d]	0.521	3.519[c]	0.191	23.1	83.272[e]	10.231	12.893[c]	0.391	15.5
Cherry tomatoes	0.830[c,f]	0.062	0.121[b,c]	0.014	14.6	11.859[c]	0.432	2.926[b,d]	0.249	24.7	64.771[c,d]	11.158	12.938[c]	0.628	20.0

The data are expressed in mg/kg of samples and standard deviation (SD).
[a–f] Different letters show a significant difference ($p < 0.05$) among the different typologies of canned tomatoes.

vs. digested samples) of each investigated carotenoid was also displayed. In the assayed canned tomato samples here, lycopene was found as the most commonly quantified carotenoid, with concentrations ranging from 50.894 to 222.061 mg/kg. As shown in **Table 10**, after GI digestion, the amount of lycopene is recorded in the range between 9.3 and 20.0% of the non-digested analyzed samples. As far as β-carotene was concerned, the levels of this important carotenoid were quantified in the assayed canned tomato samples at a concentration range of 9.313 to 52.404 mg/kg. After GI digestion, significant decrease in β-carotene was observed, ranging between 72.4 (crushed tomatoes) to 86.1% (triple concentrate). On the other hand, lutein was the less relevant carotenoid, being quantified in the initial canned tomato samples with a concentration range of 0. 654–4.018 mg/kg. In line with the other assayed carotenoids, decreased levels of lutein were observed after gastrointestinal digestion when compared with values of the non-digested samples.

DISCUSSION

This study aimed to provide valuable insights into the content of active compounds of different typologies of canned tomatoes. Although many scientific studies have reported several beneficial effects of tomato consumption against various chronic diseases, the bioaccessibility of compounds released during GI digestion has been barely studied to date. The protocol employed to replicate human GI digestion was established recently in the INFOGEST network (29).

Quantification and Retrospective Analysis of Polyphenol Compounds in Canned Tomatoes by UHPLC-Q-Exactive HRMS

Analysis of hydroxycinnamic acids (chlorogenic, caffeic, caffeic O-hexoside, and ferulic acids), flavonols (kaempferol-3-O-glucoside, rutin, and quercetin), flavanones (naringenin), and phenolic acids (protocatechuic acid) by liquid chromatography coupled to mass spectrometry was performed on the seven typologies of canned tomatoes.

Our results are in line with previous findings. Hydroxycinnamic acid derivatives have been found in tomatoes; in particular, chlorogenic acid is the most commonly reported. Rutin and naringenin have been reported as the main flavonoids found in different varieties of tomato. A recent study conducted by (34) investigated the polyphenol profile of tomatoes crude extract. The results showed that the predominant phenolic acid was represented by chlorogenic acid, with a reported concentration range of 6.77–8.65 mg/kg dry material. Rutin content was reported at a concentration range of between 21.07 and 191.18 mg/kg of dry material. Among other polyphenols, ferulic (0.26–1.96 mg/kg dry material), *p*-coumaric (0.11–0.43 mg/kg dry material), and vanillic acids were also found in the analyzed extracts at a lower concentration.

According to other investigations, (35) reported rutin as the most abundant polyphenol in tomatoes, followed by naringenin, and (36) reported an average range of 119.82 and 36.46 mg/kg fresh weight for rutin and naringenin in tomatoes, respectively. Furthermore, (36) also reported the identification of phenolic acid-O-hexosides, cinnamic acids and derivatives, di- and tricaffeoylquinic acid isomers in tomato. Neochlorogenic and cryptochlorogenic acids, naringenin C-hexoside, apigenin-C-hexoside-pentoside, were also found.

In another study conducted by (37), the polyphenol content of tomatoes was investigated. Chlorogenic acid content fell within the range of 0.79–21.8 mg/kg fresh weight, and naringenin was reported to have a concentration range of 0.5 to 6.9 mg/kg fresh weight.

Moreover, (4) monitored and identified phenolic compounds in tomatoes and different types of processed tomatoes sauce. From this study, it emerges that naringenin is increased in the different types of sauce: from 0.12 to 2.38 mg/100 g dry weight. Rutin content significantly increased in industrial processed sauce when compared to fruit, from 24.8 to 33.8 mg/100 g dry weight (36%). Furthermore, (38) validated a UHPLC–QqQ-MS method for analysis of hydroxybenzoic and hydroxycinnamic acid derivatives, flavonols, and flavanones in various typologies of tomato: fruits, sauce, and juice. Cherry tomatoes had the highest levels of rutin and naringenin. The highest content of naringenin was found in tomatoes sauce, with a concentration level of 206

mg/kg fresh weight. Tomato juice extracts had a lower amount of phenolic compounds than cherry tomatoes and sauce extracts.

During canning or drying process, temperature and processstepscould have an impact on the ultimate content of polyphenols, reducing their concentration in processed meals. Total phenolic and flavonoid content could be released into the surrounding medium. On the other hand, canning could also result in the development of several beneficial substances that are not naturally present in raw foods (39).

Bioaccessibility of Polyphenol Compounds in Canned Tomatoes by UHPLC-Q-Exactive HRMS

It is widely reported that plant-derived foods are a rich source of phytocompounds with high nutritional value. Certainly, the biological function of the human body depends on the real concentration that reaches the site of absorption. Health benefits of phenolic compounds are dependent on how they are released from the matrix, absorbed in the GI tract, and available for metabolism once consumed (40).

Bioaccessibility, the percentage of compounds liberated from the food matrix during GI digestion and rendered available for absorption in the small intestine, can be estimated using an *in vitro* gastrointestinal digestion technique that includes evaluation of the oral, gastric, and intestinal stages. The bioaccessibility of phenolic compounds is influenced by various factors such as their molecular structure. Polyphenols are subjected to various metabolism reactions such as methylation, glucuronidation, and sulfation after being absorbed (41). Polymeric or glycosylated phenolic compounds must be converted before being absorbed in the small or large intestine. The large intestine represents the site of absorption of some parts of polyphenols. With the exception of flavan-3-ols, which are primarily present in their oligomeric or polymeric forms, most flavonoids are glycosylated in their native form. Flavonoid glycosides are excessively hydrophilic to be absorbed in the small intestine directly by passive diffusion. As a result, they are deglycosylated in the small intestine lumen and passively diffused into enterocytes. The small intestine absorbs dietary phenolic acids, with the rest being changed and absorbed in the colon. Bioactive chemicals are then destroyed by the colon microbiota's esterases, resulting in additional absorbable metabolites (19, 42, 43).

In a study conducted by (44), the bioaccessibility of polyphenolic compounds after digestion was determined. A significant decrease in flavonols (26%) was observed; instead, chlorogenic acid increased (24%). Also, (45) reported an increase ($<$ 10%) in total phenolics and flavonoids after the intestinal stage. On the other hand, (46) observed a decrease in all classes of polyphenols during intestinal digestion. (47) reported that after the gastric phase there was a significant decrease in total polyphenol content, and that after the duodenal phase, further increase in total polyphenol content was observed, possibly due to structural transformation of polyphenols.

Even though the amount of phenolic compounds in food is widely recognized, to date, research on the impact of food processing on bioaccessibility is lacking.

Polyphenolic Content and *in vitro* Bioaccessibility

In order to clarify the polyphenol bioaccessibility of different canned tomato products, in this study, a Folin–Ciocalteu assay was performed following *in vitro* GI digestion, and the results were compared with the initial values.

Our results are consistent with those previously reported by (48), which highlighted higher TPC and antioxidant values in concentrated canned tomato products than canned tomato and juices, due to the higher dry matter found in concentrated tomato products. The data revealed that, with the exception of chopped tomato samples, the TPC values recorded following GI digestion were significantly lower ($p = 0.05$) than the initial values in all assayed samples. Our data are consistent with the findings reported by (49), who observed a decrease in polyphenol bioaccessibility ranging between 12 and 96% compared to the initial values in tomato-based products. Moreover, (50) reported an increase of polyphenol bioaccessibility in cherry tomatoes as a result of cooking treatment, suggesting that the thermal process may increase the release of phenolic compounds from the matrix.

On the other hand, the antioxidant activity of the canned tomato samples under investigation was measured in both the digested and non-digested samples. The results showed that the samples subjected to *in vitro* GI digestion had significantly lower antioxidant capacity than the non-digested samples. Similar findings have also been observed by (4), who reported that tomato fruits and industrial and home types of processed sauce showed less antioxidant activity against DPPH, ABTS, and FRAP radical oxidation in the small intestine compared to the initial values. Furthermore, the findings revealed strong correlations between the antioxidant capacity data obtained from the spectrophotometric assays, namely, DPPH, ABTS, and FRAP and the TPC results recorded following simulated GI digestion, highlighting that the performed methods provide valid insights into the active molecules released by the different tomatoes products after the simulated gastrointestinal process.

Carotenoid Content and *in vitro* Bioaccessibility

A wide range of scientific studies highlighted that consumption of regular tomatoes and tomato products may display protective actions against the incidence of a host of conditions, such as cognitive dysfunction, osteoporosis, cardiovascular disease, and light-induced skin damage (51, 52). The bioaccessibility of tomato carotenoids by *in vitro* GI digestion has been studied for different typologies of canned tomatoes by the HPLC-DAD method. Our findings showed that lycopene, β-carotene, and lutein content in the triple concentrate was significantly higher than that in the other assayed products, which is due to the lower moisture content found in the triple concentrate samples than in the other typologies of canned tomatoes (**Table 1**). Moreover, the data clearly indicate that the GI process could affect the bioaccessibility of carotenoid. In this study, carotenoid bioaccessibility varied from 9.3 to 20.0, 13.9 to 27., and 9 to 16.8% for lycopene, β-carotene, and lutein, respectively.

Similar outcomes were highlighted by (14) who reported that lycopene bioaccessibility from canned tomatoes was about 21%, whereas higher bioaccessibility was reported in fresh tomatoes (about 28%) and sun-dried tomatoes (about 58%). However, (53) investigated the lycopene content in processed and crude tomatoes and found that both samples had very low lycopene bioaccessibility, with concentration levels ranging from 0.1 to 1.6%. On the other hand, the reported bioaccessibility values of β-carotene and lutein in fresh tomatoes vary in the literature; the percentage of bioaccessibility observed by (54) was 15.5 and 58.6% for β-carotene and lutein, respectively. In contrast, (55) reported *in vitro* bioaccessibility of β-carotene from tomatoes paste, and their findings highlighted that the bioaccessibility of β-carotene was approximately 100%.

CONCLUSION

Tomato represents a rich source of dietary nutrients, such as flavonoids, phenolic acids, and represents the major source of lycopene linked with many health benefits, such as anticancer activity and cardiovascular protection effects. The results highlighted the high amount of rutin, naringenin, and chlorogenic acid in the analyzed canned tomato samples. Lycopene content was in the range of 50.894–222.061 mg/kg. Although there is not a daily value for lycopene assumption, based on data from epidemiologic investigations, a regular intake of around 6 mg of lycopene could provide protection. According to these data, consumption of three servings (180 g) of canned tomatoes can contribute approximately to this required intake.

Moreover, the data highlighted that the digestion process affects the active compounds present in the assayed samples, resulting in decreased antioxidant activity, total polyphenol content, and recovery of the analyzed compounds. Until now, only few scientific studies have evaluated the bioaccessibility of phenolic compounds from tomatoes. To our knowledge, this is the first study that investigated the bioaccessibility of polyphenolic compounds in Italian canned tomatoes.

In conclusion, the consumption of tomato and canned derived products could be a valid support to the intake of bioactive compounds. Tomato is an excellent source of nutrients useful in disease prevention and maintaining good health. Since limited information on changes in the bioaccessibility of bioactive compounds during GI digestion of canned tomatoes was reported, our study could be a good support to evaluate which content of bioactive compounds may be really bioaccessible to exercise beneficial effects on human health. Therefore, future *in vivo* studies are needed to confirm the real bioavailability of plasma and tissue concentrations of active compounds, and to confirm the *in vitro* results.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LI and AR: conceptualization. SL: methodology and formal analysis. LI, AG, and LC: investigation. AR: resources, project administration, and funding acquisition. LI and LC: writing original draft preparation. MG and AR: writing review and editing and supervision. All the authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

The authors would like to acknowledge Dr. Riccardo Quintili, director of Il Salvagente and Cira Tullio, for her technical support.

REFERENCES

1. Jamshidzadeh A, Baghban M, Azarpira N, Bardbori AM, Niknahad H. Effects of tomato extract on oxidative stress induced toxicity in different organs of rats. *F Chem Toxicology*. (2008) 4612:3612–5. doi: 10.1016/j.fct.2008.09.006
2. Rickman JC, Bruhn CM, Barrett DM. Nutritional comparison of fresh, frozen, and canned fruits and vegetables II. Vitamin A and carotenoids, vitamin E, minerals and fiber. *J Sci Food Agric.* (2007) 87:1185–96. doi: 10.1002/jsfa.2824
3. FAO/WHO/GIFT. *Global Individual Food consumption data Tool INRAN SCAI 2005-2006.* Available online at: https://www.fao.org/gift-individual-food-consumption/en/
4. Tomas M, Beekwilder J, Hall RD, Sagdic O, Boyacioglu D, Capanoglu E. Industrial processing versus home processing of tomato sauce: Effects on phenolics, flavonoids and *in vitro* bioaccessibility of antioxidants. *Food Chem.* (2017) 220:51–8. doi: 10.1016/j.foodchem.2016.09.201
5. Kamiloglu S, Boyacioglu D, Capanoglu E. The effect of food processing on bioavailability of tomato antioxidants. *J Berry Res.* (2013) 3:65–77. doi: 10.3233/JBR-130051
6. Amrani-Allalou H, Boulekbache-Makhlouf L, Izzo L, Arkoub-Djermoune L, Freidja ML, Mouhoubi K, et al. Phenolic compounds from an Algerian medicinal plant (Pallenis spinosa): Simulated gastrointestinal digestion, characterization, and biological and enzymatic activities. *Food Funct.* (2021) 12:1291–304. doi: 10.1039/D0FO01764G
7. Castaldo L, Narváez A, Izzo L, Graziani G, Gaspari A, Di Minno G, et al. Red wine consumption and cardiovascular health. *Molecules.* (2019) 24:3626. doi: 10.3390/molecules24193626
8. Izzo L, Castaldo L, Narváez A, Graziani G, Gaspari A, Rodríguez-Carrasco Y, et al. Analysis of phenolic compounds in commercial Cannabis sativa L. inflorescences using UHPLC-Q-Orbitrap HRMS. *Molecules.* (2020) 25:631. doi: 10.3390/molecules25030631
9. Abbas M, Saeed F, Anjum FM, Afzaal M, Tufail T, Bashir MS, et al. Natural polyphenols: an overview. *Int J Food Prop*. (2017) 20:1689–99. doi: 10.1080/10942912.2016.1220393
10. Martí R, Roselló S, Cebolla-Cornejo J. Tomato as a source of carotenoids and polyphenols targeted to cancer prevention. *Cancers.* (2016) 8:58. doi: 10.3390/cancers8060058
11. Tsao R. Chemistry and biochemistry of dietary polyphenols. *Nutrients.* (2010) 2:1231–46. doi: 10.3390/nu2121231
12. Thakur N, Raigond P, Singh Y, Mishra T, Singh B, Lal MK, et al. Recent updates on bioaccessibility of phytonutrients. *Trends Food Sci Technol.* (2020) 97:366–80. doi: 10.1016/j.tifs.2020.01.019
13. Fernández-García E, Carvajal-Lérida I, Jarén-Galán M, Garrido-Fernández J, Pérez-Gálvez A, Hornero-Méndez D. Carotenoids bioavailability from foods:

From plant pigments to efficient biological activities. *Food Res Int.* (2012) 46:438–50. doi: 10.1016/j.foodres.2011.06.007
14. Karakaya S, Yilmaz N. Lycopene content and antioxidant activity of fresh and processed tomatoes and *in vitro* bioavailability of lycopene. *J Sci Agri.* (2007) 87:2342–7. doi: 10.1002/jsfa.2998
15. Vallverdú-Queralt A, Jáuregui O, Di Lecce G, Andrés-Lacueva C, Lamuela-Raventós RM. Screening of the polyphenol content of tomato-based products through accurate-mass spectrometry (HPLC–ESI-QTOF). *Food Chem.* (2011) 129:877–83. doi: 10.1016/j.foodchem.2011.05.038
16. Motilva M-J, Macià A, Romero M-P, Labrador A, Domínguez A, Peiró L. Optimisation and validation of analytical methods for the simultaneous extraction of antioxidants: Application to the analysis of tomato sauces. *Food Chem.* (2014) 163:234–43. doi: 10.1016/j.foodchem.2014.04.096
17. Shahidi F, Peng H. Bioaccessibility and bioavailability of phenolic compounds. *JFB*. (2018) 4:11–68. doi: 10.31665/JFB.2018.4162
18. Waisundara VY. Assessment of bioaccessibility: a vital aspect for determining the efficacy of superfoods. *Superfoods Croatia: InTech Open*. (2018) 67–80. doi: 10.5772/intechopen.73152
19. Angelino D, Cossu M, Marti A, Zanoletti M, Chiavaroli L, Brighenti F, et al. Bioaccessibility and bioavailability of phenolic compounds in bread: A review. *Food Funct.* (2017) 8:2368–93. doi: 10.1039/C7FO00574A
20. Gómez-Romero M, Arráez-Román D, Segura-Carretero A, Fernández-Gutiérrez A. Analytical determination of antioxidants in tomato: typical components of the Mediterranean diet. *J Sep Sci.* (2007) 30:452–61. doi: 10.1002/jssc.200600400
21. Leontopoulos S, Skenderidis P, Kalorizou H, Petrotos K. Bioactivity potential of polyphenolic compounds in human health and their effectiveness against various food borne and plant pathogens. *A review J Food Bio Eng.* (2017) 7:1–19.
22. Motilva M-J, Serra A, Macià A. Analysis of food polyphenols by ultra high-performance liquid chromatography coupled to mass spectrometry: An overview. *J Chromatogr A*. (2013) 1292:66–82. doi: 10.1016/j.chroma.2013.01.012
23. Martinez-Huélamo M, Tulipani S, Torrado X, Estruch R, Lamuela-Raventós RM, chemistry f. Validation of a new LC-MS/MS method for the detection and quantification of phenolic metabolites from tomato sauce in biological samples. *J Agric Food Chem*. (2012) 60:4542–9. doi: 10.1021/jf205266h
24. Laganà A, Cavaliere C. High-resolution mass spectrometry in food and environmental analysis. *Anal Bioanal Chem.* (2015) 407:6235–6. doi: 10.1007/s00216-015-8837-5
25. Alvarez-Rivera G, Ballesteros-Vivas D, Parada-Alfonso F, Ibañez E, Cifuentes A. Recent applications of high resolution mass spectrometry for the characterization of plant natural products. *TrAC.* (2019) 112:87–101. doi: 10.1016/j.trac.2019.01.002
26. Lucci P, Saurina J, Núñez O. Trends in LC-MS and LC-HRMS analysis and characterization of polyphenols in food. *TrAC.* (2017) 88:1–24. doi: 10.1016/j.trac.2016.12.006
27. Izzo L, Rodríguez-Carrasco Y, Pacifico S, Castaldo L, Narváez A, Ritieni A. Colon bioaccessibility under in vitro gastrointestinal digestion of a red cabbage extract chemically profiled through UHPLC-Q-Orbitrap HRMS. *Antioxidants.* (2020) 9:955. doi: 10.3390/antiox9100955
28. Kyriacou MC, El-Nakhel C, Graziani G, Pannico A, Soteriou GA, Giordano M, et al. Functional quality in novel food sources: genotypic variation in the nutritive and phytochemical composition of thirteen microgreens species. *Food Chem.* (2019) 277:107–18. doi: 10.1016/j.foodchem.2018.10.098
29. Minekus M, Alminger M, Alvito P, Ballance S, Bohn T, Bourlieu C, et al. standardised static in vitro digestion method suitable for food–an international consensus. *Food Funct.* (2014) 5:1113–24. doi: 10.1039/C3FO60702J
30. Castaldo L, Narváez A, Izzo L, Graziani G, Ritieni A. In vitro bioaccessibility and antioxidant activity of coffee silverskin polyphenolic extract and characterization of bioactive compounds using UHPLC-Q-Orbitrap HRMS. *Molecules.* (2020) 25:2132. doi: 10.3390/molecules25092132
31. Izzo L, Pacifico S, Piccolella S, Castaldo L, Narváez A, Grosso M, et al. Chemical analysis of minor bioactive components and cannabidiolic acid in commercial hemp seed oil. *Molecules.* (2020) 25:3710. doi: 10.3390/molecules25163710
32. Luz C, Izzo L, Graziani G, Gaspari A, Ritieni A, Mañes J, et al. Evaluation of biological and antimicrobial properties of freeze-dried whey fermented by different strains of Lactobacillus plantarum. *Food Funct.* (2018) 9:3688–97. doi: 10.1039/C8FO00535D
33. Castaldo L, Lombardi S, Gaspari A, Rubino M, Izzo L, Narváez A, et al. *In vitro* bioaccessibility and antioxidant activity of polyphenolic compounds from spent coffee grounds-enriched cookies. *Foods.* (2021) 10:1837. doi: 10.3390/foods10081837
34. Błaszczak W, Jez M, Szwengiel A. Polyphenols and inhibitory effects of crude and purified extracts from tomato varieties on the formation of advanced glycation end products and the activity of angiotensin-converting and acetylcholinesterase enzymes. *Food Chem.* (2020) 314:126181. doi: 10.1016/j.foodchem.2020.126181
35. Georgé S, Tourniaire F, Gautier H, Goupy P, Rock E, Caris-Veyrat C. Changes in the contents of carotenoids, phenolic compounds and vitamin C during technical processing and lyophilisation of red and yellow tomatoes. *Food Chem.* (2011) 124:1603–11. doi: 10.1016/j.foodchem.2010.08.024
36. Vallverdú-Queralt A, Jáuregui O, Medina-Remón A, Lamuela-Raventós RM. Evaluation of a method to characterize the phenolic profile of organic and conventional tomatoes. *J Agric Food Chem.* (2012) 60:3373–80. doi: 10.1021/jf204702f
37. Vallverdu-Queralt A, Medina-Remon A, Martínez-Huélamo M, Jauregui O, Andres-Lacueva C, Lamuela-Raventos RM. Phenolic profile and hydrophilic antioxidant capacity as chemotaxonomic markers of tomato varieties. *J Agric Food Chem.* (2011) 59:3994–4001. doi: 10.1021/jf104400g
38. Di Lecce G, Martinez-Huelamo M, Tulipani S, Vallverdu-Queralt A, Lamuela-Raventós RM. Setup of a UHPLC–QqQ-MS method for the analysis of phenolic compounds in cherry tomatoes, tomato sauce, and tomato juice. *J Agric Food Chem.* (2013) 61:8373–80. doi: 10.1021/jf401953y
39. Arfaoui L. Dietary plant polyphenols: effects of food processing on their content and bioavailability. *Molecules.* (2021) 26:2959. doi: 10.3390/molecules26102959
40. Wojtunik-Kulesza K, Oniszczuk A, Oniszczuk T, Combrzyński M, Nowakowska D, Matwijczuk A. Influence of in vitro digestion on composition, bioaccessibility and antioxidant activity of food polyphenols—A non-systematic review. *Nutrients.* (2020) 12:1401. doi: 10.3390/nu12051401
41. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. *Am J Clin Nutr.* (2004) 79:727–47. doi: 10.1093/ajcn/79.5.727
42. Crozier A, Jaganath IB, Clifford MN. Dietary phenolics: chemistry, bioavailability and effects on health. *Nat Prod Rep.* (2009) 26:1001–43. doi: 10.1039/b802662a
43. Catalkaya G, Venema K, Lucini L, Rocchetti G, Delmas D, Daglia M, et al. Interaction of dietary polyphenols and gut microbiota: Microbial metabolism of polyphenols, influence on the gut microbiota, and implications on host health. *Food Front.* (2020) 1:109–33. doi: 10.1002/fft2.25
44. Bermúdez-Soto M-J, Tomás-Barberán F-A, García-Conesa M-T. Stability of polyphenols in chokeberry (Aronia melanocarpa) subjected to in vitro gastric and pancreatic digestion. *Food Chem.* (2007) 102:865–74. doi: 10.1016/j.foodchem.2006.06.025
45. Bouayed J, Hoffmann L, Bohn T. Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. *Food Chem.* (2011) 128:14–21. doi: 10.1016/j.foodchem.2011.02.052
46. Tagliazucchi D, Verzelloni E, Bertolini D, Conte A. In vitro bio-accessibility and antioxidant activity of grape polyphenols. *Food Chem.* (2010) 120:599–606. doi: 10.1016/j.foodchem.2009.10.030
47. Chen G-L, Hu K, Zhong N-J, Guo J, Gong Y-S, Deng X-T, et al. Antioxidant capacities and total polyphenol content of nine commercially available tea juices measured by an in vitro digestion model. *Eur Food Res Technol.* (2013) 236:303–10. doi: 10.1007/s00217-012-1897-2
48. Podsedek A, Sosnowska D, Anders B. Antioxidative capacity of tomato products. *Eur Food Res Technol.* (2003) 217:296–300. doi: 10.1007/s00217-003-0751-y
49. Kamiloglu S, Demirci M, Selen S, Toydemir G, Boyacioglu D, Capanoglu E. Home processing of tomatoes (Solanum lycopersicum): effects on *in vitro* bioaccessibility of total lycopene, phenolics, flavonoids, and

antioxidant capacity. *J Sci of Food Agric.* (2014) 94:2225–33. doi: 10.1002/jsfa.6546
50. Bugianesi R, Salucci M, Leonardi C, Ferracane R, Catasta G, Azzini E, et al. Effect of domestic cooking on human bioavailability of naringenin, chlorogenic acid, lycopene and β-carotene in cherry tomatoes. *Eur J Nutr.* (2004) 43:360–6. doi: 10.1007/s00394-004-0483-1
51. Burton-Freeman B, Reimers K. Tomato consumption and health: Emerging benefits. *Am J Lifestyle Med.* (2011) 5:182–91. doi: 10.1177/1559827610387488
52. Salehi B, Sharifi-Rad R, Sharopov F, Namiesnik J, Roointan A, Kamle M, et al. Beneficial effects and potential risks of tomato consumption for human health: An overview. *Nutrition.* (2019) 62:201–8. doi: 10.1016/j.nut.2019.01.012
53. Reboul E, Richelle M, Perrot E, Desmoulins-Malezet C, Pirisi V, Borel P. Bioaccessibility of carotenoids and vitamin E from their main dietary sources. *J AgriFood Chem.* (2006) 54:8749–55. doi: 10.1021/jf061818s
54. Jeffery JL, Turner ND, King SR. Carotenoid bioaccessibility from nine raw carotenoid-storing fruits and vegetables using an in vitro model. *J Sci Food Agri.* (2012) 92:2603–10. doi: 10.1002/jsfa.5768
55. Granado-Lorencio F, Olmedilla-Alonso B, Herrero-Barbudo C, Pérez-Sacristán B, Blanco-Navarro I, Blázquez-García S. Comparative in vitro bioaccessibility of carotenoids from relevant contributors to carotenoid intake. *J Agri Food Chem.* (2007) 55:6387–94. doi: 10.1021/jf070301t
56. Cavallo P, Dini I, Sepe I, Galasso G, Fedele FL, Sicari A, et al. An innovative olive pâté with nutraceutical properties. *Antioxidants.* (2020) 9:581. doi: 10.3390/antiox9070581

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Izzo, Castaldo, Lombardi, Gaspari, Grosso and Ritieni. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

REVIEW
published: 15 March 2022
doi: 10.3389/fnut.2022.849841

Scientific Evidence of the Beneficial Effects of Tomato Products on Cardiovascular Disease and Platelet Aggregation

Montaña Cámara[1]*, *Virginia Fernández-Ruiz*[1], *María-Cortes Sánchez-Mata*[1], *Rosa M. Cámara*[1], *Laura Domínguez*[1] and *Howard D. Sesso*[2]

[1] Department of Nutrition and Food Science, Faculty of Pharmacy, Complutense University of Madrid, Madrid, Spain, [2] Harvard Medical School, Brigham and Women's Hospital, Boston, MA, United States

OPEN ACCESS

Edited by:
José Pinela, Polytechnic Institute of Bragança (IPB), Portugal

Reviewed by:
Yi-Sook Jung, Ajou University, South Korea
Peng Yin, Chinese Center for Disease Control and Prevention, China
Feng-Qing Yang, Chongqing University, China

***Correspondence:**
Montaña Cámara
mcamara@ucm.es

Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *06 January 2022*
Accepted: *07 February 2022*
Published: *15 March 2022*

Citation:
Cámara M, Fernández-Ruiz V, Sánchez-Mata M-C, Cámara RM, Domínguez L and Sesso HD (2022) Scientific Evidence of the Beneficial Effects of Tomato Products on Cardiovascular Disease and Platelet Aggregation. Front. Nutr. 9:849841. doi: 10.3389/fnut.2022.849841

Cardiovascular disease (CVD) includes a group of disorders of the heart and blood vessels that includes numerous problems, many of which are related to the process called atherosclerosis. The present work is aimed to analyze the most relevant studies examining the potentially beneficial effects of tomato products on both CVD prevention and antiplatelet aggregation as well as an European Food Safety Authority health claims evaluation on tomato and tomato products. To date, only one health claim has been approved for a concentrated extract of tomato soluble in water (WSTC) marketed under the patented name of Fruitflow® with two forms of presentation: WSTC I and II, with the following claim "helping to maintain normal platelet aggregation, which contributes to healthy blood flow." Other studies also demonstrate similar beneficial effects for fresh tomatoes, tomato products and tomato pomace extracts.

Keywords: cardiovascular disease, platelet, antiplatelet, tomato, lycopene, health claims

INTRODUCTION

The World Health Organization (WHO) defines cardiovascular disease (CVD) as disorders of the heart and blood vessels originated from a chronic inflammatory vascular process that affects the wall of medium-sized arteries and ends up producing endothelial dysfunction and atherosclerosis. An important intermediate consequence of CVD is endothelial dysfunction, an alteration characterized by the functional loss of the vascular system that precedes the morphological changes characteristic of atherogenesis (1). Longer-term clinical trials examining clinical cardiology outcomes often define major cardiovascular events to include non-fatal myocardial infarction, non-fatal stroke, and CVD death.

According to the latest study published in 2020 by the American College of Cardiology Foundation on the global burden of CVD (Global Burden Disease, GBD) and its risk factors, CVD is the leading cause of death and disability in the world. Despite improvements in our knowledge of the primary prevention of CVD, progress remains muted–particularly among second- and third-world countries with limited access to preventive services. As a result, in the last 30 years (1990–2019), there has been a marked and worrying increase in the number of cases (48.2%), deaths (35.7%) and disability (48.5%) due to CVD (2).

Although CVD is multifactorial, one major risk factor is high plasma concentrations of low-density lipoprotein (LDL). Oxidative stress is another important risk factor because of the imbalance between the body's oxidation-antioxidant processes. In this situation, the endogenous defense system is overcome by the formation of reactive oxygen species (ROS) that interact with different biomolecules (carbohydrates, lipids, proteins, amino acids, and nucleic acids) and cause cellular damage. Platelets also play a relevant role in these conditions, since it has been shown that platelet hyperaggregability is associated with an increased risk of coronary heart disease (1, 3).

For this reason, the role of diet is crucial in the development and prevention of CVD. Recommendations from national and international guidelines are to follow a diet low in saturated fats and rich in bioactive compounds such as antioxidants; to achieve this goal the inclusion of fresh fruit and vegetables, as tomato fruit and tomato products, is a valuable strategy (4).

HEALTH CLAIMS RELATED TO TOMATOES AND ITS BIOACTIVES

Health claims in the labeling, presentation and/or advertising of food products are regulated in Europe by Regulations (EC) No. 1924/2006 of the European Parliament and of the Council; and Commission Regulation (EU) No. 432/2012. According to this legislation, scientific evidence on the role of a food, nutrient and/or compound in a nutritional or physiological function is not sufficient to justify the claim of beneficial effects. The substance must be present in the final product in sufficient quantities and, in addition, the amount of food that is necessary to consume to obtain the nutritional or physiological effect must be reasonable and easily achievable within the context of a balanced diet (5, 6). EFSA establishes that to consider a food or one of its constituents as subject of any health claim, it must demonstrate a beneficial effect. In this case, it would correspond to the maintenance and/or improvement of cardiovascular function, or the reduction of a risk factor for the development of this disease (7).

For lycopene, the main bioactive compound in tomato fruits, EFSA has published five scientific opinions on the approval of claims in relation to cardiovascular health and its risk factors, two of which refer to a water soluble tomato concentrate (WSTC), and three scientific opinions on lycopene (8, 9).

The first opinion was published in 2009 and evaluated the possible cause-effect relationship between the intake of a tomato extract preparation containing lycopene and whey proteins (lycopene-whey complex), and the reduction of the risk of atherosclerotic plaque formation by preventing oxidation of plasma lipoproteins. This beneficial effect was attributed to lycopene, which was sufficiently characterized. Eighty publications were evaluated, including six intervention studies, 22 observational studies, and eight reviews. The Panel reported several limitations in the design of these studies (sample size, duration, dose, absence of control groups, etc.), so a cause-effect relationship between lycopene intake and effect could not be established (7).

The second opinion was published in 2011 and evaluated the possible cause-effect relationship between the intake of lycopene and the protection of DNA, proteins, and lipids against oxidative damage due to its antioxidant capacity, as well as its contribution to normal cardiac function. According to the NDA Panel, lycopene was sufficiently characterized, and its main dietary sources corresponded to tomato and its derivatives. While a large number of scientific studies were provided in support of the health claim, many studies did not include original data since they were narrative reviews and consensual opinions. Other studies included results not related to the alleged effects or were focused on other bioactives such as carotenoids or antioxidant vitamins, alone or in combination with lycopene. In addition, none of the studies demonstrated a significant effect of lycopene on reliable markers of oxidative damage vs. controls. Finally, there was inconsistency in the provided studies that showed either no, negative, or positive associations between lycopene (either intake and/or plasma concentrations) with the risk of CVD. For all of these reasons, EFSA concluded that a cause-effect relationship could not be established between the intake of lycopene and its proposed beneficial effects (5).

The third opinion was published in 2015 and evaluated the possible cause-effect relationship between a lycopene preparation (named L-tug) obtained from an oleoresin extract from ripe tomato fruits mixed with other ingredients, and the reduction of the concentrations of plasma LDL-cholesterol. As it is a novel formulation, very limited information was provided from eight unpublished human intervention studies. The NDA Panel reported several limitations in the design of these studies, such as the absence of randomization and the lack of consideration of certain important methodological aspects, so that a cause-effect relationship could not be established between the intake of the lycopene preparation L-tug and the alleged effect (10).

Consequently, and according to the EFSA scientific opinions described above and published in 2009, 2011, and 2015, there has been insufficient scientific evidence to corroborate the alleged effects of lycopene on cardiovascular health and its risk factors for translation to published health claims on CVD and related outcomes to date.

This review is therefore aimed to review and analyse the scientific evidence for tomato products on CVD prevention and its anti-platelet effects in the context of its potential health claims according to EFSA requirements (**Figure 1**):

- Provide information proving that the consumption of the food/constituent (tomato-based product) reduces (or beneficially affects) platelet aggregation.
- Human clinical trials performance in subjects with platelet activation during sustained exposure to the food/constituent (at least 4 weeks).
- Use of valid markers: the percentage of inhibition in platelet aggregation should be measured using light transmission aggregometry (LTA) according to well-accepted and standardized protocols.

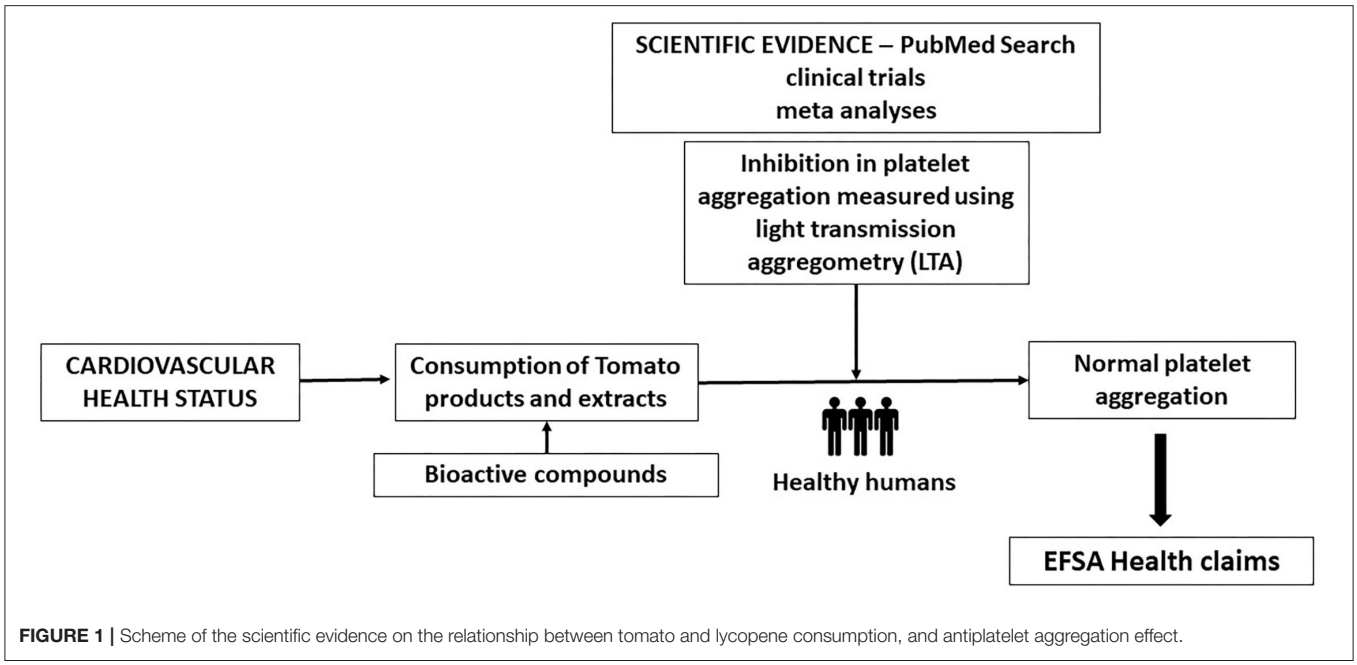


FIGURE 1 | Scheme of the scientific evidence on the relationship between tomato and lycopene consumption, and antiplatelet aggregation effect.

METHODOLOGY: LITERATURE SEARCH

An extensive literature search in the following platforms and databases was performed in the years ranging from 2011 to 2021:

- Official digital platforms of the organizations involved in European food legislation: European Commission (European Commission, CE); European Parliament and Council of the European Union (European Parliament and Council of the European Union) and European Food Safety Authority (European Food Safety Authority, EFSA).
- PubMed database [https://pubmed.ncbi.nlm.nih.gov] following EFSA's approach was conducted. In a first search, the selected keywords were "tomato" and "cardiovascular disease," in the second search the keywords used were "tomato" as well as the WHO disorder classification: "coronary heart disease," "cerebrovascular disease," "peripheral arterial disease," "rheumatic heart disease," "congenital heart disease" and "deep vein thrombosis." In the third search, the following keywords were used: "tomato," "platelet" and "antiplatelet."

EFSA TOMATO AND LYCOPENE HEALTH CLAIMS REQUIREMENTS AND STATUS

In order to demonstrate the beneficial effects of tomato product on lipid oxidative damage (lipid peroxidation), EFSA requires *in vivo* studies performance. In addition, the measurement of the following markers is required: changes in F2-isoprostanes in 24-h urine samples; measurement of oxidized LDL particles in the blood using immunological methods (antibodies) with appropriate specificity and the quantification of phosphatidylcholine hydroperoxides (PCOOH), measured in the blood or tissue by High-Performance Liquid Chromatography (HPLC) (1, 5).

According to the official database of the European Commission "EU Register on nutrition and health claims," EFSA has evaluated 30 requests for approval of health claims referred to tomatoes and/or lycopene, both as a specific and individual compound, component of a food or constituent of a mixture in a commercial product. The great majority of the requests (24 of 30 requests) correspond to article 13.1 "general function," while most other requests refer to article 13.5 "new function" (four of 30 requests) based on the latest scientific evidence and/or under the data protection, and article 14.1.a (two of 30 requests) relating to reducing the risk of illness.

Of all the submitted applications, 15 of 30 requests refer to lycopene as a component of tomato extracts or some derivatives (juice, pulp, and sauces) and 11 of 30 requests, relate lycopene with cardiovascular health and/or its risk factors (oxidative damage, high plasma cholesterol concentrations, and formation of atherosclerotic plaques) (11).

Of the 30 applications submitted, 29 obtained an unfavorable scientific opinion and only one health claim was approved, under article 13.5. The approved health claim is for a concentrated extract of tomato soluble in water (WSTC). This extract is marketed under the patented name of Fruitflow® (FF) with two forms of presentation: WSTC I and II. The effect claimed and approved by EFSA consisted of "helping to maintain normal platelet aggregation, which contributes to healthy blood flow" (11). Scientific evidence for Fruitflow ® included 15 studies (8 in humans and 7 in animals) which demonstrated that 37 compounds present in both forms of concentrated tomato extract (mainly nucleoside derivatives, conjugated phenolic compounds, and flavonoid derivatives) could significantly inhibit platelet aggregation (1, 12). Importantly, the 37 bioactive compounds

identified in WSTC are naturally found in the starting product (that is, in tomato) and the concentration of soluble solids contained in WSTC roughly corresponds to the existing content in 2.5 tomatoes (13). To verify this information, O'Kennedy et al. (12) quantified the content of the bioactive compounds responsible for the alleged effect and that are present both in Fruitflow® and in tomato extracts and other derivatives (juice, tomato paste). The concentration of nucleoside derivatives and conjugated phenolic compounds was found to be higher in tomato (8,095.6 μg/g and 410.3 μg/g, respectively) compared to the patented WSTC extract (7,874.6 μg/g and 389.2 μg/g). The content corresponding to flavonoid derivatives was slightly lower in tomato (1,802.3 μg/g compared to 2,141.3 μg/g found in WSTC). According to O'Kennedy et al., other products, such as tomato juice and paste, showed slightly lower contents than those quantified in tomato extracts and Fruitflow®.

SCIENTIFIC EVIDENCE ON THE RELATIONSHIP BETWEEN CONSUMPTION OF TOMATOES AND ITS BIOACTIVE DERIVATIVES AND CVD PREVENTION

In our first literature search, with the selected keywords "tomato" and "cardiovascular disease," we identified a total of 330 studies (167 and 92 in the last 10 and 5 years, respectively). These included four meta-analyses as the studies with the highest level of scientific evidence (14–17) which refer to 17 and 10 clinical trials in the last 10 and 5 years, respectively, as well as 6 systematic reviews (14–19).

The meta-analyses and systematic review carried out by Cheng et al. (14, 15), include 32 studies in humans to examine the possible relationship between plasma levels of lycopene and the risk of CVD. These studies also confirmed a significant reduction in systolic blood pressure (up to 5.66 mmHg) after supplementation with lycopene. Furthermore, Cheng et al. (15) reported that individuals with the highest plasma lycopene concentrations had a 26 and 37% lower risk of suffering a myocardial infarction and CVD mortality, respectively.

Li et al. (16) performed an umbrella review to collectively and systematically integrate individual study data, evaluate information from multiple meta-analyses on all health outcomes, and provide a wide view of the evidence landscape on tomatoes and lycopene. One hundred and seventy four articles were initially found, but only 17 articles with 20 health outcomes were identified based upon stringent eligibility criteria. Results showed that tomato intake is inversely associated with coronary heart disease mortality and CVD. The authors concluded that tomato or lycopene intake was generally safe and beneficial for multiple health outcomes in humans, but the quality of the evidence was not high.

Finally, Rattanavipanon et al. (17) conducted a systematic review and network meta-analysis on the effects of tomato intake, lycopene intake, and related food products on blood pressure in eight studies ($N = 617$ individuals), including seven trials ($N = 501$ individuals) in the analysis of systolic and diastolic blood pressure outcomes, respectively. Tomato products included standardized tomato extracts, a tomato- containing product without lycopene, and synthetic lycopene. Results showed that a standardized tomato extract significantly decreased systolic blood pressure compared to placebo, whereas the effect on diastolic blood pressure was not significant. In addition, other tomato products did not show consistent and significant effects on both systolic and diastolic blood pressure.

We then performed a second literature search using keywords like "tomato" as well as the WHO disorder classification (**Table 1**) results in: "coronary heart disease" (44 studies), "cerebrovascular disease" (28 studies), "peripheral arterial disease" (two studies) and "deep vein thrombosis" (one study).

TABLE 1 | Summary of the studies found in PubMed by using the key words: "tomato" and the different types of CVD, according to WHO classification.

Keywords	Total number of studies	Type of study	Studies in the last 10 years	Studies in the last 5 years	Relevant studies
Tomato and coronary heart disease	44	1 meta-analysis; 7 clinical trials; 12 reviews	Total: 16; 1 meta-analysis; 2 clinical trials	Total: 9; 1 meta-analysis; 2 clinical trials	Meta-analysis: (16); Clinical trials: (18, 20)
Tomato and cerebrovascular disease	28	1 meta-analysis; 1 clinical trials	Total: 16; 1 Meta-analysis; 1 clinical trials	Total: 6; 1 Meta-analysis; 1 clinical trials	Meta-analysis: (16); Clinical trials: (21).
Tomato and peripheral arterial disease	2	Research articles	Total: 2	Total: 1	–
Tomato and deep vein thrombosis	1	Research article	Total: 1	–	–

SCIENTIFIC EVIDENCE ON THE RELATIONSHIP BETWEEN TOMATO AND LYCOPENE CONSUMPTION, AND ANTIPLATELET AGGREGATION EFFECT

The literature search conducted in this review using the official Pubmed database and the keywords: "tomato," "platelet" and "antiplatelet" resulted in a total of 29 studies. Most of them were reviews and included only seven original clinical trials, (12, 20, 22–26) (**Table 2**).

The information included in the most recent reviews (1, 27–31), and the clinical trials are discussed below.

Regarding the evidence on the beneficial effects of tomato products consumption in healthy people, Sesso et al. (32) conducted a prospective cohort study examining the intake of tomato and tomato juice in 27,267 healthy women free of baseline CVD or cancer. Results indicated that those subjects with a weekly consumption equal to or >10 servings of tomato and tomato products had lower concentrations of

TABLE 2 | Characteristics of the clinical trials found in PubMed by using the keywords: "tomato," "platelet," "antiplatelet," and its compliance with EFSA requirements for anti-platelet effect health claim.

References	Type of tomato product	Study performed on healthy subjects	Duration of the study: 4 weeks of exposure (28 days)	Analytical EFSA valid markers.
(20)	Tomato extract	NO (high-risk hypertensive patients)	YES	NO
(22)	Tomato juice	NO	–	–
(23)	Tomato extract (2 different extract-supplemented treatment drinks)	YES	NO	YES
(24)	Tomato extract (2 treatment supplement drinks using orange juice as a vehicle)	YES	NO	YES
(12)	Water-soluble tomato extract, Fruitflow (FF)	YES	YES	YES
(25)	Tomato pomace (byproduct of tomato pomace) extract (1 g, 2.5 g or placebo)	YES (male, *n* = 99)	NO	YES
(26)	Tomato extract Fruitflow®	NO	NO	NO

total triglycerides, low-density lipoprotein (LDL) cholesterol, and glycated hemoglobin (HbA1c), biological markers of cardiovascular risk. Li et al. (33) conducted a study with 25 young women (20–30 years old) who consumed 280 ml of tomato juice per day for 2 months. A significant reduction in plasma cholesterol levels was observed in all women, as well as an increase in the concentration of adiponectin, a hormone with anti-inflammatory and antiatherogenic properties that modulates the synthesis of nitric oxide (essential in endothelial function) and the proliferation of smooth muscle cells (present in blood vessels, among others). In addition, adiponectin protects against LDL oxidation. An *in vivo* study carried out by Hsu et al. (34) showed that the consumption of tomato paste for 8 weeks contributed to the reduction of plasma concentrations of total and LDL cholesterol, as well as an increase in plasma high-density lipoproteins (HDL) cholesterol and the activity of antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase.

Burton-Freeman et al. (35) suggested that the consumption of tomato products could also attenuate the oxidation of LDL. This beneficial effect was observed in 25 individuals after eating foods with a high fat content. Postprandial oxidative stress was mitigated by the consumption of these tomato derivatives. Similarly, a decrease in lipid peroxidation, as well as an improvement in the general antioxidant status, was observed by García-Alonso et al. (36) in 18 healthy women who consumed tomato juice for 2 weeks. Xaplanteris et al. (37) also reported this effect in 19 individuals who consumed 70 grams of tomato paste during the same period. The results demonstrated a reduction in oxidative stress and an improvement in endothelial function; the latter is essential to maintain an adequate functioning of the cardiovascular system.

Different scientific studies have suggested that lycopene, as main bioactive compound in the tomato, can exert different beneficial physiological effects for improvements in cardiovascular health *via* platelet aggregation and related vascular mechanisms. For example, Hsiao et al. (38) systematically examined the effects of lycopene in the prevention of platelet aggregation and thrombus formation and proposed two mechanisms of action through the inhibition of the activation of the enzyme phospholipase C and the synthesis of cyclic guanosine monophosphate (GMP-c). Fuentes et al. (39) verified *in vitro* that tomato product intake with a higher concentration of lycopene increased the inhibition of platelet activity induced by various aggregating agents such as adenosine diphosphate (ADP), collagen, arachidonic acid, and the thrombin receptor activator peptide-6 (TRAP-6).

Several studies additionally support an association between plasma and tissue levels of lycopene and both pre-clinical and clinical cardiovascular outcomes. Kong et al. (40) also suggested a beneficial effect of lycopene in the early stages of development and progression of atherosclerosis, as well as in the thickness of the intima-media layer of the carotid artery, a parameter that allows quantifying the level of arterial thickening in preclinical phases of cardiovascular disease. Müller et al. (41) highlighted the powerful antioxidant activity of lycopene that could protect endothelial cells against oxidative stress and prevent the formation of foam cells in the early development of atheroma plaque. Sawardekar et al. (42) indicated that lycopene can exert an antiplatelet effect. Different concentrations of lycopene (4–12 μmol/L) were able to *in vitro* significantly reduce platelet aggregation induced by two aggregating agents, ADP, and collagen. This observed effect was comparable to that exerted by one of the best-known antiplatelet drugs, aspirin. The combination of 4 μmol lycopene/L with 140 μmol aspirin/L showed better results than a single dose of 140 μmol aspirin/L. Phang et al. (43) found an inverse association between plasma and tissue levels of lycopene and the incidence of acute coronary disorders, development of early atherosclerosis, and mortality from heart disease. In addition, Thies et al. (44) showed that subjects with higher lycopene concentrations had a lower risk of suffering a myocardial infarction (59%) and showed a significant improvement in HDL functionality enhancing HDL-antiatherogenic properties.

Clinical trials have also supported the potential beneficial cardiovascular effects attributed to lycopene described above. Klipstein-Grobusch et al. (45), Verghese et al. (46), Kim et al. (47), and Riccioni et al. (48) suggested that lycopene may reduce the risk of atherosclerosis, either directly by attenuating LDL oxidation or indirectly by acting on other cardiovascular risk factors, such as cholesterol. Gajendragadkar et al. (49) conducted

a study with 72 individuals, half of them healthy and the other half-undergoing drug treatment because of CVD. There was an improvement in endothelial function in those patients with previous pathologies who ingested a daily amount of 7 mg of lycopene for 2 months. Kim et al. (50) also observed a similar improvement among 37 men with a daily intake of 15 mg of lycopene during the same 8-week follow-up period. These effects for lycopene were attributed to the ability of this bioactive to significantly mitigate oxidative stress and reduce systolic blood pressure.

Other authors have reported a decrease in various cardiovascular risk factors after supplementation with tomato extracts and their derivatives. McEwen (51), Rodríguez-Azúa et al. (52), Palomo et al. (53), Fuentes et al. (39), and Yamamoto et al. (54) suggested that supplementation with tomato extracts may have an antiplatelet effect *in vitro*, *in vivo* and/or in humans; or even a thrombolytic activity in some tomato variety studied. Both observed activities are very important to avoid the formation of thrombin and, if they have already formed, their dissolution to prevent more serious cardiovascular accidents such as embolisms.

As for WSTC, Uddin et al. (26) carried out a clinical trial with 12 prehypertensive patients who were administered a 150 mg dose of WSTC per day. After 24 h, a significant reduction in blood pressure and platelet aggregation was observed compared to the control group. Likewise, Krasińska et al. (20) indicated a significant hypotensive effect for 213 mg/day of standardized tomato extract administered for 4 weeks in 32 patients with at least high cardiovascular risk based on the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH) in 2013 (55). More recently, O'Kennedy et al. (29) found that daily supplementation with Fruitflow® tomato extract reduces platelet aggregation in humans in response to different cofactors, molecules, and enzymes (ADP, collagen, arachidonic acid, and thrombin) involved in platelet activation. Furthermore, the authors suggested a possible beneficial effect of this tomato extract on some cardiovascular risk factors after intense physical activity that promotes a strong inflammatory response and platelet activation. The effect appeared more pronounced among 6 untrained individuals that had Fruitflow® 90 min before the performance of intense physical activity and significantly reduced markers of inflammation, coagulation, and platelet aggregation compared with controls.

Palomo et al. (25) conducted a pilot study to test whether a tomato pomace extract (by-product) affected platelet aggregation in healthy humans. Tomato pomace extract contains flavonoids as coumaric acid, floridzin, floretin, procyanidin B2, luteolin-7-O-glucoside, kaempferol, and quercetin; as well as nucleosides (adenosine, inosine, and guanosine). The study showed that the daily consumption of 1 g of aqueous extract of tomato pomace for 5 days exerted an inhibitory activity on platelet aggregation.

More recently, investigators have focused their efforts on the role of nutrients and bioactive compounds in helping the immune system to fight against COVID-19 through the diet (56). O'Kennedy and Duttaroy (57) suggest that targeting platelet hyperactivity in the early stages of COVID-19 infection may reduce the immunothrombotic complications of COVID-19 and subdue the systemic inflammatory response. As a result, we believe that the bioactive compounds contained in tomatoes, tomato food products or extracts could meaningfully contribute and promote antioxidant and antiplatelet effects in the human body to complement existing established pharmacologic interventions for the primary and secondary prevention of CVD.

CONCLUSIONS

With regard to the association between tomato products, cardiovascular disease prevention and antiplatelet aggregation, in order to obtain EFSA approval for a related health claim, main research gaps are related to the lack of intervention studies on healthy humans (those with no history of serious disease or hemostatic disorders). At present, with the exception of WSTC studies, most of the clinical trials are performed on individuals with some CV risk factor. With independence of study duration which can be easily fit the 4 weeks required, other difficulty is the use of valid markers to prove the percentage of inhibition in platelet aggregation according to EFSA which should be measured using light transmission aggregometry (LTA) using well-accepted and standardized protocols. Finally, as all components found in the tomato extracts are originally in fresh tomato and tomato products, very convenient and appreciated food products by consumers, future directions on this research topic could be focused on the study of mechanism by which tomatoes and tomato products contribute to cardiovascular health to be considered valued as functional foods.

AUTHOR CONTRIBUTIONS

MC, VF-R, and LD conceived the project and design the protocol. MC, VF-R, LD, and RC performed the bibliographic search. MC, VF-R, LD, RC, and M-CS-M performed results analysis. MC and LD wrote the manuscript. HS performed critical review of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

The authors thank support by UCM ALIMNOVA Research Group (GRFN17/21) and Project OTRI Art. 83 Ref: 317-2020, UCM-Fundación Sabor y Salud. LD is grateful to her PhD grant (UCM-Santander; Ref: CT42/18-CT43/18).

ACKNOWLEDGMENTS

Authors are grateful to the RCC Harvard for the supporting of the Study Group on Functional and Novel Foods, https://rcc.harvard.edu/funcitonal-and-novel-foods.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.849841/full#supplementary-material

REFERENCES

1. Cámara M, Fernández-Ruiz V, Sánchez-Mata MC, Domínguez Díaz L, Kardinaal A, Van Lieshout M. Evidence of antiplatelet aggregation effects from the consumption of tomato products, according to EFSA health claim requirements. *Crit Rev Food Sci Nutr.* (2019) 60:1515–22. doi: 10.1080/10408398.2019.1577215
2. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global burden of cardiovascular diseases and risk factors, 1990–2019. Update from the GBD 2019 study. *J Am Coll Cardiol.* (2020) 76:2982–3021. doi: 10.1016/j.jacc.2020.11.010
3. Cámara M, Sánchez-Mata MC, Fernández-Ruiz V, Cámara RM, Manzoor S, Cáceres JO. Lycopene: A Review of Chemical and Biological Activity Related to Beneficial Health Effects en Atta-ur Rahman (Ed.), *Studies in Natural Products Chemistry (1st ed., Vol. 40)*. Amsterdam: Elsevier. (2013) p. 383–426. doi: 10.1016/B978-0-444-59603-1.00011-4
4. Willcox JK, Catignani GL, Lazarus S. Tomatoes and cardiovascular health. *Crit Rev Food Sci Nutr.* (2003) 43:1–18. doi: 10.1080/10408690390826437
5. EFSA. *Scientific Opinion on the substantiation of health claims related to lycopene and protection of DNA, proteins and lipids from oxidative damage (ID 1608, 1609, 1611, 1662, 1663, 1664, 1899, 1942, 2081, 2082, 2142, 2374), protection of the skin from UV-induced (including photo-oxidative) damage (ID 1259, 1607, 1665, 2143, 2262, 2373), contribution to normal cardiac function (ID 1610, 2372), and maintenance of normal vision (ID 1827) pursuant to Article 13(1) of Regulation (EC) No 1924/2006.* (2011). Available online at: https://www.efsa.europa.eu/en/efsajournal/pub/2031.
6. European Parliament and Council of the European Union. *Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods.* (2006). Available online at: http://eur-lex.europa.eu/legal-content/EN/ALL/?uri¼CELEX%3A02006R1924-20121129.
7. EFSA. *Scientific Opinion on Lycopene-whey complex (bioavailable lycopene) and risk of atherosclerotic plaques. Scientific substantiation of a health claim related to Lycopene-whey complex (bioavailable lycopene) and reduction of the risk of atherosclerotic plaques pursuant to Article 14 of Regulation (EC) No 1924/2006.* (2009) Available online at: https://www.efsa.europa.eu/es/efsajournal/pub/1179
8. Cámara M, Fernández-Ruiz V, Fernández Redondo D, Sánchez-Mata MC, Cámara RM, Gervás C, et al. scientific requirements related to lycopene as antioxidant prevention of oxidative damage and cardiovascular health claims. *Acta Hortic.* (2015) 1081:303–7. doi: 10.17660/ActaHortic.2015.1081.39
9. Cámara M, Fernández-Ruiz V, Domínguez L, Cámara RM, Sánchez-Mata MC. (2018) Lycopene: Regulatory status on its antioxidant health claims en. In: Venketeshwer Rao A, Young GL, Rao LG, editors. *Lycopene and Tomatoes in Human Health and Nutrition (1st ed.)*. London: Taylor & Francis. p. 179–96. doi: 10.1201/9781351110877-10
10. EFSA. Scientific Opinion on the substantiation of a health claim related to "L-tug lycopene" and reduction of blood LDL-cholesterol pursuant to Article 14 of Regulation (EC) No 1924/2006. (2015). Available online at: https://www.efsa.europa.eu/es/efsajournal/pub/4025.
11. European Commission. *EU Register of nutrition and health claims made on food.* (2021). Available online at: http://ec.europa.eu/food/safety/labelling_nutrition/claims/register/public/?event=register.home.
12. O'Kennedy N, Raederstorff D, Duttaroy AK. Fruitflow®: the first European food safety authority-approved natural cardioprotective functional ingredient. *Eur J Nutr.* (2017) 56:461–82. doi: 10.1007/s00394-016-1265-2
13. EFSA. *Scientific Opinion on water-soluble tomato concentrate (WSTC I and II) and platelet aggregation. Scientific substantiation of a health claim related to water-soluble tomato concentrate (WSTC I and II) and platelet aggregation pursuant to Article 13(5) of Regulation (EC) No 1924/2006.* (2009). Available online at: https://www.efsa.europa.eu/en/efsajournal/pub/1101. doi: 10.2903/j.efsa.2009.1101
14. Cheng HM, Koutsidis G, Lodge JK, Ashor A, Siervo M, Lara J. Tomato and lycopene supplementation and cardiovascular risk factors: A systematic review and Meta-analysis. *Atherosclerosis.* (2017) 257:100–8. doi: 10.1016/j.atherosclerosis.2017.01.009
15. Cheng HM, Koutsidis G, Lodge JK, Ashor AW, Siervo M, Lara Lycopene J. and tomato and risk of cardiovascular diseases: a systematic review and meta-analysis of epidemiological evidence. *Crit Rev Food Sci Nutr.* (2019) 11:1–18. doi: 10.1080/10408398.2017.1362630
16. Li N, Wu X, Zhuang W, Xia L, Chen Y, Wu C, et al. Tomato and lycopene and multiple health outcomes: umbrella review. *Food Chem.* (2021) 343:128396. doi: 10.1016/j.foodchem.2020.128396
17. Rattanavipanon W, Nithiphongwarakul C, Sirisuwansith P, Chaiyasothi T, Thakkinstian A, Nathisuwan S, et al. Effect of tomato, lycopene and related products on blood pressure: a systematic review and network meta-analysis. *Phytomedicine.* (2021) 153512. doi: 10.1016/j.phymed.2021.153512
18. Tierney AC, Rumble CE, Billings LM, George ES. Effect of Dietary and Supplemental Lycopene on Cardiovascular Risk Factors: a systematic review and meta-analysis. *Adv Nutrit.* (2020) 11:1453–88. doi: 10.1093/advances/nmaa069
19. Rouhi-Boroujeni H, Heidarian E, Rouhi-Boroujeni H, Deris F, Rafieian-Kopaei M. Medicinal plants with multiple effects on cardiovascular diseases: a systematic review. *Curr Pharm Des.* (2017) 23:999–1015. doi: 10.2174/1381612822666161021160524
20. Krasińska B, Osińska A, Krasińska A, Osiński M, Rzymski P, Tykarski A, et al. Favourable hypotensive effect after standardised tomato extract treatment in hypertensive subjects at high cardiovascular risk: a randomised controlled trial. *Kardiol Pol.* (2018) 76:388–95. doi: 10.5603/KP.a2017.0215
21. Droste DW, Iliescu C, Vaillant M, Gantenbein M, De Bremaeker N, Lieunard C, et al. Advice on lifestyle changes (diet, red wine and physical activity) does not affect internal carotid and middle cerebral artery blood flow velocity in patients with carotid arteriosclerosis in a randomized controlled trial. *Cerebrovascular Dis.* (2014) 37:368–75. doi: 10.1159/000362535
22. Lazarus SA, Bowen K, Garg ML. Tomato juice and platelet aggregation in type 2 diabetes. *J Am Med Assoc.* (2004) 292:805–6. doi: 10.1001/jama.292.7.805
23. O'Kennedy N, Crosbie L, Van Lieshout M, Broom JI, Webb DJ, Duttaroy AK. Effects of antiplatelet components of tomato extract on platelet function *in vitro* and *ex vivo*: a timecourse cannulation study in healthy humans. *Am J Clin Nutr.* (2006) 84:570–9. doi: 10.1093/ajcn/84.3.570
24. O'Kennedy N, Crosbie L, Whelan S, Luther V, Horgan G, Broom JI, et al. Effects of tomato extract on platelet function: A double-blinded crossover study in healthy humans. *Am J Clin Nutr.* (2006) 84:561–9. doi: 10.1093/ajcn/84.3.561
25. Palomo I, Concha-Meyer A, Lutz M, Said M, Sáez B, Vásquez A, et al. Chemical characterization and antiplatelet potential of bioactive extract from tomato pomace (Byproduct of Tomato Paste). *Nutrients.* (2019) 11:456. doi: 10.3390/nu11020456
26. Uddin M, Biswas D, Ghosh A, O'Kennedy N, Duttaroy AK. Consumption of fruitflow® lowers blood pressure in prehypertensive males: A randomised, placebo controlled, double blind, cross-over study. *Int J Food Sci Nutr.* (2018) 69:494–502. doi: 10.1080/09637486.2017.1376621
27. Fuentes E, Trostchansky A, Reguengo LM, Junior MRM, Palomo I. Antiplatelet effects of bioactive compounds present in tomato pomace. *Curr Drug Targets.* (2021) 22:1716–24. doi: 10.2174/1389450122999210128180456
28. Mozos I, Stoian D, Caraba A, Malainer C, Horbanczuk JO, Atanasov AG. Lycopene and vascular health. *Front Pharmacol.* (2018) 9:521. doi: 10.3389/fphar.2018.00521
29. O'Kennedy N, Duss R, Duttaroy AK. Dietary Antiplatelets: A New Perspective on the Health Benefits of the Water-Soluble Tomato Concentrate Fruitflow®. *Nutrients.* (2021) 13:2184. doi: 10.3390/nu13072184
30. Olas B. Anti-aggregatory potential of selected vegetables—promising dietary components for the prevention and treatment of cardiovascular disease. *Adv Nutrit.* (2019) 10:280–290. doi: 10.1093/advances/nmy085
31. Tang G, Meng X, Li Y, Zhao C, Liu Q, Li H. Effects of vegetables on cardiovascular diseases and related mechanisms. *Nutrients.* (2017) 9:857. doi: 10.3390/nu9080857
32. Sesso HD, Wang L, Ridker PM, Buring JE. Tomato-based food products are related to clinically modest improvements in selected coronary biomarkers in women. *J Nutr.* (2012) 142:326–33. doi: 10.3945/jn.111.150631
33. Li YF, Chang YY, Huang HC, Wu YC, Yang MD, Chao PM. Tomato juice supplementation in young women reduces inflammatory adipokine levels independently of body fat reduction. *Nutrition.* (2015) 31:691–6. doi: 10.1016/j.nut.2014.11.008

34. Hsu YM, Lai CH, Chang CY, Fan CT, Chen CT, Wu CH. Characterizing the lipid-lowering effects and antioxidant mechanisms of tomato paste. *Biosci Biotechnol Biochem.* (2008) 72:677–85. doi: 10.1271/bbb.70402
35. Burton-Freeman B, Talbot J, Park E, Krishnankutty S, Edirisinghe I. Protective activity of processed tomato products on postprandial oxidation and inflammation: A clinical trial in healthy weight men and women. *Mol Nutr Food Res.* (2012) 56:622–31. doi: 10.1002/mnfr.201100649
36. García-Alonso FJ, Jorge-Vidal V, Ros G, Periago MJ. Effect of consumption of tomato juice enriched with n-3 polyunsaturated fatty acids on the lipid profile, antioxidant biomarker status, and cardiovascular disease risk in healthy women. *Eur J Nutr.* (2012) 51:415–24. doi: 10.1007/s00394-011-0225-0
37. Xaplanteris P, Vlachopoulos C, Pietri P, Terentes-Printzios D, Kardara D, Alexopoulos N, et al. Tomato paste supplementation improves endothelial dynamics and reduces plasma total oxidative status in healthy subjects. *Nutrit Res.* (2012) 32:390–4. doi: 10.1016/j.nutres.2012.03.011
38. Hsiao G, Wang Y, Tzu NH, Fong TH, Shen MY, Lin KH, et al. Inhibitory effects of lycopene on *in vitro* platelet activation and *in vivo* prevention of thrombus formation. *J Lab CliniMed.* (2005) 146:216–26. doi: 10.1016/j.lab.2005.03.018
39. Fuentes E, Carle R, Astudillo L, Guzman L, Gutierrez M, Carrasco G, et al. Antioxidant and antiplatelet activities in extracts from green and fully ripe tomato fruits (*Solanum lycopersicum*) and pomace from industrial tomato processing. *Evid Based Complementary Altern.* (2013) 867578. doi: 10.1155/2013/867578
40. Kong KW, Khoo HE, Prasad KN, Ismail A, Tan CP, Rajab NF. Revealing the power of the natural red pigment lycopene. *Molecules.* (2010) 15:959–87. doi: 10.3390/molecules15020959
41. Müller L, Caris-Veyrat C, Lowe G, Böhm V. Lycopene and its antioxidant role in the prevention of cardiovascular diseases—a critical review. *Crit Rev Food Sci Nutr.* (2016) 56:1868–79. doi: 10.1080/10408398.2013.801827
42. Sawardekar SB, Patel TC, Uchil D. Comparative evaluation of antiplatelet effect of lycopene with aspirin and the effect of their combination on platelet aggregation: an *in vitro* study. *Indian J Pharmacol.* (2016) 48:26–31. doi: 10.4103/0253-7613.174428
43. Phang M, Lazarus S, Wood LG, Garg M. Diet and thrombosis risk: nutrients for prevention of thrombotic disease. *Semin Thromb Hemost.* (2011) 37:199–208. doi: 10.1055/s-0031-1273084
44. Thies F, Mills LM, Moir S, Masson LF. Cardiovascular benefits of lycopene: fantasy or reality? *Proc Nutr Soc.* (2017) 76:122–9. doi: 10.1017/S0029665116000744
45. Klipstein-Grobusch K, Launer LJ, Geleijnse JM, Boeing H, Hofman A, Witteman JC. Serum carotenoids and atherosclerosis. The Rotterdam study. *Atherosclerosis.* (2000) 148:49–56. doi: 10.1016/S0021-9150(99)00221-X
46. Verghese M, Richardson JE, Boateng J, Shackelford LA, Howard C, Walker LT, et al. Dietary lycopene has a protective effect on cardiovascular disease in New Zealand male rabbits. *J Biological Sci.* (2008) 8:268–77. doi: 10.3923/jbs.2008.268.277
47. Kim OY, Yoe HY, Kim HJ, Park JY, Kim JY, Lee SH, et al. Independent inverse relationship between serum lycopene concentration and arterial stiffness. *Atherosclerosis.* (2010) 208:581–6. doi: 10.1016/j.atherosclerosis.2009.08.009
48. Riccioni G, Scotti L, Di Ilio E, Bucciarelli V, Ballone E, De Girolamo M, et al. Lycopene and preclinical carotid atherosclerosis. *J Biol Regul Homeost Agents.* (2011) 25:435–41. Available online at: https://www.researchgate.net/publication/51739032_Lycopene_and_preclinical_carotid_atherosclerosis
49. Gajendragadkar PR, Hubsch A. Mäki-Petäjä, KM, Serg M, Wilkinson IB, Cheriyan J. *Effects of oral lycopene supplementation on vascular function in patients with* Cardiovascular disease and healthy volunteers: a randomised controlled trial. *PLoS ONE.* (2014) 9:e99070. doi: 10.1371/journal.pone.0099070
50. Kim JY, Paik JK, Kim OY, Park HW, Lee JH, Jang Y, et al. Effects of lycopene supplementation on oxidative stress and markers of endothelial function in healthy men. *Atherosclerosis.* (2011) 215:189–95. doi: 10.1016/j.atherosclerosis.2010.11.036
51. McEwen BJ. The influence of diet and nutrients on platelet function. *Semin Thromb Hemost.* (2014) 40:214–26. doi: 10.1055/s-0034-1365839
52. Rodríguez-Azúa R, Treuer A, Moore-Carrasco R, Cortacans D, Gutiérrez M, Astudillo L, et al. Effect of tomato industrial processing (different hybrids, paste, and pomace) on inhibition of platelet function *in vitro*, *ex vivo*, *in vivo*. *J Med Food.* (2014) 17:505–11. doi: 10.1089/jmf.2012.0243
53. Palomo I, Fuentes E, Padro T, Badimon L. Platelets and atherogenesis: platelet antiaggregation activity and endothelial protection from tomatoes (*Solanum lycopersicum* L.). *Exp Ther Med.* (2012) 23:109–11. doi: 10.3892/etm.2012.477
54. Yamamoto J, Taka TK, Yamada Y, Ijiri M, Murakami Y, Hirata A, et al. Tomatoes have natural anti-thrombotic effects. *Br J Nutrit.* (2003) 90:1031–8. doi: 10.1079/BJN2003988
55. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J.* (2013) 34:2159–219. doi: 10.1093/eurheartj/eht151
56. Cámara M, Sánchez-Mata MC, Fernández-Ruiz V, Cámara RM, Cebadera E, Domínguez L, et al. Review of the role of micronutrients and bioactive compounds on immune system supporting to fight against the COVID-19 disease. *Foods.* (2021) 10:1088. doi: 10.3390/foods10051088
57. O'Kennedy N, Duttaroy AK. Platelet hyperactivity in COVID-19: can the tomato extract fruitflow® be used as an antiplatelet regime? *Med Hypotheses.* (2021) 147:110480. doi: 10.1016/j.mehy.2020.110480

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Cámara, Fernández-Ruiz, Sánchez-Mata, Cámara, Domínguez and Sesso. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 22 March 2022
doi: 10.3389/fnut.2022.830186

Changes in Greenhouse Grown Tomatoes Metabolite Content Depending on Supplemental Light Quality

Ina Alsina[1], *Ieva Erdberga*[1]*, *Mara Duma*[2], *Reinis Alksnis*[3] *and Laila Dubova*[1]

[1] *Faculty of Agriculture, Institute of Soil and Plant Sciences, Latvia University of Life Sciences and Technologies, Jelgava, Latvia,* [2] *Department of Chemistry, Faculty of Food Technology, Latvia University of Life Sciences and Technologies, Jelgava, Latvia,* [3] *Department of Mathematics, Faculty of Information Technologies, Latvia University of Life Sciences and Technologies, Jelgava, Latvia*

Tomatoes (*Solanum lycopersicum* L.) are good source of several biologically active compounds and antioxidants, especially lycopene, phenolic compounds, and vitamins. Tomatoes are found all over the world and are cultivated in a wide variety of environmental conditions. Light-emitting diode (LED) lamps are increasingly being used in the cultivation of tomatoes due to their cost-effectiveness and wide range of possibilities to adapt the spectrum of light emitted to the needs of plants. The aim of this study is to evaluate the effect of different additional lighting used in the greenhouse on the accumulation of biologically active compounds in different varieties of tomato fruit. Chemical composition—content of organic acids, lycopene, total carotenoids, total phenolics and flavonoids as well as dry matter, soluble solids content, and taste index were determined in five tomato cultivars ("Bolzano F1," "Chocomate F1," "Diamont F1," "Encore F1," and "Strabena F1"), which were cultivated in greenhouse in an autumn-spring season by using additional lighting with 16 h photoperiod. Three different lighting sources were used: LED, induction (IND) lamp, and high-pressure sodium lamp (HPSL). Experiments were performed during 3 years. Results showed that tomato varieties react differently to the supplemental lighting used. Cultivars, such as "Encore" and "Strabena," are the most unresponsive to supplemental light. Experiments have shown that HPSL stimulates the accumulation of primary metabolites in tomato fruit. In all the cases, soluble solids content was 4.7–18.2% higher as compared to other lighting sources. As LED and IND lamps emit about 20% blue-violet light, the results suggest that blue-violet light of the spectrum stimulates the accumulation of phenolic compounds in the fruit by 1.6–47.4% under IND and 10.2–15.6% under LED compared to HPSL. Red fruit varieties tend to synthesize more β-carotene under supplemental LED and IND light. An increase of blue promotes the synthesis of secondary metabolites.

Keywords: tomatoes, LED, HPSL, lycopene, taste index, phenols, flavonoids

OPEN ACCESS

Edited by:
José Pinela,
Polytechnic Institute of Bragança (IPB), Portugal

Reviewed by:
Ilahy Riadh,
Institut National de la Recherche Agronomique de Tunisie (INRAT), Tunisia
Mauricio Hunsche,
University of Bonn, Germany

***Correspondence:**
Ieva Erdberga
ieva.erdberga@llu.lv

Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *06 December 2021*
Accepted: *20 January 2022*
Published: *22 March 2022*

Citation:
Alsina I, Erdberga I, Duma M, Alksnis R and Dubova L (2022) Changes in Greenhouse Grown Tomatoes Metabolite Content Depending on Supplemental Light Quality. Front. Nutr. 9:830186. doi: 10.3389/fnut.2022.830186

INTRODUCTION

As understanding of the importance of diet in ensuring quality and sustainability of human life grows, the pressure on the agricultural sector as a basic element in securing food quality is increasing. Tomatoes, as the second most grown vegetable [according to the Food and Agriculture Organization (FAO) statistics for 2019], are an important part of the cuisine of almost every nation.

The limited caloric supply, relatively high fiber content, and presence of mineral elements, vitamins, and phenols, such as flavonoids, make the tomato fruit an excellent "functional food" providing many physiological benefits and basic nutritional requirements (1). The biochemically active substances found in tomatoes, mainly due to their high antioxidant capacity, are recognized not only for the general improvement of health, but also as a therapeutic option against various diseases, such as diabetes, heart diseases, and toxicities (2–4). Ripe tomato fruit contains an average 3.0–8.88% dry matter, which consists of 25% fructose, 22% glucose, 1% sucrose, 9% citric acid, 4% malic acid, 8% mineral elements, 8% protein, 7% pectin, 6% cellulose, 4% hemicellulose, 2% lipids, and the remaining 4% are amino acids, vitamins, phenolic compounds, and pigments (5, 6). The composition of these compounds varies depending on genotype, growing conditions, and fruit development stage. Tomato plants are highly sensitive to environmental factors, such as light conditions, temperature, and the amount of water in the substrate, which lead to changes in plant metabolism, which, in turn, affect the quality and chemical composition of the fruit (7). Environmental conditions affect both the tomato physiology and the synthesis of secondary metabolites. Plants grown under stress conditions react by increasing their antioxidant properties (8).

The origin of tomatoes as a species is linked to the Central American region (9) and techniques, such as the construction of greenhouses to supply the necessary temperature and light for tomatoes, are often required to provide the necessary agroclimatic conditions, especially in the temperate climatic zone and during the winter season. Under such conditions, light is often the limiting factor for tomato development. Supplementary lighting during winter and early spring seasons allows producing high-quality tomatoes during the low solar irradiance period (10). The use of lamps with different wavelengths cannot only ensure a sufficient tomato yield, but also change the biochemical composition of tomato fruit. For the last 60 years, high-pressure sodium lamps (HPSLs) have been used in the greenhouse industry due to their long operating life and low acquisition costs (11). However, in the last years, light-emitting diodes (LEDs) have become increasingly popular as a more energy-saving alternative (12). Supplemental LED has been used as an efficient light source to meet the demand for tomatoes production. Lycopene and lutein contents in tomatoes were 18 and 142% higher when they were exposed to the supplemental LED lighting. However, β-carotene content did not differ between the light treatments (12). LED blue and red light increased lycopene and β-carotene content (13), resulting in the early ripening of tomato fruit (14). Soluble sugar contents of the ripe tomato fruit were decreased by longer far-red (FR) light durations (15). Analogous conclusions were drawn in the study by Xie: red light induces lycopene accumulation, but FR light reverses this effect (13). There is less information on the effects of blue light on tomato fruit development, but studies show that blue light has a lesser effect on the amount of biochemical compounds in tomato fruit, but more on process stability. For example, Kong and others have found that blue light is better used to prolong the shelf-life of tomatoes, as blue light significantly increases the firmness of the fruit (16), which essentially means that blue light slows down the ripening process, which leads to an increase in amount of sugars and pigments. The use of greenhouse coverings as a means of regulating the composition of light proves a similar pattern. The use of a coating with a higher red and lower blue light transmission increases the lycopene content by about 25%. In combination with a photoperiod increased from 11 to 12 h, the amount of lycopene increases by about 70% (17). It is not always possible in studies to accurately distinguish the effect of factors on changes in the chemical composition of tomato fruit. Especially, in greenhouse conditions, the composition of the fruit can be increased by elevated temperatures or reduced water levels. In addition, these factors may correlate with the genotype-specific to the variety and development stage (1, 18). Water deficit may benefit tomatofruit quality due to increased levels of total soluble solids (sugars, amino acids, and organic acids), which are major compounds accumulated in fruit. A rise of soluble solids improves the quality of fruits because it affects the flavor and taste (8).

Despite the reported effects of light spectrum on the accumulation of plant metabolites, the wider knowledge of different spectrum effects for improving the quality of tomatoes is required. Accordingly, the aim of this study is to evaluate the effect of additional lighting used in the greenhouse on the accumulation of primary and secondary metabolites in different tomatoes varieties. Changes in the spectral content of lighting system can alter the composition of primary and secondary metabolites in tomato fruit. The acquired knowledge will improve the understanding of the effect of light on the relationship between yield and its quality.

MATERIALS AND METHODS

Plant Material and Growing Conditions

Experiments were conducted in greenhouse (4 mm cell polycarbonate) of the Institute of Soil and Plant Sciences, Latvia University of Life Sciences and Technologies 56°39'N 23°43'E during 2018/2019, 2019/2020, and 2020/2021 late autumn-early spring seasons.

Commercially grafted tomato (*Solanum lycopersicum* L.) cultivars "Bolzano F1" (fruit color—orange), "Chocomate F1" (fruit color—red-brown), and red fruit cultivars "Diamont F1," "Encore F1," and "Strabena F1" were used. Each plant had two leading heads and during growth, it was trellised on a high–wire system. Obtained plants, first, were transplanted in black 5 L plastic containers with "Laflora" peat substrate KKS-2, pH_{KCl} 5.2–6.0, and fraction size 0–20 mm, PG mixture (NPK 15-10-20) 1.2 kg m^{-3}, Ca 1.78%, and Mg 0.21%. When plants reached anthesis, they were transplanted into 15 L black plastic containers with the same "Laflora" peat substrate KKS-2. Plants were fertilized once a week with 1% solution of Kristalon Green (NPK 18-18-18) with Mg, S, and microelements during the vegetative phase of plant growth and with Kristalon Red (NPK 12-12-36) with microelements or 1% $Ca(NO_3)_2$ during the reproductive phase, in proportion 300 ml per L of substratum.

The water content in the vegetation containers was maintained at 50–80% of the full water holding capacity. Average day/night temperatures were 20–22°C/17–18°C.

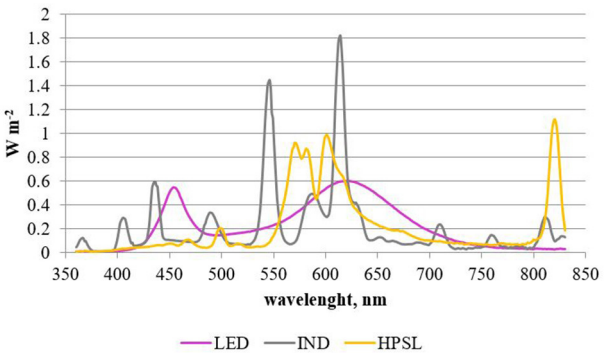


FIGURE 1 | Spectrum of lighting sources.

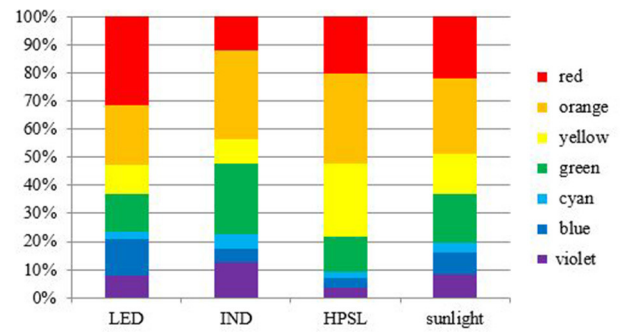


FIGURE 2 | Spectral distribution of lighting source.

Maximal temperature during the day (March) did not exceed 32°C and minimal temperature (November) during the night was not <12°C. Temperature has also been measured under the lamps at the distance 50, 100, and 150 cm from the luminaire. It was detected that under the HPSL 50 cm from the luminaire, temperature was 1.5°C higher than under the others. Temperature differences at the fruit level were not detected.

Lighting Conditions

Tomatoes were cultivated in autumn-spring seasons by using additional lighting with a 16 h photoperiod. Three different lighting sources were used: Led cob Helle top LED 280 (LED), induction (IND) lamp, and HPSL Helle Magna (HPSL). At the apex height, plants received 200 ± 30 μmol m^{-2} s^{-1} under LED and HPSL and 170 ± 30 μmol m^{-2} s^{-1} under IND lamps. Distribution of light radiance is shown in **Figures 1**, **2**. Light intensity and spectral distribution were detected by handheld spectral light meter MSC15 (Gigahertz Optik GmbH, Türkenfeld, Germany, UK).

The used lamps differed in their light spectral distribution. The most similar to sunlight in the red part (625–700 nm) of the spectrum was HPSL. The IND lamp in this part of the spectrum gave 23.5% less light, but LED was close to 2 times more. Orange light (590–625 nm) was emitted mostly by HPSL, green light (500–565 nm) was emitted mostly by IND, blue light (450–485 nm) was emitted mostly by LED, but purple light (380–450 nm) was emitted mostly by IND lamp. When comparing the whole spectrum of visible light, the LED light source should be considered as the closest to sunlight and the IND should be considered as the most inappropriate in terms of spectrum.

Extraction and Determination of Phytochemicals

Tomato fruits were harvested on the full ripeness stage. Fruits were harvested once a month starting in the middle of November and ending in March. All the fruits were counted and weighted. At least, 5 fruits from each variant (for cv "Strabena" −8–10 fruits) were sampled for analyses. Tomato fruits were ground into a puree by using a hand blender. For each evaluated parameter, three replications were analyzed.

Determination of Lycopene and β-Carotene

To determine the concentration of lycopene and β-carotene, a sample of 0.5 ± 0.001 g from the tomato puree was then weighed into a tube and 10 mL of tetrahydrofuran (THF) was added (19). The tubes were sealed and kept at room temperature for 15 min, shaking occasionally, and finally centrifuged for 10 min at 5,000 rpm. The absorbance of the supernatants obtained was determined spectrophotometrically by measuring the absorbance at 663, 645, 505, and 453 nm and then the lycopene and β-carotene contents (mg 100 mL^{-1}) were calculated according to the following equation.

$$C_{lyc} = -0.0458 \times A_{663} + 0.204 \times A_{645} + 0.372 \times A_{505} - 0.0806 \times A_{453} \quad (1)$$

$$C_{car} = 0.216 \times A_{663} - 1.22 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453} \quad (2)$$

where A663, A645, A505, and A453—absorption at corresponding wavelength (20).

The lycopene and β-carotene concentrations are expressed as mg g_{FM}^{-1}.

Determination of Total Phenols

A sample of 1 ± 0.001 g from the tomato puree was weighed into a graduated tube and 10 ml of solvent (methanol/distilled water/hydrochloric acid 79:20:1) was added. The graduated tubes were sealed and shaken for 60 min at 20°C in the dark and then centrifuged for 10 min at 5,000 rpm. The total phenol concentration was determined by using the Folin–Ciocalteu spectrophotometric method (21) with some modifications: Folin–Ciocalteu reagent (diluted 10-fold in distilled water) was added to 0.5 ml of the extract and after 3 min add 2 mL of sodium carbonate (Na_2CO_3) (75 gL^{-1}). The sample was mixed and after 2 h incubation at room temperature in the dark, the absorbance at 760 nm was measured. The concentration of total phenolic compounds was calculated by using the calibration curve and obtained equation 3, and expressed as gallic acid equivalent (GAE) per 100 g of fresh tomato mass.

$$Phe = \frac{0.556 \times (A_{760} + 0.09) \times 100}{m} \quad (3)$$

where A_{760}–absorption at corresponding wavelength and m—mass of the sample.

Determination of Flavonoids

A sample of 1 ± 0.001 g from the tomato puree was weighed into a graduated tube and 10 mL ethanol was added. The graduated tubes were sealed and shaken for 60 min at 20°C in the dark and then centrifuged for 10 min at 5,000 rpm. The colorimetric method (22) was used to determine flavonoids with minor changes: 2 mL of distilled water and 0.15 mL of 5% sodium nitrite ($NaNO_2$) solution were added to 0.5 mL of the extract. After 5 min, a 0.15-mL of 10% solution of aluminum chloride ($AlCl_3$) was added. The mixture was allowed to stand for another 5 min and 1 mL 1 M sodium hydroxide (NaOH) solution was added. The sample was mixed and after 15 min at room temperature, the absorbance at 415 nm was measured. The total flavonoid concentration was calculated by using calibration curve and Equation 4 and expressed as the amount of catechin equivalents (CEs) per 100 g of fresh tomato weight.

$$Fla = \frac{0.444 \times A_{415} \times 100}{m} \quad (4)$$

where A_{415}–absorption at corresponding wavelength and m—mass of the sample.

Determination of Dry Matter and Soluble Solids

Dry matter was determined by drying samples in the thermostat at 60°C.

The total soluble solids content (expressed as °Brix) was measured with a refractometer (A.KRÜSS Optronic Digital Handheld Refractometer Dr301-95) calibrated at 20°C with distilled water.

Determination of Titratable Acidity (TA)

A sample of 2 ± 0.01 g from the tomato puree was weighed into a graduated tube and distilled water was added till 20 mL. The graduated tubes were sealed and shaken for 60 min at room temperature and then centrifuged for 10 min at 5,000 rpm. 5 mL aliquots were titrated with 0.1 M NaOH in the presence of phenolphthalein.

$$TA = \frac{V_{NaOH} \times V_t}{V_s \times m} \quad (5)$$

where V_{NaOH}–volume of used 0.1 M NaOH, Vt—total volume (20 mL), and Vs—sampled volume (5 mL).

Results are expressed as mg of citric acid per 100 g of fresh tomato weight. 1 mL 0.1 M NaOH corresponds to 6.4 mg citric acid.

Determination of Taste Index (TI)

A TI was calculated by using equation 6 (23).

$$TI = \frac{^{\circ}\text{Brix}}{20 \times TA} + TA \quad (6)$$

Statistical Analyses

The normality and homogeneity of the descriptive statistics were tested for 354 observations. The Shapiro–Wilk test was used for the evaluation of normality within each combination of variety and lighting treatment. To estimate homogeneity of variances, Levene's test was conducted. The Kruskal–Wallis test was used to examine the differences between lighting conditions. When statistically significant differences were identified, the Wilcoxon *post-hoc* test with Bonferroni corrections was used for pairwise comparisons. The significance level used in the text, tables and graphs is $\alpha = 5\%$, unless stated otherwise.

RESULTS

Tomato fruit size and fruit biochemical parameters are genetically determined parameters, but cultivation conditions have a significant impact on these features. The largest fruits are harvested from "Diamont" (88.3 ± 22.9 g) and the smallest fruits are harvested from "Strabena" (13.0 ± 3.8 g), which are a variety of cherry tomatoes. The size of the fruit within the variety also varied from the time of harvest. The largest fruits were harvested at the beginning of production and the size of the tomatoes decreased as the plants grew. However, it should be noted that with the increased proportion of natural light at the end of March, tomatoes size slightly increased.

In all three years, the highest tomato yield was harvested using HPSL as additional lighting. The yield decrease under LED's was 16.0%, and under IND - 17.7% compared with HPSL. Different varieties of tomatoes reacted differently to supplemental lighting. Yield increase, although statistically insignificant, were observed for the cv "Strabena", "Chocomate" and "Diamont" under LEDs. For cv "Bolzano" neither LED nor IND additional lighting was suitable, the reduction of total yield by 25–31% was observed.

In average, larger tomato fruits contain less dry matter and soluble solids, they are not so tasty, and contain less carotenoids and phenols. The factor that is least affected by fruit size is the acid content. A high correlation is observed between the dry matter and soluble solids content and the TI ($r_{n=195} > 0.9$). The correlation coefficient between the dry matter or soluble solids content and the carotenoid (lycopene and carotene) and the phenol content ranges between 0.7 and 0.8 (**Figure 3**).

Experiments have shown that, although the differences in the studied parameters between the lights used are sometimes large, there are few such parameters that would change significantly under the influence of the light source used during the whole growing season and taking into account the variety and three growing seasons (**Table 1**). It can be stated that tomatoes of all the varieties grown under HPSL have more dry matter (**Table 1** and **Figure 5**).

Fresh Weight, Dry Matter, and Soluble Solids

The weight and size of the fruit depend significantly on the growing conditions of the plant. Although there were differences between the varieties, the average fruit of tomatoes growing under induction lamps was 12% smaller than under HPSL

or LED. Different varieties seem to react differently to the supplementary LED light. Larger fruits are formed under the LEDs by "Chocomate" and "Diamont," but the fresh weight of "Bolzano" is on average only 72% of the weight of tomato under HPSL. Fruits of "Encore" and "Strabena" grown under LED and IND supplementary lighting are similar in weight and are 10 and 7% smaller, respectively, than tomatoes grown under HPSL (**Figure 4**).

Dry matter content is one of the indicators of fruit quality. It correlates with the soluble solids content and influences tomatoes taste. In our experiments, the dry matter content of tomatoes varied between 46 and 113 mg g^{-1}. The highest dry matter content (on average 95 mg g^{-1}) was found for cherry variety "Strabena." Among other tomatoes cultivars, the highest dry matter content (on average 66 mg g^{-1}) was found in "Chocomate" (**Figure 5**).

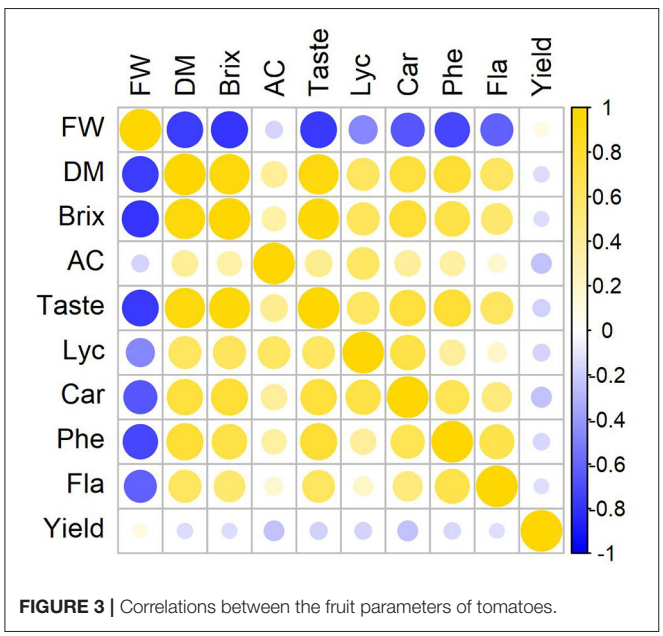


FIGURE 3 | Correlations between the fruit parameters of tomatoes.

During the experiment, the organic acid content, expressed as citric acid (CA) equivalent in tomatoes, averaged from 365 to 640 mg 100 g^{-1}. The highest organic acid content was found in the cherry tomato cv "Strabena," an average of 596 ± 201 mg CA 100 g^{-1}, but the lowest organic acid content was found in the yellow fruit cv "Bolzano," an average of 545 ± 145 mg CA 100 g^{-1}. Organic acid content varied greatly not only between varieties, but also between sampling times; however, on average, higher organic acid content was found in tomatoes grown under IND lamps (exceeding HPSL and LED by 10.2%).

On average, the highest dry matter content was found in fruits grown under HPSL. Under the IND lamp, the dry matter content of tomato fruit decreases by 4.7–16.1%, below the LED of 9.9–18.2%. The varieties used in the experiments are differently sensitive to light. The smallest decrease in the dry matter under different light conditions was observed for cv "Strabena" (5.8% for IND and 11.1% for LED, respectively) and the largest decrease in the dry matter under different light conditions was observed for cv "Diamont" (16.1% and 18.2% respectively).

On average, soluble solids content varied between 3.8 and 10.2 °Brix. Similarly, for dry matter, the highest soluble solids content was detected in cherry tomatoes cultivar "Strabena" (on average 8.1 ± 1.0 °Brix). The tomato cv "Diamont" was the least sweet (on average 4.9 ± 0.4 °Brix).

Supplemental lighting significantly affected soluble solids content of tomato cultivars "Bolzano," "Diamont," and "Encore." Under LED light, soluble solids content in these varieties significantly decreased in comparison with HPSL. The effect of the IND lamp was less. Under this lighting conditions, growing tomatoes of cv "Bolzano" and "Strabena" had on average 4.7 and 4.3% more sugar than under HPSL grown. Unfortunately, this increase is not statistically significant (**Figure 6**).

Tomatoes TI varies from 0.97 to 1.38. The tastiest was tomatoes of cv "Strabena," on average TI was 1.32 ± 0.1 and the less tastiest was tomatoes of cv "Diamont," on average TI was only 1.01 ± 0.06. High TI has tomato cultivar "Bolzano," on average TI (1.12 ± 0.06), followed by "Chocomate," on average TI (1.08 ± 0.06).

On average, the TI is not significantly affected by lighting source, except for cv "Strabena," where the fruits under IND lamp

TABLE 1 | *P*-values (Kruskal-Wallis test) of the effects of different supplementary lightings on tomato fruit quality ($n = 118$).

Parameter	"Bolzano"	"Chocomate"	"Encore"	"Diamont"	"Strabena"
Fruit weight	0.013*	0.008**	0.110	0.400	0.560
Dry matter	0.022*	0.013*	0.011*	0.001**	0.015*
Soluble solids	0.027*	0.030	0.030*	0.001**	0.270
Acidity	0.078	0.022	0.160	0.001**	0.230
Taste index	0.370	0.140	0.600	0.001**	0.023*
Lycopene	0.052	0.290	0.860	0.160	0.920
β-carotene	<0.001***	0.007**	0.940	0.110	0.700
Phenols	0.097	0.750	0.450	0.800	0.420
Flavonoids	0.430	0.035*	0.720	0.440	0.170

*Significance levels "***" 0.001, "**" 0.01, and "*" 0.05.*

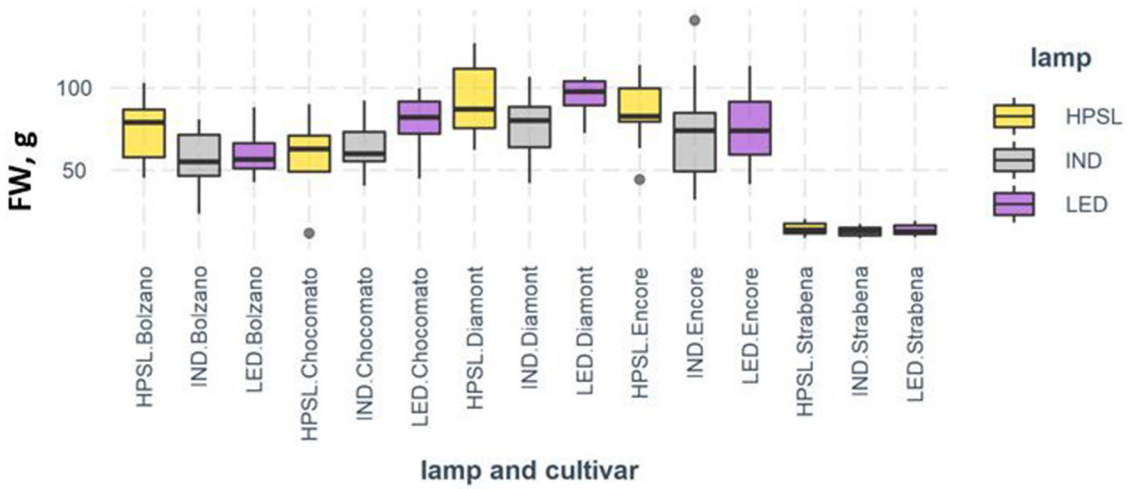


FIGURE 4 | Fresh weight (g) of tomato fruits grown under different supplemental light sources.

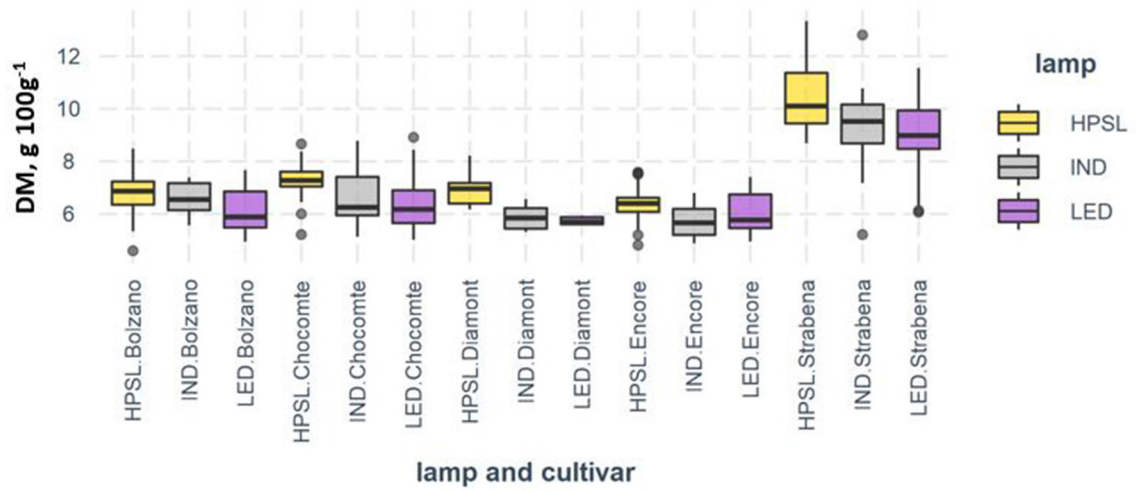


FIGURE 5 | Dry matter (g 100 g^{-1}) of tomato fruits grown under different supplemental light sources.

have the TI increase in comparison with HPSL by 7.4% (LED by 4.2%) in comparison with HPSL and cv "Diamont" under both the previously mentioned lighting conditions decrease by 5.3 and 8.4%, respectively, was detected.

Carotenoids Content

Lycopene concentration in tomatoes varied from 0.07 (cv "Bolzano") to 7 mg 100 g^{-1} FM ("Strabena"). Slightly higher lycopene content in comparison with "Diamont" (4.40 ± 1.35 mg 100 g^{-1} FM) and "Encore" (4.23 ± 1.33 mg 100 g^{-1} FM) was found in brownish red-colored fruits of "Chocomate" (4.74 ± 1.48 mg 100 g^{-1} FM).

On average, fruits from plants grown under IND lamps contain 17.9% more lycopene in comparison with HPSL. LED lighting has also promoted lycopene synthesis, but to a lesser extent, by an average of 6.5%. The effect of light sources has varied depending on the cultivar. The largest differences in lycopene biosynthesis were observed for "Chocomate." The increase of lycopene content under IND compared to HPSL was 27.2% and below LED by 13.5%. "Strabena" was the least sensitive, with changes of 3.2 and −1.6%, respectively, compared to HPSL (**Figure 7**). Despite the relatively convincing results, the mathematical processing of the data does not confirm its reliability (**Table 1**).

During the experiment, β-carotene content in tomatoes averaged from 4.69 to 9.0 mg 100 g^{-1} FM. The highest β-carotene content was found in the cherry tomato cv "Strabena," an average of 8.88 ± 1.58 mg 100 g^{-1} FM, but the lowest β-carotene content was found in the yellow fruit cv "Bolzano," an average of 5.45 ± 1.45 mg 100 g^{-1} FM.

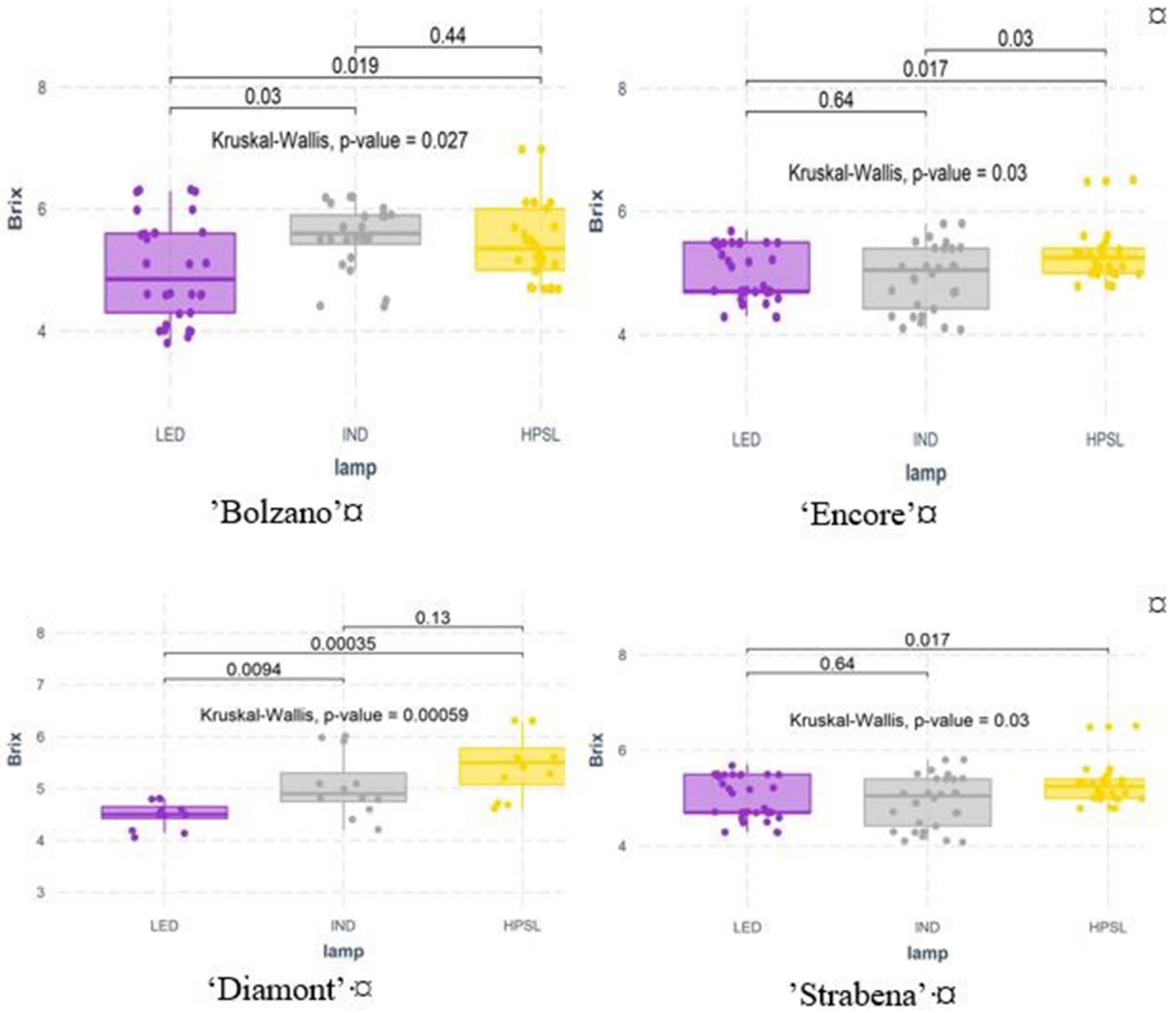


FIGURE 6 | Soluble solids (Brix) in tomato fruits grown under different supplemental light sources.

The significant differences in carotene content were found between varieties grown under different supplemental lighting. Cv "Bolzano" grown under LED shows a significant decrease in carotene content (by 18.5% compared to HPSL), while "Chocomate" has the lowest carotene content just below HPSL in tomato fruit (5.32 ± 1.08 mg 100 g FM^{-1}) and it was increased by 34.3% under LED and 46.4 % under IND lamps (**Figure 8**).

Total Phenolics and Flavonoids Content

The phenol content of tomato fruits varies on average from 27.64 to 56.26 mg GAE 100 g^{-1} FM (**Table 2**). The highest phenol content is observed for the variety "Strabena" and the lowest phenol content is observed for the variety "Diamont." The phenol content of tomatoes varies according to the ripening season of the fruit, so there are large fluctuations between different sampling times. This leads to the fact that the differences between the tomatoes grown under different lamps are not significant.

Although significant differences between the supplemental light variants appear only in the case of the cv "Chocomate," the average flavonoid content of fruits grown under the lamp is by 33.3%, but below the LED by 13.3% higher. Under IND lamps, large differences between varieties are observed, but below LED the variability is in the range of 10.3–15.6%.

Experiments have shown that different tomato varieties react differently to the supplemental lighting used.

It is not recommended to grow cv "Bolzano" under LED or IND lamp because in this lighting, the parameters are similar to those obtained under HPSL or significantly lower. Under LED lamps, the weight of one fruit, dry matter, soluble solids content, and carotene are significantly reduced (**Figure 9**).

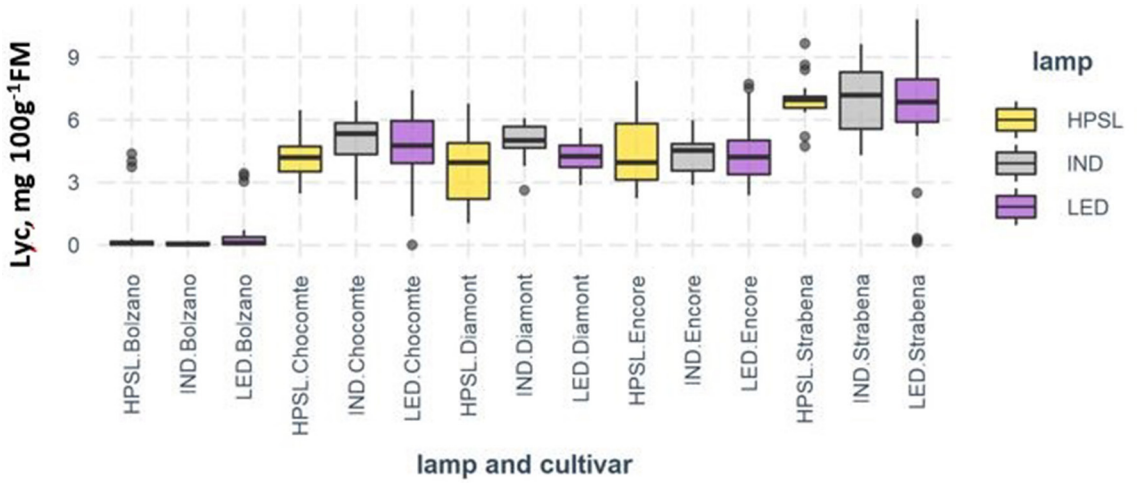


FIGURE 7 | Lycopene content (mg 100 g^{-1} FM) in tomato fruits grown under different supplemental light sources.

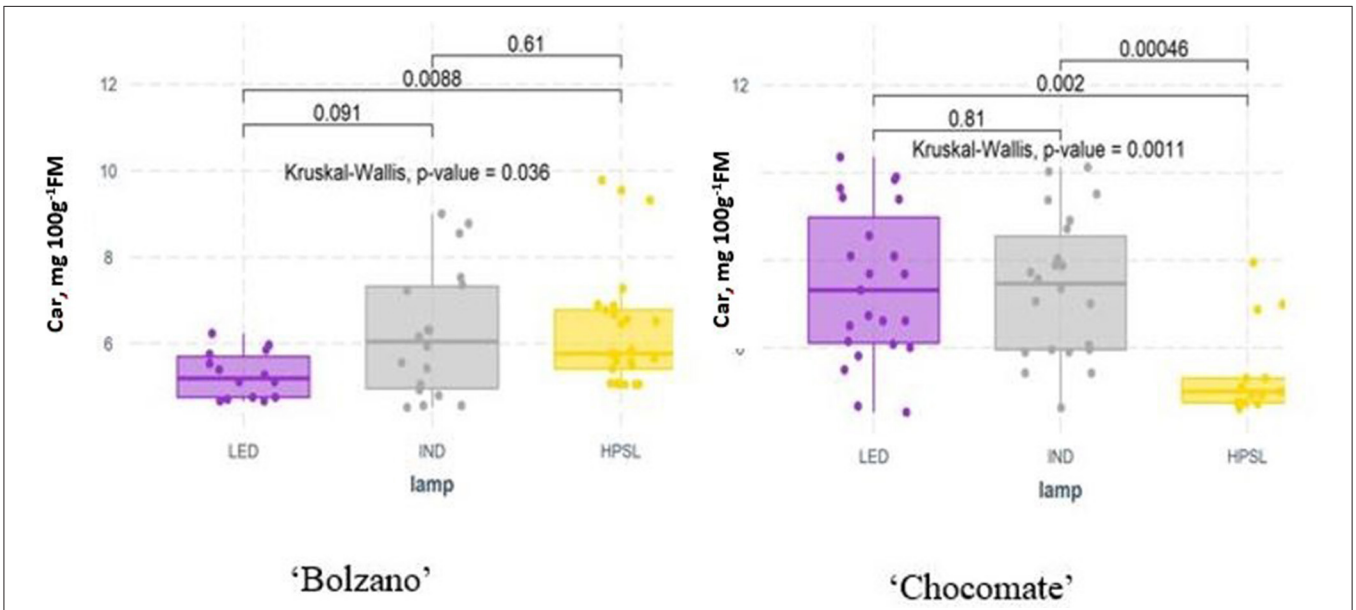


FIGURE 8 | β-carotene content (mg 100 g^{-1} FM) in tomatoes cv 'Bolzano' and 'Chocomate' fruits grown under different supplemental light sources.

TABLE 2 | Content of total phenolics [mg gallic acid equivalent (GAE) 100 g^{-1} FM] and flavonoids [mg citric acid (CA) 100 g^{-1} FM] in the tomato fruits grown under different supplemental lighting.

Parameter	"Bolzano"	"Chocomate"	"Encore"	"Diamont"	"Strabena"
Phenols					
HPSL	36.33 ± 5.34	31.23 ± 5.67	27.64 ± 7.12	30.26 ± 5.71	48.70 ± 11.24
IND	33.21 ± 4.05	34.77 ± 6.39	31.00 ± 6.02	30.63 ± 5.11	56.26 ± 13.59
LED	36.16 ± 6.41	31.70 ± 6.80	30.44 ± 3.01	30.98 ± 6.52	52.57 ± 10.41
Flavonoids					
HPSL	4.50 ± 1.32	3.78 ± 0.65a	2.65 ± 1.04	2.57 ± 1.15	5.17 ± 2.33
IND	4.57 ± 0.75	5.24 ± 0.79b	4.96 ± 1.46	2.84 ± 0.67	6.65 ± 1.64
LED	4.96 ± 1.08	4.37 ± 1.18ab	3.02 ± 1.04	2.88 ± 1.08	5.91 ± 1.20

Significantly different means are labeled with different letters.

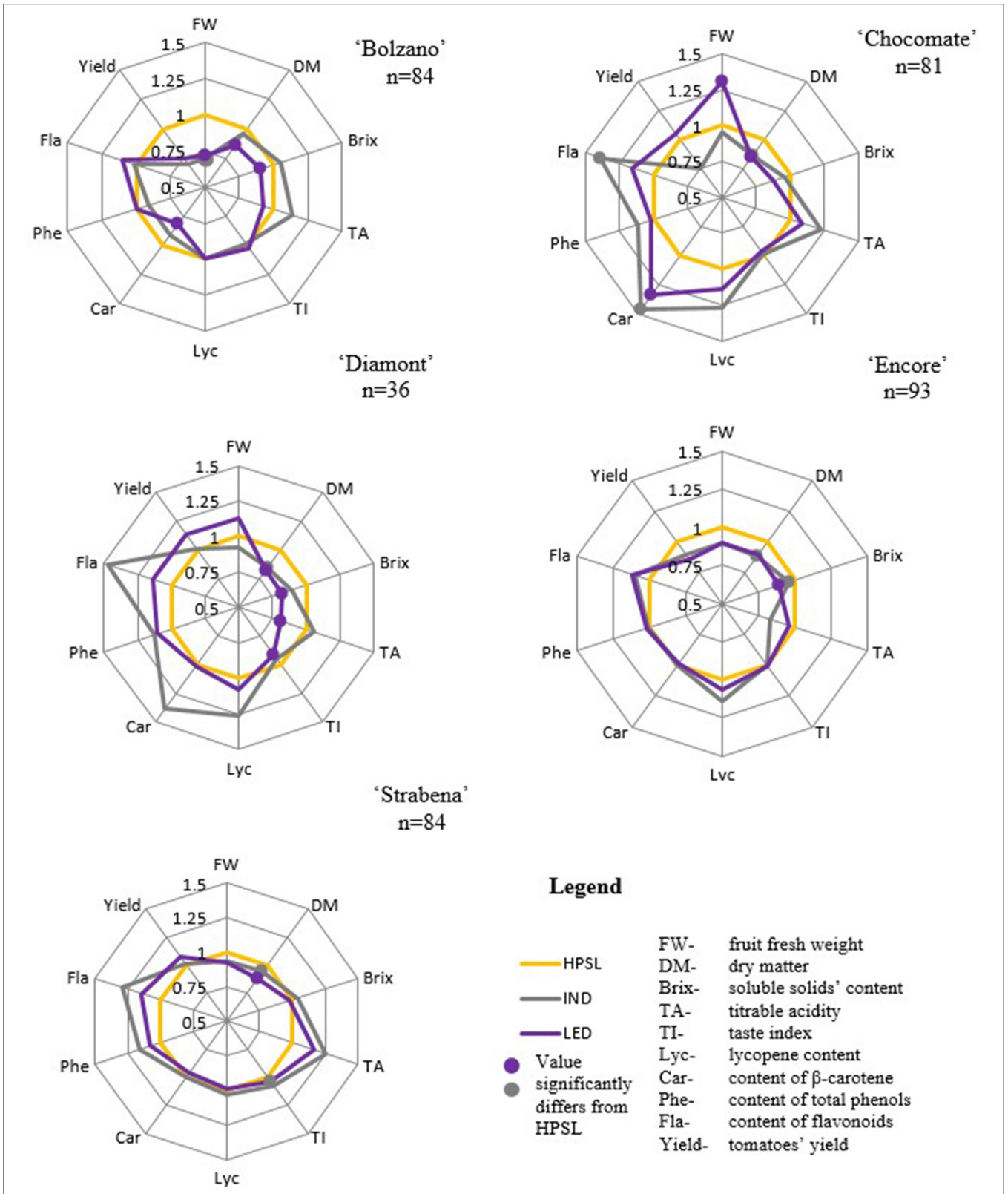


FIGURE 9 | Ratio of the obtained parameters under supplemental light-emitting diode (LED) and induction (IND) lamps to high-pressure sodium lamp (HPSL).

Unlike "Bolzano," "Chocomate" under LED lighting increases the weight of one fruit and the amount of carotene increases. Other parameters excluded dry matter and soluble solids content are also higher than in fruits obtained under HPSL. In the case of this variety, the induction lamp also shows good results (**Figure 9**).

For the cv "Diamont," the indicators that determine the taste properties are significantly reduced under LED light, but the content of pigments and flavonoids is increased (**Figure 9**).

Cultivars "Encore" and "Strabena" are the most unresponsive to supplemental light treatment. For "Encore," the only parameter significantly affected by the LED light spectrum is the soluble solids content. "Strabena" is also relatively tolerant on the changes in the spectral composition of light. This could be due to the genetic characteristics of the variety, as this was the only cherry tomato variety included in the experiment. It was characterized by significantly higher all the studied parameters. Therefore, it was not possible to detect changes in the studied parameters under the influence of light (**Figure 9**).

DISCUSSION

The average weight of the tomato fruit correlates with the intended weight of the variety; though, it is not achieved. This could be due to the cultivation method rather than the quality of the lighting, as less water can be used in a peat substrate, which may reduce the weight of the fruit, but increase the concentration of the active substances and improve the saturation of the taste (24). The smallest fluctuation of the average fruit weight of the "Encore F1" as a result of the lighting source could indicate a tolerance of this variety to quality of lighting. This corresponds with the review of the subject (25). The yield and quality of tomatoes are affected not only by the intensity of the supplemental light used, but also by its quality. Results show that lesser yield formed under IND lamps. However, it could be possible that lesser results showed due to smaller intensity of induction lamps in spite of the fact that main feature of induction lamps is broader green waves band. The data shows that the increase in the amount of red light contributes to the increase in the fresh weight of the tomatoes, but does not affect the increase in the dry matter content. It seems that the red light has stimulated the increase in the water content in the tomatoes. In contrast, the increase in blue light reduces the dry matter content of all tomato varieties. The least sensitive is yellow tomatoes cultivar "Balzano". Several researches showed that photosynthesis under a combination of red and blue light tends to be higher than under HPS lighting, but fruit yield is equal (12). Olle and Virsile (26) found that red LEDs enhance tomatoes yield and that underlines findings of our research that states that generally with higher addition of red waves increases yield. In similar opinion, Zhang et al. (14) defines that even adding FR light in combination with red LEDs and HPSL increases total fruit number. Supplemental blue and red LED light resulted in the early ripening of tomato fruit. This could indicate that reason for higher fruit mass under LEDs for "Chocomate F1" and "Diamont F1" cultivars, since early ripening led to earlier setting of new fruits. In terms of yield, our data show that it is not the increase in red light that is more important in increasing yields, but the increased proportion of red light over blue light.

Since one of the beloved trait of tomato of the customer is sweetness, it is important to understand the possible ways of enhancing this feature. Nevertheless, it is usually altered by various environmental factors (27). There are evidences that the qualitative composition of light also affects the biochemical content of tomato fruit. Soluble sugar contents of the ripe tomato fruit were decreased by longer FR light durations (15). Kong et al. (16) results showed that blue light treatment significantly led to more total soluble solids. Sugar contents in plants are increased by green, blue and red light (28). Our experiments do not confirm that, because increasing of both blue and red light separately reduced the soluble solids content in most cases. Our results showed that the highest level of soluble sugars were found under HPSL which brings the largest proportion of red light than other lamps and also raises the temperature near the lamps. This correspondences with earlier researches where studies of Erdberga et al. (29) showed that content of soluble sugars, organic acids increase with increasing red waves doses. Similar results were obtained in other studies. A higher mean tomato fruit weight was obtained in plants supplementary lighted with HPS lamps as compared to plants from LED lamps (8.7–12.2% depending on cultivar) (30).

However, studies of Dzakovich et al. (31) proved that supplemental light quality (HPSL *via* LEDs) did not significantly affect the physicochemical (total soluble solids, titratable acidity, ascorbic acid content, pH, total phenolics, and prominent flavonoids and carotenoids) or sensory properties of greenhouse-grown tomatoes. This shows that the amount of soluble sugars in fruits can be affected not only by individual factors, but also by their combinations. Also in our experiments it was not possible to find regularities between influences of light on the acid content. In particular, future research should focus not only on the relationship between species and light, but also on the relationship between cultivar and light. Dry matter content was higher in "Chocomate F1" and "Strabena F1." This corresponds with Kurina et al. (6), where on average, the red-brown accessions accumulated more dry matter (6.46%). Studies of Duma et al. (32) showed that when comparing fruits mass and TI, it is observed that higher TI is for smaller or bigger tomatoes. Experiments of Rodica et al. (23) showed that cherry and brownish red-colored tomatoes contain more soluble solids. In this study, it is underlined that quantity of the organic compounds determining the fruit taste depends on the yield of the cultivar.

The exposure to supplementary red and blue LED lighting increases the lycopene and β-carotene content (13, 29, 33, 34). Dannehl et al. (12) studies have shown that lycopene and lutein contents in tomatoes were 18 and 142% higher when they were exposed to the LED fixture. However, β-carotene content was not different between the light treatments. Ntagkas et al. (35) showed that zeaxanthin, the product of β-carotene conversion,

increases in tomato fruits under blue and white light. In this study, these statements partly are true only in case of "Bolzano F1" where significantly larger amount of lycopene were found under LED treatment, but β-carotene did respond negatively to this treatment. This could be due to genetic features since "Bolzano F1" is only orange-fruited cultivar in this study. In other studies, with red-fruited and brown cultivars, highest amount of lycopene and β-carotene were found under Induction lamps which do not confirm the trends of previous years (29). Our experiments showed that the lycopene content of all red fruit tomato cultivars increased with increasing of blue light. In contrast, changes in carotene content in different cultivars fail to establish regularities common to all tomato cultivars used in the experiments. This discrepancy points to the need for additional testing of subject in the future. Same pattern of response to light due to cultivar features was observed with amount of phenols and flavonoids. All the red-fruited and brown-fruited cultivars showed better results under IND lamps, while "Bolzano F1" responded with higher results to HPSL and LED lamps with no significant difference. This study corresponds with the findings of Kong: the blue light treatment significantly led to more concentration of individual phenolic compounds (chlorogenic acid, caffeic acid, and rutin) (16). Continuous red light significantly increased lycopene, β-carotene, total phenolic content, total flavonoid concentration, and antioxidant activity in tomatoes (36). In our earlier studies, flavonoids changed fluctuating; therefore, no effects of light wavelength should be noted as significant.

The amount of phenols increased with the growing proportion of blue light provided by LED lamps (29), this corresponds also with our research. It is mentioned in other researchers' works that exposure to either UV or LED light had no effect on total phenolic compounds, despite the fact that both the light treatments are known to modulate the expression of an array of genes involved in the biosynthesis of phenolic compounds and carotenoids (36). There should be mentioned that similarly with the weight of the fruit, there are no significant differences in chemical compounds in "Encore F1" due to light treatment. This allows to declare that cultivar "Encore F1" could be tolerant to composition of light. Our experiments confirm the literature data that the synthesis of secondary metabolites is enhanced by both the quantitative amount of blue light and the increased proportion of blue light in the overall lighting system.

The results obtained show that the chemical components, including the acid-soluble sugars and their ratio, which are responsible for the characteristic taste of the variety, depend primarily on the genetics of the variety. The good taste of tomatoes is characterized not only by the combination of species-specific pigments and biologically active substances, but also by their amount. In particular, the ratio and quantity of acids and sugars characterize the saturated and high-quality taste. In this study, the positive correlation between soluble sugars and titratable acids is ∼0.4, which is correlated with research of Hernández Suárez, where the positive correlation between the two indicators was found to be 0.39 (37). In studies of Dzakovich et al. (31), tomatoes were profiled for total soluble solids, titratable acidity, ascorbic acid content, pH, total phenolics, and prominent flavonoids and carotenoids. Their studies indicated that greenhouse tomato fruit quality was only marginally affected by supplemental light treatments. Moreover, consumer sensory panel data indicated that tomatoes grown under different lighting treatments were comparable across the lighting treatments tested. Study suggested that the dynamic light environment inherent to greenhouse production systems may nullify the effects of wavelengths of light used in their studies on specific aspects of fruit secondary metabolism (31). This is partly in line with this study, as the figures obtained do not show clear and unambiguous trends, which allow us to say that one of the lighting is more useful for tomatoes than the others. However, certain lamps may be used for certain varieties, for example, HPSL lamps would be more suitable for "Bolzano F1" and LED lighting is recommended for "Chocomate F1." This corresponds with study were effect of different geographical latitudes on the chemical properties of tomatoes was studied. Bhandari et al. (38) clarified that while the combination of the position of the sun toward the sky and, consequently, the combination of visible light waves, it plays an important role in changing the chemical composition of tomatoes; there are varieties that are immune to these processes. All these conclusions allow to underline that chemical composition of tomato is primarily dependent on genotype, since cultivars relationships with growing factors, particularly with lighting, are genetically predisposed.

CONCLUSION

Different tomato varieties react differently to the supplemental lighting used. Cultivars "Encore" and "Strabena" are the most unresponsive to supplemental light. For "Encore," the only parameter significantly affected by the LED light spectrum is the soluble solids content. "Strabena" is also relatively tolerant on the changes in the spectral composition of light. This could be due to the genetic characteristics of the variety, as this was the only cherry tomato variety included in the experiment. It is not recommended to grow orange color fruit cv "Bolzano" under LED or IND lamp because in this lighting, the parameters are at the level of HPSL or significantly worse. Under LED lamps, the weight of one fruit, dry matter, soluble solids content, and β-carotene are significantly reduced. The one fruit weight and the amount of β-carotene of red-brown color fruit cv "Chocomate" under LED lighting significantly increases. Other parameters excluded dry matter and soluble solids content are also higher than in fruits obtained under HPSL.

Experiments have shown that HPSL stimulates the accumulation of primary metabolites in tomato fruit. In all the cases, soluble solids content was 4.7–18.2% higher as compared to other lighting sources.

As LED and IND lamps emit about 20% blue-violet light, the results suggest that this part of the spectrum stimulates the accumulation of phenolic compounds in the fruit by 1.6–47.4% compared to HPSL. The content of carotenoids as secondary metabolites depends on both the variety and the light source. Red fruit varieties tend to synthesize more β-carotene under supplemental LED and IND light.

The blue part of the spectrum plays a greater role in ensuring crop quality. An increase or quantification of its proportion in the total spectrum promotes the synthesis of secondary metabolites (lycopene, phenols and flavonoids), leading to a decrease in dry matter and soluble solids content.

Given the large effect of genotypic variability in the tomatoes and light relations, further study should continue to focus on the combinations of cultivars and different supplemental light spectra to increase the content of biologically active compounds.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

IE was incharge of tomatoes cultivation and sampling, laboratory work, compounds quantification, and also contributed to the writing of the manuscript. IA brought up the idea, contributed to the study conception and design, was incharge of tomatoes sampling, laboratory work, compounds quantification, and also contributed to the writing of the manuscript. MD contributed to the study conception and design, optimization of analytical methods, analyzed the samples in the laboratory, and made recommendations and suggestions. RA contributed to the statistical analysis, interpretation of data, and made recommendations and suggestions regarding the manuscript. LD contributed to the study conception and design, was incharge of tomatoes sampling, laboratory work, compounds quantification, and made recommendations and suggestions regarding the manuscript. All authors contributed to the article and approved the submitted version of the manuscript.

FUNDING

This study was funded by the Latvian Rural Development Program 2014-2020 Cooperation, call 16.1 project Nr. 19-00-A01612-000010 Investigation of innovative solutions and new method development for efficiency and quality increase in Latvian greenhouse sector (IRIS).

REFERENCES

1. Vijayakumar A, Shaji S, Beena R, Sarada S, Sajitha Rani T, Stephen R, et al. High temperature induced changes in quality and yield parameters of tomato (Solanum lycopersicum L) and similarity coefficients among genotypes using SSR markers. *Heliyon.* (2021) 7:e05988. doi: 10.1016/j.heliyon.2021.e05988
2. Duzen IV, Oguz E, Yilmaz R, Taskin A, Vuruskan A, Cekici Y, et al. Lycopene has a protective effect on septic shock-induced cardiac injury in rats. *Bratisl Med J.* (2019) 120:919–23. doi: 10.4149/BLL_2019_154
3. Dogukan A, Tuzcu M, Agca CA, Gencoglu H, Sahin N, Onderci M, et al. tomato lycopene complex protects the kidney from cisplatin-induced injury via affecting oxidative stress as well as Bax, Bcl-2, and HSPs expression. *Nutr Cancer.* (2011) 63:427–34. doi: 10.1080/01635581.2011.535958
4. Warditiani NK, Sari PMN, Wirasuta MAG. Phytochemical and Hypoglycemia Effect of Tomato Lycopene Extract (TLE). *Sys Rev Pharm.* (2020) 11:509–14. doi: 10.31838/srp.2020.4.77
5. Ando A. "Taste compounds in tomatoes". In: Higashide T, editor. *Solanum Lycopersicum: Production, Biochemistry and Health Benefits*. New York, Nova Science Publishers (2016). p. 179–187.
6. Kurina AB, Solovieva AE, Khrapalova IA, Artemyeva AM. Biochemical composition of tomato fruits of various colors. *Vavilovskii Zhurnal Genet Selektsii.* (2021) 25:514–27. doi: 10.18699/VJ21.058
7. Murshed R, Lopez-Lauri F, Sallanon H. Effect of water stress on antioxidant systems and oxidative parameters in fruits of tomato (Solanum lycopersicon L, cvMicro-tom). *Physiol Mol Biol Plants.* (2013) 19:363–78. doi: 10.1007/s12298-013-0173-7
8. Klunklin W, Savage G. Effect of quality characteristics of tomatoes grown under well-watered and drought stress conditions. *Foods.* (2017) 6:56. doi: 10.3390/foods6080056
9. Chetelat RT, Ji Y. Cytogenetics and evolution. *Genetic Improv Solanaceous Crops.* (2007) 2:77–112. doi: 10.1201/b10744-4
10. Wang W, Liu D, Qin M, Xie Z, Chen R, Zhang Y. Effects of supplemental lighting on potassium transport and fruit coloring of tomatoes grown in hydroponics. *Int J Mol Sci.* (2021) 22:2687. doi: 10.3390/ijms22052687
11. Ouzounis T, Giday H, Kjær KH, Ottosen CO. LED or HPS in ornamentals? A case study in roses and campanulas. *Eur J Hortic Sci.* (2018) 83:166–72. doi: 10.17660/eJHS.2018/83.3.6
12. Dannehl D, Schwend T, Veit D, Schmidt U. Increase of yield, lycopene, and lutein content in tomatoes grown under continuous PAR spectrum LED lighting. *Front Plant Sci.* (2021) 12:611236. doi: 10.3389/fpls.2021.611236
13. Xie BX, Wei JJ, Zhang YT, Song SW, Su W, Sun GW, et al. Supplemental blue and red light promote lycopene synthesis in tomato fruits. *J Integr Agric.* (2019) 18:590–8. doi: 10.1016/S2095-3119(18)62062-3
14. Zhang JY, Zhang YT, Song SW, Su W, Hao YW, Liu HC. Supplementary red light results in the earlier ripening of tomato fruit depending on ethylene production. *Environ Exp Bot.* (2020) 175:10404. doi: 10.1016/j.envexpbot.2020.104044
15. Zhang Y, Zhang Y, Yang Q, Li T. Overhead supplemental far-red light stimulates tomato growth under intra-canopy lighting with LEDs. *J Integr Agric.* (2019)18:62–9. doi: 10.1016/S2095-3119(18)62130-6
16. Kong D, Zhao W, Ma Y, Liang H, Zhao X. Effects of light-emitting diode illumination on the quality of fresh-cut cherry tomatoes during refrigerated storage. *Int J Food Sci Technol.* (2021) 56: 2041–52. doi: 10.1111/ijfs.14836
17. Jarquín-Enríquez L, Mercado-Silva EM, Maldonado JL, Lopez-Baltazar J. Lycopene content and colour index of tomatoes are affected by the greenhouse cover. *Sc Horticulturae.* (2013) 155:43–8. doi: 10.1016/j.scienta.2013.03.004
18. Wahid A, Gelani S, Ashraf M, Foolad MR. Heat tolerance in plants: an overview. *Environ Exp Bot.* (2007) 61:199–223. doi: 10.1016/j.envexpbot.2007.05.011
19. Duma M, Alsina I. The content of plant pigments in red and yellow bell peppers. *Sci Pap B Horticulture.* (2012) 56:105–8.
20. Nagata M, Yamashita I. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *J Jpn Food Sci Technol.* (1992) 39:925–8. doi: 10.3136/nskkk1962.39.925
21. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* (1999) 299:152–78. doi: 10.1016/S0076-6879(99)99017-1
22. Kim D, Jeond S, Lee C. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem.* (2003) 81:321–6. doi: 10.1016/S0308-8146(02)00423-5
23. Rodica S, Maria D, Alexandru-Ioan A, Marin S. The evolution of some nutritional parameters of the tomato fruit during the

harvesting stages. *Hort Sci.* (2019) 46:132–7. doi: 10.17221/222/2017-HORTSCI
24. Máté MD, Szalókiné Zima I. Development and yield of field tomato under different water supply. *Res J Agric Sci.* (2020) 52:167–77.
25. Mauxion JP, Chevalier C, Gonzalez N. Complex cellular and molecular events determining fruit size. *Trends Plant Sci.* (2021) 26:1023–38. doi: 10.1016/j.tplants.2021.05.008
26. Olle M, Alsina I. Influence of wavelength of light on growth, yield and nutritional quality of greenhouse vegetable. *Proc Latvian Acad Sci B.* (2019) 73:1–9. doi: 10.2478/prolas-2019-0001
27. Kawaguchi K, Takei-Hoshi R, Yoshikawa I, Nishida K, Kobayashi M, Kushano M, et al. Functional disruption of cell wall invertase inhibitor by genome editing increases sugar content of tomato fruit without decrease fruit weight. *Sci Rep*. (2021) 11:1–12. doi: 10.1038/s41598-021-00966-4
28. Olle M, Viršile A. Influence of wavelenght of light on growth, yield and nutritional quality of greenhouse vegetables. *Agricult Food Sci.* (2013) 22:223–34. doi: 10.23986/afsci.7897
29. Erdberga I, Alsina I, Dubova L, Duma M, Sergejeva D, Augšpole I, et al. Changes in the biochemical composition of tomato fruit under the influence of illumination quality. *Key Eng Mater.* (2020) 850:172–8. doi: 10.4028/www.scientific.net/KEM.850.172
30. Gajc-Wolska J, Kowalczyk K, Metera A, Mazur K, Bujalski D, Hemka L. Effect of supplementary lighting on selected physiological parameters and yielding of tomato plants. *Folia Horticulturae.* (2013) 25:153–9. doi: 10.2478/fhort-2013-0017
31. Dzakovich M, Gómez C, Ferruzzi MG, Mitchell CA. Chemical and sensory properties of greenhouse tomatoes remain unchanged in response to red, blue, and far red supplemental light from light-emitting. *Hortscience.* (2017) 52:1734–41. doi: 10.21273/HORTSCI12469-17
32. Duma M, Alsina I, Dubova L, Augspole I, Erdberga I. Suggestions for consumers about suitability of differently coloured tomatoes in nutrition. In: *FoodBalt 2019: Proceedings of 13th Baltic Conference on Food Science and Technology; 2019 May 2-3*. Jelgava, Latvia: LLU (2019). p. 261–4.
33. Ngcobo BL, Bertling I, Clulow AD. Preharvest illumination of cherry tomato reduces ripening period, enhances fruit carotenoid concentration and overall fruit quality. *J Hortic Sci Biotechnol.* (2020) 95:617–27. doi: 10.1080/14620316.2020.1743771
34. Nájera C, Guil-Guerrero JL, Enríquez LJ, Álvaro JE, Urrestarazu M. LED-enhanced dietary and organoleptic qualities in postharvest tomato fruit. *Postharvest Biol Technol.* (2018) 145:151–6. doi: 10.1016/j.postharvbio.2018.07.008
35. Ntagkas N, de Vos RC, Woltering EJ, Nicole C, Labrie C, Marcelis L F. Modulation of the tomato fruit metabolome by LED light. *Metabolites.* (2020) 10:266. doi: 10.3390/metabo10060266
36. Baenas N, Iniesta C, González-Barrio R, Nuñez-Gómez V, Periago MJ, García-Alonso FJ. Post-Harvest Use of Ultraviolet Light (UV) and Light Emitting Diode (LED) to enhance bioactive compounds in refrigerated tomatoes. *Molecules.* (2021) 26:1847. doi: 10.3390/molecules26071847
37. Hernández Suárez M, Rodriguez ER, Romero CD. Analysis of organic acid content in cultivars of tomato harvested in Tenerife. *Eur Food Res Technol.* (2008) 226:423–35. doi: 10.1007/s00217-006-0553-0
38. Bhandari HR, Srivastava K, Tripathi MK, Chaudhary B, Biswas S. Shreya Environment×Combining ability interaction for quality traits in tomato (Solanum lycopersicum L.). *Int J Bio-Resour Stress Manage.* (2021) 12:455–62. doi: 10.23910/1.2021.2276

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Alsina, Erdberga, Duma, Alksnis and Dubova. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers | Frontiers in Nutrition

MINI REVIEW
published: 06 April 2022
doi: 10.3389/fnut.2022.868492

Functional and Nutraceutical Compounds of Tomatoes as Affected by Agronomic Practices, Postharvest Management, and Processing Methods: A Mini Review

Giuseppina Pace Pereira Lima[1], Héctor Alonzo Gómez Gómez[2], Santino Seabra Junior[3], Marcelo Maraschin[4], Marco Antonio Tecchio[5] and Cristine Vanz Borges[6]*

[1] Laboratory of Plant Biochemistry, Institute of Biosciences, São Paulo State University (UNESP), Botucatu, Brazil, [2] Academic Department of Food, Faculty of Technological Sciences, National University of Agriculture, Catacamas, Honduras, [3] Department of Agronomy, Mato Grosso State University (UNEMAT), Nova Mutum, Brazil, [4] Plant Morphogenesis and Biochemistry Laboratory, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil, [5] Department of Horticulture, School of Agriculture, São Paulo State University (UNESP), Botucatu, Brazil, [6] Department of Health Sciences, Universidade Alto Vale do Rio do Peixe (UNIARP), Caçador, Brazil

OPEN ACCESS

Edited by:
José Pinela, Polytechnic Institute of Bragança (IPB), Portugal

Reviewed by:
Shimeles Tilahun, Kangwon National University, South Korea
László Csambalik, Hungarian University of Agricultural and Life Sciences, Hungary

***Correspondence:**
Giuseppina Pace Pereira Lima
pace.lima@unesp.br

Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *02 February 2022*
Accepted: *08 March 2022*
Published: *06 April 2022*

Citation:
Lima GPP, Gómez HAG, Seabra Junior S, Maraschin M, Tecchio MA and Borges CV (2022) Functional and Nutraceutical Compounds of Tomatoes as Affected by Agronomic Practices, Postharvest Management, and Processing Methods: A Mini Review. Front. Nutr. 9:868492. doi: 10.3389/fnut.2022.868492

Tomatoes and their by-products are indisputable sources of substances with antioxidants properties. Several factors limit the production and influence the nutritional and antioxidant quality of tomato fruit. However, consumers can benefit from the effects of environmental factors, such as water and hydric stress, UV radiation, agronomic practices, among others, which lead to changes in the content of secondary metabolites in tomatoes. Molecules as phenolic compounds, carotenoids, and biogenic amines are often formed in response to environmental adversities. In this way, the consumption of tomato fruits or their by-products with higher levels of antioxidants may be important adjuvants in the prevention or reduction of diseases. In this mini-review, we will present how pre- and postharvest conditions may influence the content of some bioactive compounds in tomatoes. Furthermore, we will present how some heat processing methods may change the antioxidant content, as well as, the functional and nutritional properties of the final product.

Keywords: carotenoids, phenolic compounds, biogenic amines, postharvest, waste, by-products

INTRODUCTION

Tomatoes are widely used for food and beverages. The tomato crop is the largest vegetable crop in the world after potatoes and sweet potatoes. Tomatoes are an excellent source of nutrients and bioactive compounds important for human health, including phenolic compounds and carotenoids. Factors, including genetics, environmental conditions, ripeness, and postharvest conditions may influence the chemical composition and levels of phytochemicals (1).

Carotenoids, phenolic compounds and nitrogen-containing compounds are classified as non-nutritive phytochemicals. Carotenoids have a structure consisting of 40-carbon (C-40) isoprene units. Isoprenoids constitute a very representative group, which includes lycopene, β-carotene, g-carotene, z-carotene, among others (2) (**Figure 1**). Lycopene is responsible for the red color, and it has higher singlet oxygen quenching potential than β-carotene and α-tocopherol (3).

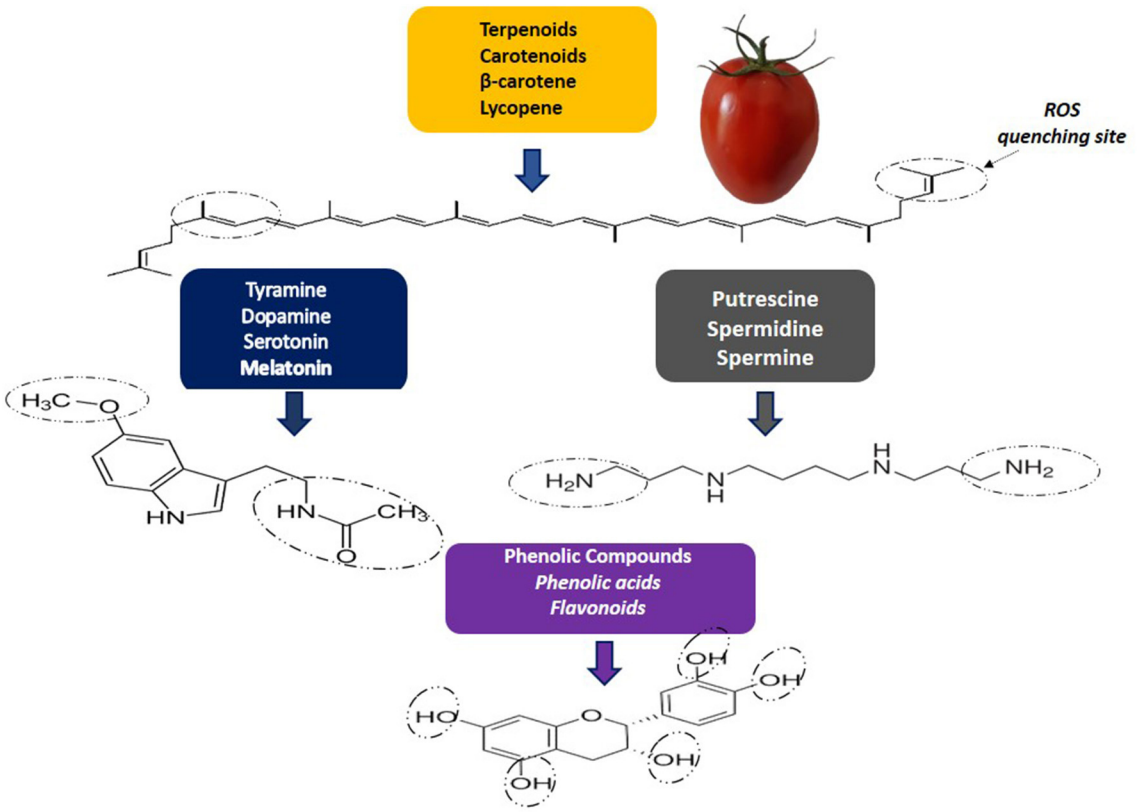


FIGURE 1 | Reactive oxygen species (ROS) by phytochemical quenching.

Phenolic compounds have one or more aromatic rings with one or more hydroxyl groups and during ripening, chlorogenic acid and quercetin have been described as major compounds in tomatoes. In ripened tomato fruit, naringenin chalcone was described as the major polyphenol (4), while caffeic acid is present in higher levels in red and yellow tomatoes (5). The content and profile of phenolic compounds are different in relation to tomato morphology, that is, phenolic acids are found in all tissues, while flavonoids and derivatives are described in the epidermis (6). The consumption of phenolic compounds is important for health, as they act as anti-atherogenic, anti-inflammatory, anti-allergenic, cardioprotective compounds (7) (**Figure 2**).

Biogenic amines (BAs) stand out for their antioxidant activity (**Figure 1**). Some Bas, as spermidine (Spd) and spermine (Spm), can play a protective role against different stresses and are related to the shelf life and quality of tomato fruits (8). Monoamines such as serotonin have also been detected in tomatoes and it was shown content ranging from 132.47 to 266.87 mg/kg, depending on the genotype (9). However, BAs in foods can also have negative impacts on health, especially due to histamine (His) and tyramine (Tyr) content. In tomatoes, His and Tyr may occur at levels ranging from not detected to 17.1 mg/kg and not detected to 6.38 mg/kg, respectively (10). Both His and Tyr were detected in cherry tomatoes but at levels below those considered harmful (His: 0.16–2.26 mg/kg; Tyr: 1.13–1.82 mg/kg) (11). Although other BAs, such as putrescine and cadaverine, do not have a direct toxic effect, they can potentiate the effects of tyramine and histamine, competing with detoxifying enzymes, in addition to being related to the formation of nitrosamines (12).

PREHARVEST FACTORS

The environment in which the crop grows, the relative humidity, temperature, photoperiod, irradiance, soil, and water in combination with seasonal changes play a definitive role in determining the content and the profile of phytochemicals (**Figure 2**). Preharvest factor may promote changes in the postharvest quality in tomatoes. The selection of tomato genotypes with higher nutritional and phytochemical quality can be obtained as a function of the genotype-environment interaction (13). In contrast, in "TY Megaton" and "Yureka," there was a reduction of phenolic compounds at the vine- and postharvest-ripened red stages (14). Variations in the profile of phenolic compounds can also occur under vine- and postharvest-ripening conditions, as well as the concentration of some specific phenolic compounds are dependent on the cultivar (15). In hydroponically grown cherry tomatoes ripened from breaker stage there was a rapid decrease in the flavonoids content (55%) compared to on-vine red ripe (16). Furthermore, the level of antioxidant compounds is different depending on the stage of

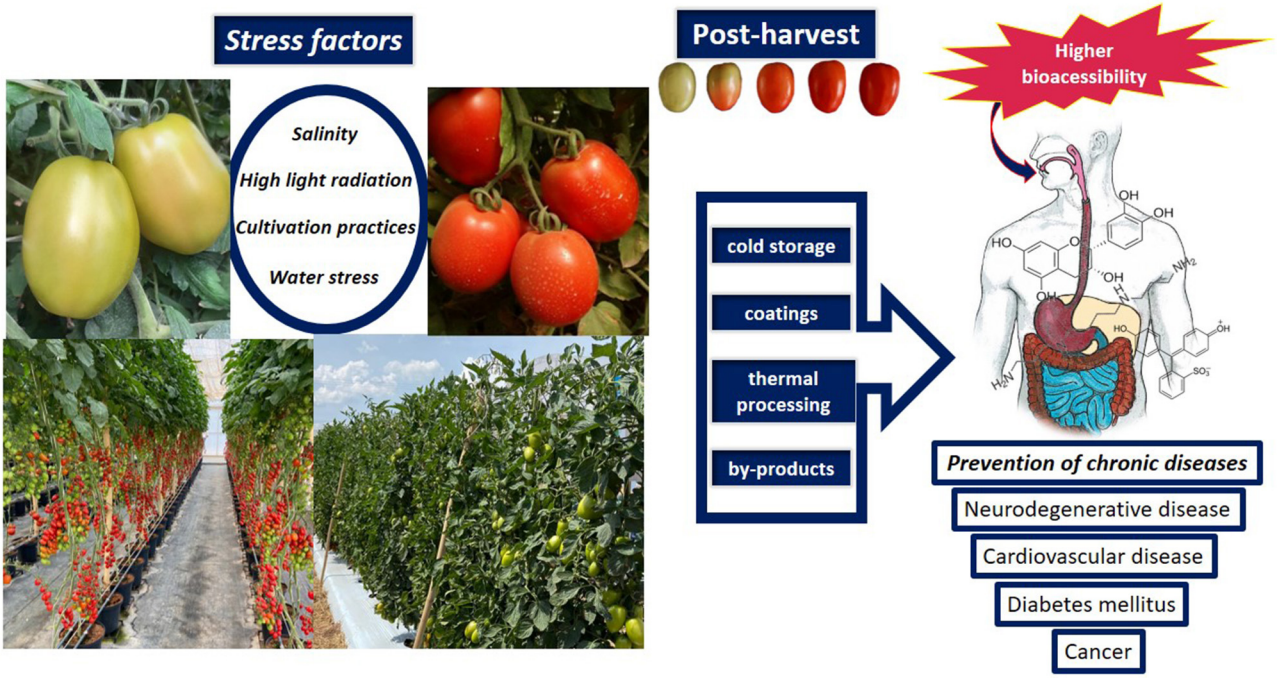


FIGURE 2 | Influence of biotic and abiotic factors on the bioaccessibility and bioavailability of tomato bioactive compounds.

maturity. After the breaker stage, the lycopene content increases reaching maximum values at the ripe-red stage, depending on the cultivar (17).

UV radiation can influence the content of phytochemicals with high antioxidant potential. UV-B (280–315 nm) induces DNA damage and leads to the production of reactive oxygen species. As a defense, the plant may also trigger the production of antioxidant compounds (18). UV-B can alter the citric acid cycle, an important pathway that provides the substrate for the synthesis of phenolic compounds through the phenylpropanoid pathway (19). In greenhouses, UV-B is usually filtered through plastics (opaque to incident UV-B). UV exclusion under a polyvinylchloride (PVC) cover may be responsible for low levels of phenolic compounds in fruits produced in greenhouses (20). Tomatoes grown under a covering that allowed solar UV transmission accumulated more phenols than those grown under a covering material that excluded solar UV (21). In tomatoes grown with doses of supplemental radiation, there was a stimulation of rutin content. However, the use of UV-A or UV-A+B did not influence other phenolic compounds or carotenoids (22).

The temperature during cultivation is a key factor for the levels of some bio-actives. Temperatures during cultivation above 30°C and below 12°C may influence lycopene levels. At higher temperatures, inhibition of lycopene formation can occur and increase the synthesis of other carotenoids, changing the color of the fruits which become more yellow (21, 22). During tomato fruit ripening in high temperature conditions (>30°C) there is an increase in rutin and caffeic acid glucoside (23). In thermotolerant saladette-type tomatoes cultivated at high temperature and in protected cultivation (24), detected high levels of lycopene (up to 2.73 mg/100 g) and β-carotene (1.33 mg/100 g). The consumption of some genotypes may amount to 54.6 and 66.5% of the daily needs of lycopene and β-carotene, considering the minimum recommended (2 mg/day) (25, 26).

Variations in vapor pressure (VP) can affect the growth, flowering and quality of tomato fruits. Low VP (deficit) results in a decrease in growth, a consequence of increased transpiration, in addition to variations in the water potential of the stem, affecting the xylem influx. VP deficit may induce fruit cracking due to the variations in fruit growth and water influx and high VP reduce fruit cracking and promote blossom-end-rot (26). Water stress also directly influences the content of secondary metabolites. It is well-argued that climate change will contribute to increasing water scarcity, and available water resources will need to be used more efficiently. In water deficit, plants tend to present an increase in reactive oxygen species (ROS) and inactivation of enzymes. In water-stressed tomatoes, it was described a decrease in lycopene and β-carotene levels and an increase in chlorogenic acid (27).

Salt stress is currently one of the biggest problems. This occurs due to a lack of water, and it may be a cause of water deficit. In salinity tolerant tomato landraces, there may be an increase in the content of phenolic compounds and carotenoids, as there was an adaptation to the saline condition (28). In saline stress, the increase in the levels of some compounds is due to the osmoregulatory/osmo-protective effect, in addition to scavenging ROS or reactive nitrogen species (RNS) formed

in response to the overproduction of free radicals. BAs can scavenge free radicals, acting as membrane protectors against lipid peroxidation and oxidative stress (29). Tomatoes supplied with 80 or 160 mM NaCl and harvested at the ripe stage showed an increase in serotonin (radical scavenging activity) with average of 6.4 μg/g f.w. (30). In response to water stress, Spd and Spm levels increase in un-grafted, grafted, and self-grafted tomato fruits (31). Depending on the genotype, the content ranged from 0.55 to 2.18 mg/g d.w. Spd and 0.18 to 0.95 mg/g d.w. Spm. In water stress-resistant cherry tomatoes, increase in Spd and Spm levels were related to drought tolerance (32). The increase in Spm may be related to the osmotic potential, stomatal opening and closing, or the stimulation of NO production as a way to mitigate stress (33). Spd is described by having protective effect against cancer and metabolic and neurodegenerative disorders (34) and it has been described as delaying aging in humans (35). It is a "caloric restriction mimetic," inducing biochemical changes similar to caloric restrictions (34). Also, it competes with acetyl CoA for binding to the catalytic site of EP300, decreasing its activity (36) and demonstrating the anticoagulant action of Spd (37).

The content of Spd, Spm, His, and total BAs content were highest in organically grown tomatoes. Hydroponically cultivated cherry tomatoes showed higher levels of phenolic compounds (817.66 mg/kg), total BAs (415.31 mg/Kg), and BAs index (BAI) (applied to determine the loss in quality) (6.63) when compared to fruits from conventional cultivation (total BA = 409.28 mg/Kg and BAI = 1.20) and to organic cultivation (total BA = 186.62 mg/kg and BAI = 0.73) (11). Regardless of the type of cultivation, serotonin was the major BAs, ranging between 167.41 to 385.96 mg/Kg. In tomato ketchup, histamine levels are slightly elevated, i.e., 22 mg/kg (38). Comparatively, the levels of His (0.16 to 2.26 mg/kg) were below the levels considered harmful to humans (500 ppm) (39) or 25 to 50 mg of His per healthy person per meal, established using non-observed adverse effect level (NOAEL) (12). It is worth noting that food intake increases the bioavailability of His, as endogenous His is synthesized from L-histidine by enzyme-dependent histidine decarboxylation in mast cells and basophils, among others (40). When an immune or non-immune stimulus occurs, promoted by viruses or other pathogens, His is released in the body. Some studies suggest that the use of antihistamines may be useful in the treatment of patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (41). The levels of Tyr in tomatoes do not appear to be harmful to human health. Studies describe levels between 0.12 to 0.18 gm/100 g (42), while other describe contents ranging from not detected to 0.31 mg/100 g (43). For a safe diet, Tyr levels should not exceed 600 mg for healthy individuals. For those who are taking a monoamine oxidase inhibitor (MAOI), levels should not exceed 50 mg (MAOI—third generation) and 6 mg for people taking classical MAOI (10).

POSTHARVEST FACTORS

Storage and Postharvest Treatments

Removal of field heat is important as it allows longer shelf life of tomato fruits (**Figure 2**), as well as storage at low temperatures (4 to 12°C) enhance shelf-life and maintains the nutritional quality (44). Frost/freezing and chilling injuries should be avoided as they promote damage, such as low lycopene (45) and high putrescine content (46), the formation of ice crystals and damage to cell integrity, blotchy coloration (dependent on temperature and exposure time), in addition to phenolic oxidation induced by the release of polyphenoloxidase of vacuole. Storage at low or room temperatures did not influence the serotonin content (30), while exogenous melatonin in tomatoes confer chilling tolerance (47) through proline activation and NO biosynthesis (47).

The edible chitosan coating on tomato fruits induces changes in the anthocyanins and flavonoids, in addition to modifying the antioxidant capacity (48). Tomatoes treated with different concentrations of gum Arabic used as an edible coating did not show an increase in storage life, but treatment with 10% gum Arabic was efficient in maintaining the levels of phenolic compounds, carotenoids, and antioxidant activity until the 10th day of storage at 20°C (49). Due to the content and diversity of bioactives in tomatoes, they have been used as adjuvants in edible coatings. Gelatin coatings incorporated with tomato oily extracts were efficient for preserving the phytochemicals in garambullo fruits (*Myrtillocactus geometrizans*). In addition, they delayed weight loss as well as changes in pH and soluble solids, among others (50).

Natural preservative agents can be a safe option against the harmful effects of chemical residues applied in postharvest. Exogenous melatonin in postharvest may inhibit the senescence and be related to anthocyanin accumulation in ripened tomato (51), besides from inducing the endogenous melatonin levels (49, 52) and phenolic compounds (52, 53).

Thermal Processing

Heat processing increases the bioavailability and bio-accessibility of carotenoids because, as already mentioned, there is a disintegration of the cell wall and organelle membranes where carotenoids are located (54). Heat denatures the protein-carotenoid complexes that limit the bio-accessibility of carotenoids, favoring their release from the food matrix (55). Thus, thermal processing has a direct effect on the profile and amount of carotenoids in tomatoes. The content of lycopene in raw tomatoes is ∼2 mg of *t*-lycopene/g, and heating (88°C) can induce increases above 150% (52). An increase in *cis*-lycopene levels has also been described by the authors, although in a lower percentage and varying as a function of cooking time at temperatures below 100°C. At temperatures between 130 and 140°C, a decrease in *t*-lycopene content occur due to its degradation (53). It is noteworthy that in tomatoes, the bioavailability of lycopene in processed products is higher than in fresh (56).

TOMATO BY-PRODUCTS

Agriculture is one of the biggest generators of waste, representing millions of tons of lost and wasted food resulting from the production and sales for consumption *in natura* (57). Every year, tomato production generates a considerable amount of vegetable by-products of no commercial value, resulting from several stages which include tomato field waste before harvest and postharvest and from home or industrial processes. It is

currently unthinkable that such losses could occur, given the current and future needs of the human population and the finite resources our planet has. In this sense, the use of by-products is fundamental. This is especially true for tomatoes due to their nutritional and phytochemical importance. The residues could be used for the elaboration of new products, such as flours, or be used for the production of new polysaccharides with antioxidant and anticytotoxic activity and for production of biofilms (58).

For the flour, the ideal temperature *vs.* time must be used for the drying process so that the nutritional and phytochemical quality of the product is preserved. Using field waste and tomato fruit in the late stage of production, it was found that the best drying time and temperature was 55°C for 120 min using forced convection oven (59). The study also analyzed the lyophilized product. Authors detected 11.26 μg/mg of lycopene and 162.821 μg/mg of phenolic compounds. In the flour, kaempferol and lycopene were the major compounds detected (1.09 and 11.26 μg/mg, respectively). Among the phenolic compounds, those that appeared at the highest levels were naringerin (90.04 μg/mg) from oven-dried and catechin (255.03 μg/mg) from lyophilized by-product. In dried tomato waste, (60) detected ellagic and chlorogenic acids (143.4 and 76.3 mg/kg), and lycopene (510.6 mg/kg) and β-carotene (95.6 mg/kg) were the most abundant phenolic compounds and carotenoids. Syringic acid has been described in tomato processing by-products. It has antimicrobial activity against *Staphylococcus aureus* (61).

Phytochemicals may be unstable in response to processing conditions such as high temperatures, acidity, oxidation, light, solubility, among others that are used and it is important to study ways to protect the extraction or incorporation of bio-actives in foods to avoid loss of functionality (62). Microencapsulation has shown positive effects for incorporation of bioactive by-products in food. The addition of lycopene in spices increases the antioxidant activity and improves the stability of the bioactive (63).

CONCLUSION

The beneficial effects of tomato and tomato-based products are closely related to the presence and abundance of various biologically active compounds. Agricultural practices may favor the accumulation of phytochemicals. Cultivation techniques, such as the selection of genotypes with target metabolites (e.g. high lycopene or serotonin content), temperature and vapor pressure control and water stress could be used for the production of tomatoes with superior quality and, consequently, for the development of derived products with superior amounts of beneficial compounds. Simple techniques (e.g., cooking) may facilitate the extraction and increase the bio-accessibility and bioavailability of compounds related to the reduction and/or prevention of metabolic and cardiovascular diseases and improve the functioning of the immune system. The study of management techniques in the pre- and postharvest of tomatoes, as well as the by-products generated during the productive system of the culture, becomes essential to guarantee for the population safe food with ideal quality for consumption, in addition to helping to avoid and/or reduce the frequency of food and health crises.

AUTHOR'S NOTE

The mechanisms involved in tomato fruit quality have been extensively investigated by physiologists and geneticists, and the responses to climatic and cultural practices have been widely described. Yet, our ability to manage and improve fruit quality in a context of global change will rely on our capacity to integrate knowledge's and anticipate interactions among genotype, environment and cultural practices.

AUTHOR CONTRIBUTIONS

GL and CB contributed to the conception, writing and editing of the manuscript. HG, SS, MM, and MT contributed to the writing. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

GL, MM, and MT would to thank National Council for Scientific and Technological Development, Brazil (CNPq) (grants 307571/2019-0, 304657/2019-0 and 305724/2018-5) and PROPG UNESP (Edital 01/2022).

REFERENCES

1. Coyago-Cruz E, Corell M, Moriana A, Hernanz D, Benítez-González AM, Stinco CM, et al. Antioxidants (carotenoids and phenolics) profile of cherry tomatoes as influenced by deficit irrigation, ripening and cluster. *Food Chem.* (2018) 240:870–84. doi: 10.1016/j.foodchem.2017.08.028
2. Salehi B, Sharifi-Rad R, Sharopov F, Namiesnik J, Roointan A, Kamle M, et al. Beneficial effects and potential risks of tomato consumption for human health: an overview. *Nutrition.* (2019) 62:201–8. doi: 10.1016/j.nut.2019.01.012
3. Chaudhary P, Sharma A, Singh B, Nagpal AK. Bioactivities of phytochemicals present in tomato. *J Food Sci Technol.* (2018) 55:2833–49. doi: 10.1007/s13197-018-3221-z
4. Martí R, Roselló S, Cebolla-Cornejo J. Tomato as a source of carotenoids and polyphenols targeted to cancer prevention. *Cancers (Basel).* (2016) 8:1–28. doi: 10.3390/cancers8060058
5. Georgé S, Tourniaire F, Gautier H, Goupy P, Rock E, Caris-Veyrat C. Changes in the contents of carotenoids, phenolic compounds and vitamin C during technical processing and lyophilisation of red and yellow tomatoes. *Food Chem.* (2011) 124:1603–11. doi: 10.1016/j.foodchem.2010.08.024
6. Moco S, Capanoglu E, Tikunov Y, Bino RJ, Boyacioglu D, Hall RD, et al. Tissue specialization at the metabolite level is perceived during the development of tomato fruit. *J Exp Bot.* (2007) 58:4131–46. doi: 10.1093/jxb/erm271
7. Shahidi F, Varatharajan V, Oh WY, Peng H. Phenolic compounds in agri-food by-products, their bioavailability and health effects. *J Food Bioact.* (2019) 5:57–119. doi: 10.31665/JFB.2019.5178

8. Kamiab F, Tavassolian I, Hosseinifarahi M. Biologia futura: the role of polyamine in plant science. *Biol Futur.* (2020) 71:183–94. doi: 10.1007/s42977-020-00027-3
9. Ibrahim AMH, Quick JS, Kaya R, Grandgirard J, Poinsot D, Krespi L, et al. Evaluation of spring wheat genotypes for heat tolerance using cell membrane thermostability. *Crop Pasture Sci.* (2017) 2:291–6. doi: 10.1501/Tarimbil
10. Sánchez-Pérez S, Comas-Basté O, Rabell-González J, Veciana-Nogués MT, Latorre-Moratalla ML, Vidal-Carou MC. Biogenic amines in plant-origin foods: are they frequently underestimated in low-histamine diets? *Foods.* (2018) 7:1–17. doi: 10.3390/foods7120205
11. Rapa M, Ciano S, Ruggieri R, Vinci G. Bioactive compounds in cherry tomatoes (Solanum Lycopersicum var. Cerasiforme): cultivation techniques classification by multivariate analysis. *Food Chem.* (2021) 355:1–7. doi: 10.1016/j.foodchem.2021.129630
12. EFSA. Scientific Opinion on risk based control of biogenic amine formation in fermented foods. *EFSA J.* (2011) 9:2393–486. doi: 10.2903/j.efsa.2011.2393
13. Asensio E, Sanvicente I, Mallor C, Menal-Puey S. Spanish traditional tomato. Effects of genotype, location and agronomic conditions on the nutritional quality and evaluation of consumer preferences. *Food Chem.* (2019) 270:452–8. doi: 10.1016/j.foodchem.2018.07.131
14. Tilahun S, Do SP, Mu HS, Cheon SJ. Review on factors affecting the quality and antioxidant properties of tomatoes. *Afr J Biotechnol.* (2017) 16:1678–87. doi: 10.5897/AJB2017.16054
15. Tilahun S, Park DS, Taye AM, Jeong CS. Effect of ripening conditions on the physicochemical and antioxidant properties of tomato (*Lycopersicon esculentum* Mill). *Food Sci Biotechnol.* (2017) 26:473–9. doi: 10.1007/s10068-017-0065-7
16. Tsakiri S, Sofia T, Nifakos K, Tsaniklidis G, Vakros J, Delis C, et al. Comparison of on-vine and post-harvest ripening on antioxidant compounds and antioxidant activities of hydroponically grown cherry tomatoes. *Eur J Horotultural Sci.* (2020) 85:422–9. doi: 10.17660/eJHS.2020/85.6.6
17. Tilahun S, Park DS, Solomon T, Choi HR, Jeong CS. Maturity stages affect nutritional quality and storability of tomato cultivars. *CYTA - J Food.* (2019) 17:87–95. doi: 10.1080/19476337.2018.1554705
18. Huché-Thélier L, Crespel L, Gourrierec J. Le, Morel P, Sakr S, Leduc N. Light signaling and plant responses to blue and UV radiations-Perspectives for applications in horticulture. *Environ Exp Bot.* (2016) 121:22–38. doi: 10.1016/j.envexpbot.2015.06.009
19. Cavalcanti JHF, Esteves-Ferreira AA, Quinhones CGS, Pereira-Lima IA, Nunes-Nesi A, Fernie AR, et al. Evolution and functional implications of the tricarboxylic acid cycle as revealed by phylogenetic analysis. *Genome Biol Evol.* (2014) 6:2830–48. doi: 10.1093/gbe/evu221
20. Patanè C, Malvuccio A, Saita A, Rizzarelli P, Siracusa L, Rizzo V, et al. Nutritional changes during storage in fresh-cut long storage tomato as affected by biocompostable polylactide and cellulose based packaging. *Lwt.* (2019) 101:618–24. doi: 10.1016/j.lwt.2018.11.069
21. Luthria DL, Mukhopadhyay S, Krizek DT. Content of total phenolics and phenolic acids in tomato (*Lycopersicon esculentum* Mill) fruits as influenced by cultivar and solar UV radiation. *J Food Compos Anal.* (2006) 19:771–7. doi: 10.1016/j.jfca.2006.04.005
22. Dzakovich MP, Ferruzzi MG, Mitchell CA. Manipulating sensory and phytochemical profiles of greenhouse tomatoes using environmentally relevant doses of ultraviolet radiation. *J Agric Food Chem.* (2016) 64:6801–8. doi: 10.1021/acs.jafc.6b02983
23. Gautier H, Diakou-Verdin V, Bénard C, Reich M, Buret M, Bourgaud F, et al. How does tomato quality (sugar, acid, and nutritional quality) vary with ripening stage, temperature, and irradiance? *J Agric Food Chem.* (2008) 56:1241–50. doi: 10.1021/jf072196t
24. Junior SS, Casagrande JG, Toledo CA de L, Ponce F da S, Ferreira F da S, Zanuzo MR, et al. Selection of thermotolerant Italian tomato cultivars with high fruit yield and nutritional quality for the consumer taste grown under protected cultivation. *Sci Hortic (Amsterdam).* (2022) 291:110559. doi: 10.1016/j.scienta.2021.110559
25. Stinco CM, Pumilia G, Giuffrida D, Dugo G, Meléndez-Martínez AJ, Vicario IM. Bioaccessibility of carotenoids, vitamin A and α-tocopherol, from commercial milk-fruit juice beverages: contribution to the recommended daily intake. *J Food Compos Anal.* (2019) 78:24–32. doi: 10.1016/j.jfca.2019.01.019
26. Bertin N, Génard M. Tomato quality as influenced by preharvest factors. *Sci Hortic (Amsterdam).* (2018) 233:264–76. doi: 10.1016/j.scienta.2018.01.056
27. Atkinson NJ, Dew TP, Orfila C, Urwin PE. Influence of combined biotic and abiotic stress on nutritional quality parameters in tomato (*Solanum lycopersicum*). *J Agric Food Chem.* (2011) 59:9673–82. doi: 10.1021/jf202081t
28. Sumalan RM, Ciulca SI, Poiana MA, Moigradean D, Radulov I, Negrea M, et al. The antioxidant profile evaluation of some tomato landraces with soil salinity tolerance correlated with high nutraceuticaland functional value. *Agronomy.* (2020) 10:500. doi: 10.3390/agronomy10040500
29. Toscano S, Trivellini A, Cocetta G, Bulgari R, Francini A, Romano D, et al. Effect of preharvest abiotic stresses on the accumulation of bioactive compounds in horticultural produce. *Front Plant Sci.* (2019) 10:1212. doi: 10.3389/fpls.2019.01212
30. Hano S, Shibuya T, Imoto N, Ito A, Imanishi S, Aso H, et al. Serotonin content in fresh and processed tomatoes and its accumulation during fruit development. *Sci Hortic (Amsterdam).* (2017) 214:107–13. doi: 10.1016/j.scienta.2016.11.009
31. Sánchez-Rodríguez E, Romero L, Ruiz JM. Accumulation of free polyamines enhances the antioxidant response in fruits of grafted tomato plants under water stress. *J Plant Physiol.* (2016) 190:72–8. doi: 10.1016/j.jplph.2015.10.010
32. Montesinos-Pereira D, Barrameda-Medina Y, Romero L, Ruiz JM, Sánchez-Rodríguez E. Genotype differences in the metabolism of proline and polyamines under moderate drought in tomato plants. *Plant Biol.* (2014) 16:1050–7. doi: 10.1111/plb.12178
33. Hasan MM, Skalicky M, Jahan MS, Hossain MN, Anwar Z, Nie Z, et al. Spermine: its emerging role in regulating drought stress responses in plants. *Cells.* (2021) 10:1–15. doi: 10.3390/cells10020261
34. Madeo F, Pietrocola F, Eisenberg T, Kroemer G. Caloric restriction mimetics: towards a molecular definition. *Nat Rev Drug Discov.* (2014) 13:727–40. doi: 10.1038/nrd4391
35. Yang X, Zhang M, Dai Y, Sun Y, Aman Y, Xu Y, et al. Spermidine inhibits neurodegeneration and delays aging via the PINK1-PDR1-dependent mitophagy pathway in C. *elegans Aging (Albany NY).* (2020) 12:16852–66. doi: 10.18632/aging.103578
36. Pietrocola F, Lachkar S, Enot DP, Niso-Santano M, Bravo-San Pedro JM, Sica V, et al. Spermidine induces autophagy by inhibiting the acetyltransferase EP300. *Cell Death Differ.* (2015) 22:509–16. doi: 10.1038/cdd.2014.215
37. Madeo F, Carmona-Gutierrez D, Kepp O, Kroemer G. Spermidine delays aging in humans. *Aging.* (2018) 10:2209–11. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6128428/
38. Maintz L, Novak N. Histamine and histamine intolerance. *Am J Clin Nutr.* (2007) 85:1185–96. doi: 10.1093/ajcn/85.5.1185
39. Papageorgiou M, Lambropoulou D, Morrison C, Kłodzińska E, Namieśnik J, Płotka-Wasylka J. Literature update of analytical methods for biogenic amines determination in food and beverages. *TrAC Trends Anal Chem.* (2018) 98:128–42. doi: 10.1016/j.trac.2017.11.001
40. Khan MA, Khan ZA, Naeem A, Naqvi N, Srivast S, Bhargava A, et al. *Effect of Dietary Modification for Targeting Histamine Activity in Patients of Allergic Rhinitis: a Randomised Open Label Study*. Lucknow: Research Square (2020). p. 1–17. doi: 10.21203/rs.3.rs-25717/v1
41. Ennis M, Tiligada K. Histamine receptors and COVID-19. *Inflamm Res.* (2021) 70:67–75. doi: 10.1007/s00011-020-01422-1
42. Pinho L de, Almeida AC, Costa CA, Paes MCD, Glória MBA, Souza RM. Nutritional properties of cherry tomatoes harvested at different times and grown in an organic cropping. *Hortic Bras.* (2011) 29:205–11. doi: 10.1590/S0102-05362011000200012
43. Dala-Paula BM, Starling M de F V, Gloria MBA. Vegetables consumed in Brazilian cuisine as sources of bioactive amines. *Food Biosci.* (2021) 40:100856. doi: 10.1016/j.fbio.2020.100856
44. Chomchalow S, El Assi NM, Sargent SA, Brecht JK. Fruit maturity and timing of ethylene treatment affect storage performance of green tomatoes at chilling and nonchilling temperatures. *Horttechnology.* (2002) 12:271–83. doi: 10.21273/HORTTECH.12.1.104
45. Rai A, Kumari K, Vashistha P. Umbrella review on chilling injuries: post-harvest issue, cause, and treatment in tomato. *Sci Hortic (Amsterdam).* (2022) 293:110710. doi: 10.1016/j.scienta.2021.110710
46. Tsaniklidis G, Charova SN, Fanourakis D, Tsafouros A, Nikoloudakis N, Goumenaki E, et al. The role of temperature in mediating postharvest

polyamine homeostasis in tomato fruit. *Postharvest Biol Technol.* (2021) 179:111586. doi: 10.1016/j.postharvbio.2021.111586
47. Jannatizadeh A, Aghdam MS, Luo Z, Razavi F. Impact of exogenous melatonin application on chilling injury in tomato fruits during cold storage. *Food Bioprocess Technol.* (2019) 12:741–50. doi: 10.1007/s11947-019-2247-1
48. Ortega-Ortiz H, Benavides-Mendoza A, Mendoza-Villarreal R, Ramírez-rodríguez H, Romenus KDA. Enzymatic activity in tomato fruits as a response to chemical elicitors. *J Mex Chem Soc.* (2007) 51:141–4. https://www.redalyc.org/pdf/475/47551303.pdf
49. Ali A, Maqbool M, Alderson PG, Zahid N. Effect of gum arabic as an edible coating on antioxidant capacity of tomato (*Solanum lycopersicum* L.) fruit during storage. *Postharvest Biol Technol.* (2013) 76:119–24. doi: 10.1016/j.postharvbio.2012.09.011
50. López-Palestina CU, Aguirre-Mancilla CL, Raya-Pérez JC, Ramírez-Pimentel JG, Gutiérrez-Tlahque J, Hernández-Fuentes AD. The effect of an edible coating with tomato oily extract on the physicochemical and antioxidant properties of garambullo (*Myrtillocactus geometrizans*) fruits. *Agronomy.* (2018) 8:1–14. doi: 10.3390/agronomy8110248
51. Sun Q, Zhang N, Wang J, Cao Y, Li X, Zhang H, et al. A label-free differential proteomics analysis reveals the effect of melatonin on promoting fruit ripening and anthocyanin accumulation upon postharvest in tomato. *J Pineal Res.* (2016) 61:138–53. doi: 10.1111/jpi.12315
52. Dewanto V, Xianzhong W, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J Agric Food Chem.* (2002) 50:3010–4. doi: 10.1021/jf0115589
53. Colle I, Van Buggenhout S, Van Loey A, Hendrickx M. High pressure homogenization followed by thermal processing of tomato pulp: Influence on microstructure and lycopene in vitro bioaccessibility. *Food Res Int.* (2010) 43:2193–200. doi: 10.1016/j.foodres.2010.07.029
54. Cilla A, Bosch L, Barberá R, Alegría A. Effect of processing on the bioaccessibility of bioactive compounds – A review focusing on carotenoids, minerals, ascorbic acid, tocopherols and polyphenols. *J Food Compos Anal.* (2018) 68:3–15. doi: 10.1016/j.jfca.2017.01.009
55. Colle I, Lemmens L, Van Buggenhout S, Van Loey A, Hendrickx M. Effect of thermal processing on the degradation, isomerization, and bioaccessibility of lycopene in tomato pulp. *J Food Sci.* (2010) 75:C753–9. doi: 10.1111/j.1750-3841.2010.01862.x
56. Shi J, Le Maguer M. Lycopene in tomatoes: Chemical and physical properties affected by food processing. *Crit Rev Biotechnol.* (2000) 20:293–334. doi: 10.1080/07388550091144212
57. Dahiya S, Kumar AN, Shanthi Sravan J, Chatterjee S, Sarkar O, Mohan SV. Food waste biorefinery: sustainable strategy for circular bioeconomy. *Bioresour Technol.* (2018) 248:2–12. doi: 10.1016/j.biortech.2017.07.176
58. Tommonaro G, Poli A, De Rosa S, Nicolaus B. Tomato derived polysaccharides for biotechnological applications: chemical and biological approaches. *Molecules.* (2008) 13:1384–98. doi: 10.3390/molecules13061384
59. Paulino SLJ, Adrián ÁTG, Gabriela EAL, Maribel VM, Sergio MG. Nutraceutical potential of flours from tomato by-product and tomato field waste. *J Food Sci Technol.* (2020) 57:3525–31. doi: 10.1007/s13197-020-04585-1
60. Nour V, Panaite TD, Ropota M, Turcu R, Trandafir I, Corbu AR. Nutritional and bioactive compounds in dried tomato processing waste. *CYTA J Food.* (2018) 16:222–9. doi: 10.1080/19476337.2017.1383514
61. Szabo K, Dulf FV, Diaconeasa Z, Vodnar DC. Antimicrobial and antioxidant properties of tomato processing byproducts and their correlation with the biochemical composition. *LWT.* (2019) 116:108558. doi: 10.1016/j.lwt.2019.108558
62. Araújo-Rodrigues H, Santos D, Campos DA, Ratinho M, Rodrigues IM, Pintado ME. Development of frozen pulps and powders from carrot and tomato by-products: Impact of processing and storage time on bioactive and biological properties. *Horticulturae.* (2021) 7:185. doi: 10.3390/horticulturae7070185
63. Gheonea I, Aprodu I, Enachi E, Horincar G, Bolea CA, Bahrim GE, et al. Investigations on thermostability of carotenoids from tomato peels in oils using a kinetic approach. *J Food Process Preserv.* (2020) 44:1–9. doi: 10.1111/jfpp.14303

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Lima, Gómez, Seabra Junior, Maraschin, Tecchio and Borges. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 28 April 2022
doi: 10.3389/fnut.2022.844162

Effect of Individual and Selected Combined Treatments With Saline Solutions and Spent Engine Oil on the Processing Attributes and Functional Quality of Tomato (*Solanum lycopersicon* L.) Fruit: In Memory of Professor Leila Ben Jaballah Radhouane (1958–2021)

Riadh Ilahy[1], Imen Tlili[1], Zoltán Pék[2], Anna Montefusco[3], Hussein Daood[2], Mohamed Azam[4], Mohammed Wasim Siddiqui[5], Thouraya R'him[1], Miriana Durante[6], Marcello Salvatore Lenucci[3] and Lajos Helyes[2]*

[1] Laboratory of Horticulture, National Agricultural Research Institute of Tunisia (INRAT), University of Carthage, Ariana, Tunisia, [2] Horticultural Institute, Hungarian University of Agriculture and Life Sciences, Gödöllo, Hungary, [3] Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali (DiSTeBA), Università del Salento, Lecce, Italy, [4] Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan, [5] Department of Food Science and Postharvest Technology, Bihar Agricultural University, Bhagalpur, India, [6] Istituto di Scienze Delle Produzioni Alimentari (ISPA)-CNR, Lecce, Italy

The results showed that soil electrical conductivity, (EC2: 7 dS/m) increased soluble solids, lycopene content, total phenolic content, hydrophilic and lipophilic radical scavenging activities (HRSA and LRSA) by 14.2, 149, 20, 46.4, and 19.0%, respectively, compared with control. Under 0.5% spent engine oil (SEO), flavonoid content decreased by 21.7% compared with the control. HRSA and LRSA of fruits subjected to EC2/SEO1 treatment were, respectively, 45.9 and 35.5% lower than control. The a*/b* ratio was positively and significantly ($P < 0.01$) correlated with β-carotene (R = 0.78), lycopene (R = 0.68), total vitamin C (R = 0.71), α-tocopherol (R = 0.83), γ-tocopherol (R = 0.66), HRSA (R = 0.93), LRSA (R = 0.80), and soluble solids (R = 0.84) suggesting that it may be a promising indicator of fruit quality in areas affected by such constraints. The research revealed that combined stresses induce responses markedly different from those of individual treatments, which strain the need to focus on how the interaction between stresses may affect the functional quality of tomato fruits.

Keywords: carotenoids, flavonoids, phenolic, radical scavenging activity, vitamin C, salt stress, soil pollution, tocopherols

OPEN ACCESS

Edited by:
Spyridon Alexandros Petropoulos, University of Thessaly, Greece

Reviewed by:
Nahla El-Sherif, Ain Shams University, Egypt
Xiumin Fu, South China Botanical Garden (CAS), China

***Correspondence:**
Marcello Salvatore Lenucci
marcello.lenucci@unisalento.it

Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *27 December 2021*
Accepted: *21 March 2022*
Published: *28 April 2022*

Citation:
Ilahy R, Tlili I, Pék Z, Montefusco A, Daood H, Azam M, Siddiqui MW, R'him T, Durante M, Lenucci MS and Helyes L (2022) Effect of Individual and Selected Combined Treatments With Saline Solutions and Spent Engine Oil on the Processing Attributes and Functional Quality of Tomato (Solanum lycopersicon L.) Fruit: In Memory of Professor Leila Ben Jaballah Radhouane (1958–2021). Front. Nutr. 9:844162. doi: 10.3389/fnut.2022.844162

INTRODUCTION

Tomato (*Solanum lycopersicon* L.) is one of the most important vegetable crops in the world, ranking second just after potato. The fruits are excellent dietary sources of minerals, fibers, vitamins, and several antioxidants, principally the distinctive red pigment lycopene (1). This linear carotene is a powerful scavenger of free radicals, the major driving factor in the pathophysiology of various chronic and age-related diseases (2). Moreover, evidence is emerging on its role as a potential effector in the prevention and therapy of several disorders including spleen inflammation, neuropathy, and dyslipidemia, and also in exerting potential beneficial effects on skeletal muscle metabolism (3, 4). Besides lycopene, tomato also contains many other hydrophilic, and lipophilic antioxidants such as phenolic compounds (mainly phenolic acids and flavonoids), tocopherols, ascorbic, and dehydroascorbic acids contributing to protection against free radicals (5).

Salinization is a major abiotic factor limiting agricultural production worldwide. Salt pollution disturbs plant development by limiting its nutrient uptake and reducing the quality of the water available to the plant. It affects the metabolism of soil organisms and severely reduces soil fertility. High levels of salinity in soils provoke plant withering due to the increase in osmotic pressure and the toxic effects of salts (6).

The effect of saline water irrigation on tomato productivity and fruit quality has been well-defined, indicating that the thresholds of electric conductivity (EC) for the decrease of yield and plant growth are moderately high and differ among cultivars. Recently Ben Ali et al. (7), treating the cultivar (cv.) Rio Grande with salt solutions with EC of 3.5 and 7.0 deciSiemens per meter (dS/m), reported a significant reduction in plant height and root depth, but not in leaf area index, concluding that irrigation with saline is possible for tomato crop within adequate limits. Indeed, within defined EC values, an improvement of ripe tomato fruit quality and taste has been reported under salinity (8, 9). In addition to soil salinization, several pollutants increasingly threaten agricultural areas affecting crop growth, production, and quality (10). More than 50% of the main soil contaminants are petroleum hydrocarbons deriving primarily from accidental spillage during extraction, transportation, and distribution of petroleum and petroleum derivatives, but also from the illicit tapping from pipelines or dumping of spent products. These criminal activities represent a global threat concerning both developing and industrialized countries (11, 12). Actually, between 2010 and 2016, more than 20 pipeline liquid spillage incidents have been recorded each year in Europe and 399 in the United States, while 465 intentional unlawful discharge acts were reported in Italy between 2011 and 2019, which generally resulted in soil pollution (13). In the ScienceDirect database (www.sciencedirect.com), using oil spill and agriculture as keywords, the number of published research items has increased sharply from 175 to 1,353 between 1998 and 2021, suggesting the importance of carefully focusing on the deleterious effect of such problems. In particular, soil and surface water contamination with spent engine oil (SEO) is becoming a prevalent environmental issue in most developing countries (14). SEO reduces soil aeration causing root stress and chlorosis of leaves and leads to an impairment of soil microbial community with deleterious effects on fertility and crop production potential. These deleterious effects have been thoroughly documented on various crops, including maize, peanut, cowpea, bean, gombo, green amaranth, sponge gourd, fluted pumpkin, and pepper (14–20).

To the best of our knowledge, there is no detailed data regarding the effect of salinity and SEO on tomato fruits quality. Thus, this research was carried out to fill this knowledge gap by assessing the impact of individual and combined treatments with saline water and SEO on the main processing traits and functional quality of ripe tomato fruits of the cv. Rio Grande.

MATERIALS AND METHODS

Chemicals

2,20-Azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), ascorbic acid, Rutin, Gallic acid, butylated hydroxytoluene (BHT), NaOH, $NaNO_2$, $AlCl_3$ were obtained from Sigma–Aldrich, Chemical Co., Milan. Other reagents were of analytical grade and purchased from Carlo Erba (Milan, Italy).

Plant Material and Treatments

Certified tomato seeds of the cv. Rio Grande was provided by the germplasm bank of the National Agricultural Research Institute of Tunisia. Rio Grande is a determinate open-pollinated highly productive (97.0–145.4 t/ha) tomato cv. widely grown in Tunisia. It produces large fruits suitable for both fresh consumption and processing with an average weight ranging from 77.0 to 81.2 g and soluble solids content between 5.2 and 5.9°Brix (5). Sowing was carried out on plastic trays in February 2018. A total of four-week-old seedlings were individually transplanted into 20 L pots containing a clay-loamy substrate with 246.2 g/kg clay, 160 g/kg loam, and 290.7 g/kg sand and high levels of the calcareous substance, with pH (7.72), EC (0.19 mS/cm), mineral and organic composition suitable for tomato cultivation. The pots were placed under natural meteorological conditions (**Figure 1**) in the experimental field of the University of Carthage, with additional watering as required by the experimental design.

A total of 10 days after transplant, seedlings were exposed to seven different treatments (30 plants/treatment): two salinity levels (EC1: 3.5 dS/m and EC2: 7.0 dS/m, equivalent to 5.46 and 10.93 g NaCl/L, respectively); two levels of SEO [SEO1: 5 ml SEO/L (0.5%) and SEO2: 10 ml SEO/L (1%)], two combinations of saline and SEO levels (EC1/SEO2: 3.5 dS/m, 1% SEO and EC2/SEO1: 7dS/m, 0.5% SEO) and a common control treatment (0.4 dS/m EC and no SEO application). SEO was applied directly around the root zone of tomato plants before irrigation 2–3 times a week throughout seven weeks. Some combined treatments were not considered in the present research as preliminary tests showed that EC2/SEO2 treatment led to plants severely affected in growth parameters and leaves' cover with no fruit set, while EC1/SEO1 treatment gave results similar to SEO1, at least in terms of yield, average fruit weight and visual appearance of fruit cross-sections. The irrigation management

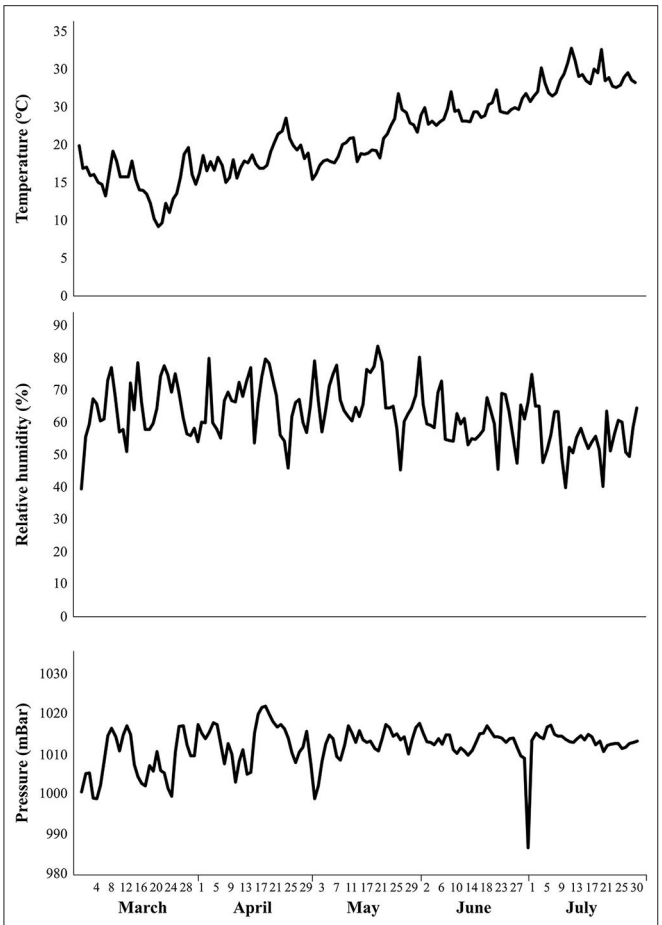


FIGURE 1 | Temperature (°C), relative humidity (%), and atmospheric pressure (mBar) data were recorded by the weather station of the National Agricultural of Tunisia (Lat 40°20′ N, Lon 18°07′) of the University of Carthage. The reported values refer to 2018 and cover the entire tomato plant growing season (March–July).

was based on monitoring the soil moisture content using a BIOS probe measuring simultaneously the moisture content and temperature. Irrigation was performed manually until the water content of the used substrate reached the field capacity value (34%).

Fruit Sampling

Tomato fruits were collected from each plant at the red stage of ripeness and yield per plant (yield/plant) was determined. A total of 18–20 healthy fresh ripe-red tomato fruits were collected from each bloc and quickly carried to the laboratory. The sampling was performed in triplicate when the red-ripe stage was attained. Bright red-ripe fruits were selected, externally washed with deionized water, cut into small pieces, and homogenized in a blender (Waring Laboratory Science, Torrington, CT, US). The homogenates were frozen at −20°C and used to determine the content of carotenoids, total phenols, flavonoids, vitamin C as well as HRSA and LRSA within the following days to prevent nutrient degradation.

Determination of Total Soluble Solids, Titratable Acidity, and Color Indexes

Brix of freshly prepared juice was used to measure the soluble solid content of tomato fruits. For this reason, some drops of filtered juice were placed on the prism of an Atago PR-100 digital refractometer equipped with automated temperature correction. Titratable acidity was calculated as a percentage of citric acid following titration of the diluted juice using 0.1 M sodium hydroxide solution until reaching 8.1 pH. A Minolta CR-400 (Minolta Corp., Osaka, Japan) was used to estimate the redness a* and b* yellowness color indexes and the a*/b* ratio was consequently calculated.

Analysis of Carotenoid Content

Carotenoids were determined according to the protocol of Daood et al. (21) slightly modified. Briefly, tomato homogenate (2.5 g aliquots) was crushed in a crucible mortar with quartz sand. Subsequently, 20 ml of methanol was added for 1–2 min, and the upper layer was poured into an Erlenmeyer flask. A volume of 50 ml of dichloroethane was then mixed with 10 ml of methanol in a graduated cylinder and mixed softly before and after adding some distilled water drops. The mixture was filtrated in a separating funnel and allowed to evaporate at 70°C until complete evaporation. A volume of 5 ml of analytical grade methanol was mixed with 5 ml of pigment eluents and poured into the same flask then mixed gently, sonicated, filtered using a 0.22 μm membrane syringe, and finally injected into the HPLC (Hitachi Chromaster, Tokyo, Japan) system consisting of a 5,110 Pump, a 5,210 Auto Sampler, a 5,430 Diode Array detector, and a 5,440 FL detector.

Determination of Total Phenolic and Flavonoid Contents

The procedure of Martínez-Valverde et al. (22) was used for the extraction and quantification of total phenolics content. In total, 5 ml of methanol 80% and 50 μl of 37 % HCl were mixed with 0.3 g of tomato homogenate and extracted for 2 h at 4°C under 300 rpm then centrifuged for 20 min at 10,000 g. The Folin–Ciocalteu reagent was used with a 50 μl supernatant sample and the absorbance was measured at 750 nm using a Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK), and total phenolics content was expressed as mg of gallic acid equivalent (GAE)/kg fw. Moreover, the procedure of Asami et al. (23) was used to correct the obtained values due to the interference of sugars with phenolic compounds.

Zhishen et al. (24) described a method that was used for the determination of flavonoids content. For this reason, a sample of 0.3 g was extracted with methanol and a volume of 50 μl was diluted with distilled water to attain 0.5 ml, and 30 μl of 5% $NaNO_2$ was poured and 60 μl of $AlCl_3$ (10%) and 200 μl of NaOH (1 M) were added after 5 and 6 min, respectively. The wavelength 510 nm was used to measure the absorbances of the samples using a Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK) and the results were expressed as mg of rutin equivalent (RE)/kg fw.

TABLE 1 | Main processing attributes (yield/plant, average fruit weight, titratable acidity, total soluble solids and a^*/b^* ratio) of red-ripe tomato fruits (cv. Rio Grande) harvested from plants subjected to control conditions (0.4 dS/m EC, no SEO), saline stress (EC1, 3.5 dS/m; EC2, 7 dS/m), spent engine oil stress (SEO1, 0.5%; SEO2, 1%), and saline/SEO combinations (EC1/SEO2, 3.5 dS/m/1% SEO; (EC2/SEO1, 7 dS/m/0.5% SEO).

Traits/treatments	Yield/plant (g)	Average fruit weight (g)	Titratable acidity (%)	Soluble solids (°Brix)	a*/b*
Control	1,201.7 ± 52.5 a	90.0 ± 3.5 a	0.37 ± 0.004 d	5.6 ± 0.06 c	1.14 ± 0.03 c
EC1	875.7 ± 12.9 b	82.33 ± 3.4 a	0.43 ± 0.05 d	6.2 ± 0.09 ab	1.41 ± 0.04 b
EC2	808.3 ± 18.8 b	73.33 ± 2.02 b	0.57 ± 0.006 c	6.4 ± 0.115 a	1.62 ± 0.07 a
SEO1	716.7 ± 8.9 c	64.33 ± 2.1 c	0.56 ± 0.01 c	6.03 ± 0.03 b	1.23 ± 0.1 c
SEO2	631.4 ± 13.3 d	59.66 ± 3.3 cd	0.63 ± 0.002 bc	5.43 ± 0.04 c	1.21 ± 0.05 c
EC1/SEO2	570.1 ± 5.8 d	53.33 ± 1.8 de	0.70 ± 0.03 b	5.16 ± 0.02 d	0.93 ± 0.02 d
EC2/SEO1	466.0 ± 11.5 e	48.66 ± 0.88 e	0.86 ± 0.03 a	5.36 ± 0.1 cd	0.85 ± 0.04 d

Values are expressed as the mean ± SD of three independent experimental replicas (n = 3).
Different letters in the same column indicate significant differences between means according to the Duncan's test at the 5% level.

Determination of Total Vitamin C Content

The extraction and quantification of total vitamin C (AsA + DHA) content were carried out according to Kampfenkel et al. (25) on triplicate samples (0.2 g) of homogeneous tomato juice. The absorbance was read at 525 nm in a spectrophotometer (Cecil BioQuest CE 2501) and expressed as mg/kg fw. The linear reading of the standard curve was from 0 to 700 μmol AsA.

Determination of Tocopherol Content

The procedure of Abushita et al. (26) was adopted for the extraction of tocopherols using n-hexane. The separation was performed on Nucleosil 5 mm (250 4.6 mm i.d.) with a mobile phase consisting of 99.5:0.5 n-hexane: ethanol and detected at 295 and 320 nm as the excitation and emission wavelength, respectively, as outlined in Duah et al. (27). α, β, γ, and δ isomers (from Sigma-Aldrich Ltd., Budapest, Hungary) were used for the identification of tocopherols.

Determination of the Radical Scavenging Activity

The radical scavenging activity of the hydrophilic and lipophilic fractions (HRSA and LRSA, respectively) was evaluated utilizing the TEAC assay. The method outlined by Miller and Rice-Evans (28) is widely used for the determination of the radical scavenging activity of fruit, vegetables, and processed products due to its reproducibility and high fidelity across complex matrices. The extraction of the antioxidants from the hydrophilic and lipophilic fractions was performed on 0.3 g of the tomato sample using methanol (50%) acetone (50%), respectively, at 4°C under constant shaking (300 rpm) during 12 h. Tomato homogenate samples were centrifuged for 7 min at 10,000 g, supernatants were used for the measurement of the radical scavenging activity at 734 nm in a Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). Radical scavenging activity was calculated and expressed as μM of Trolox/100 g of fw.

Statistical Analysis

The variations affecting the processing and functional quality of tomato fruits under the applied treatments were evaluated by one-way ANOVA. When a significant difference was detected, the means were compared using the Duncan test ($P < 0.05$). All the statistical comparisons were performed using version 6.1 of SAS software (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp., NC, USA). Correlations were examined using Pearson's correlation coefficient (r).

RESULTS AND DISCUSSION

Processing Attributes

The main processing attributes (yield/plant, average fruit weight, total soluble solids, titratable acidity, and a*/b* ratio) of the red-ripe fruits harvested from tomato plants grown under control conditions or exposed to the individual and combined treatments with saline water and SEO are reported in **Table 1** and expressed as percent variation with respect to the control in **Table 2**.

All the evaluated processing attributes were significantly affected by treatments ($P < 0.05$). Salinity and SEO applied individually and in combination, resulted in 27.1–32.7%, 40.3–47.5%, and 52.6–60.9% reduction in yield/plant, respectively, which decreased from the mean yield of 1,201.7 g/plant in the non-treated plant, down to only 466.0 g/plant upon combined exposure to 7 ds/m EC and 0.5% SEO (EC2/SEO1). Similarly, we noticed a reduction of 8–18%, 28–33%, and 40–46% in the average fruit weight, respectively, which decreased from the mean value of 90 g of a control sample; down to 48.66 g under 7 ds/m EC and 0.5% SEO combined exposure (EC2/SEO1). This may result from a drop in water balance/uptake determined by the reduction of soil water potential due to the increase of soluble salts and/or the decrease of soil water holding capacity promoted by SEO (29, 30). The treatments distinctively affected total soluble solids of tomato fruits: EC1, EC2, and SEO1, applied individually, resulted in a 7–14% increase, while, individual application of 1% SEO (SEO2) and both combined treatments (EC1/SEO2; EC2/SEO1) caused a significant (29–77%) decrease compared with the control. According to Botella et al. (8), salinity significantly increases total soluble solids and titratable acidity stimulating the synthesis of soluble sugars (glucose and fructose) and organic acids. Consistently, Yurtseven et al. (31) reported an increase in total soluble solids attaining 7.51 and 10.4 °Brix under 5 and 10 dS/m, respectively, compared with 5.43 °Brix of control fruits of the tomato cv. H2274-Oturak. Sugar and sugar derivative synthesis/accumulation may contribute to

TABLE 2 | Percent variation compared to the control in yield/pl. (Y/pl.), average fruit weight (Avg. fruit wt.), total soluble solids (TSS), titratable acidity (TA), a^*/b^* ratio, β-carotene (β-Crt), lycopene (Lyc), total phenolic compounds (TPC), total flavonoids (TF), total vitamin C (TVC), α-, β-, and γ-Tocopherol (α-, β- and γ-T), hydrophilic radical scavenging activity (HRSA) and lipophilic radical scavenging activity (LRSA) under the different applied treatments and their combinations.

Traits/ treatments	Y/pl.	Avg.fruit wt.	TSS	TA	a*/b*	β-Crt	Lyc	TPC	TF	TVC	α-T	β-T	γ-T	HRSA	LRSA
EC1	−27.1%	−8%	10%	15%	23%	31%	98%	−17%	−5%	47%	31%	93%	18%	16%	19%
EC2	−32.8%	−18%	14%	53%	40%	49%	149%	20%	−11%	66%	26%	nd	26%	26%	19.3
SEO1	−40.3%	−28%	7%	51%	7%	47%	123%	−7%	−21%	95%	−56%	nd	−24%	−28%	−7%
SEO2	−47.5%	−33%	−29%	69%	6%	90%	37%	−8%	−0.3%	−14%	−19%	nd	−86%	−16%	7.1%
EC1/SEO2	−52.6%	−40%	−77%	89%	−18%	17%	43%	6%	−2%	−27%	−2%	nd	nd	−17%	−21%
EC2/SEO1	−60.9%	−46%	−41%	131%	−24%	−30%	29%	21%	−2%	−31%	3%	nd	nd	−26%	−35%

Green cells identify increased changes, yellow cells identify reductions.
nd, not detected.

satisfy the increasing energy demand required to tolerate the adverse stress conditions and/or, acting as osmolytes, to protect cells and sustain a minimal osmotic balance under water stress conditions determined not only by salinity but also by pollution with high molecular weight polycyclic aromatic hydrocarbons (PAH) (29, 30). In this regard, Molina and Segura (32) reported that sugar metabolisms are activated by the presence of PAH with a consequential increase of glucose, mannose, galactose, raffinose, galactinol, melibiose, and sucrose contents in plant tissues. A decrease in fruit quality and sugar content was, instead, reported in peppers grown in hydroponic systems under a glasshouse exposed to 5 mS/cm salinity possibly determined by the enhancement of respiration rate of stressed fruits (33, 34), confirming that the responses to salinity stress are species specific, or even variety dependent.

With regard to SEO, no literature data are available on its effects on the total soluble solids of tomatoes, neither if applied individually, nor in combination with other stresses. Nevertheless, the observed negative effect on total soluble solids of 1% SEO treatment (SEO2), further amplified by the coexistence of saline stress, can probably be explained by a direct or indirect toxicity effect of the pollutant on the central carbon metabolism of plants. Indeed, SEO contains heavy metals [including lead (Pb), cadmium (Cd), arsenic (As), zinc (Zn), and copper (Co)] which interfere with physiological, biochemical, and molecular processes of living systems (35, 36).

EC and SEO treatments, individually and combined, resulted, respectively, in a 15–53%, 51–69%, and 89–131% increase of titratable acidity compared to control tomato fruits (**Table 2**), with values ranging from 0.37% (control), up to 0.86% (EC2/SEO1). The observed increase is possibly related to an active accumulation of ions and organic acids produced by the plant under stress conditions. Alterations in the relative concentrations of tricarboxylic acid cycle intermediates, resulting in increased citrate and malate levels but α-ketoglutarate, fumarate, oxaloacetate, pyruvate, and succinate decrease, were reported in response to PAH as a consequence of the overexpression or downregulation of the enzymes of the Krebs cycle and of the mitochondrial respiratory chain (32).

The a^*/b^* ratio is a suitable parameter to characterize the quality and maturity of tomato fruits (5, 37). EC and SEO treatments applied separately determined, respectively, a 23–40% and 6–7% increase in the a^*/b^* ratio compared with the control, while the combined treatments prompted an 18–24% decrease (**Table 2**). Accordingly, an evident discoloration of the mesocarp characterizes the cross-sections of tomato fruit samples grown under EC1/SEO2 and EC2/SEO1 (**Figure 2**).

Stress interactions can be classified as (1) additive, when the response is proportional to the sum of the single applied stresses; (2) synergistic, when it overcomes the sum of the individual stresses; (3) idiosyncratic, when the outcome differs significantly from the stresses applied individually and (4) dominant when the response is similar to that induced by one of the stresses applied individually (38). Our findings hint at differences in the responses to the two assayed stress combinations for the same trait. For average fruit weight and total soluble solids, the response to EC1/SEO2 (3.5 dS/m EC, 1% SEO) and EC2/SEO1

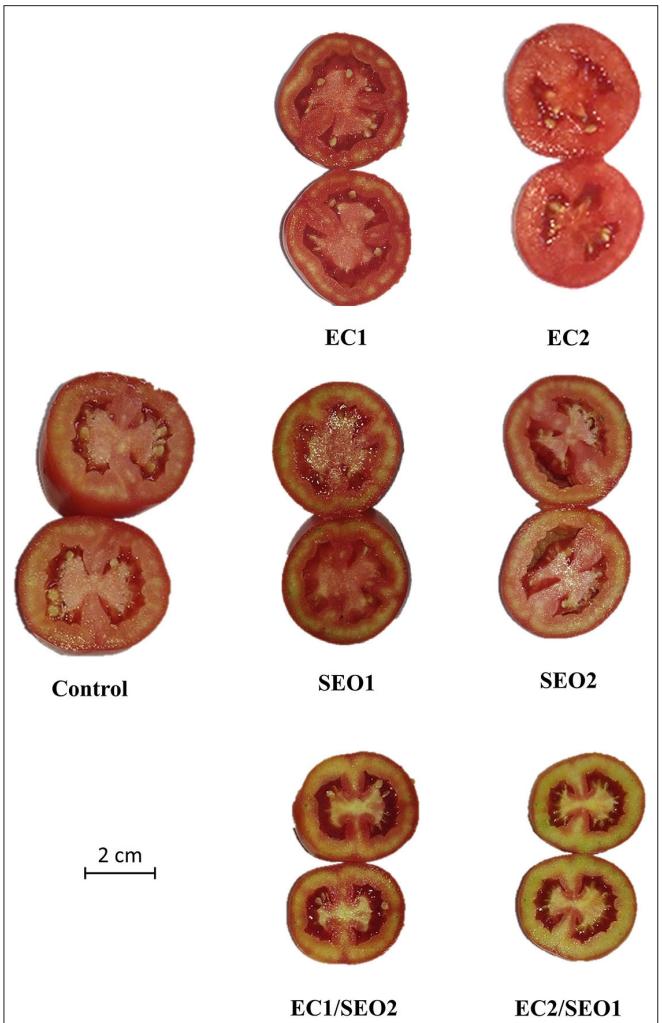


FIGURE 2 | Transverse sections of tomato fruits under control (C, 0.4 dS/m), saline (EC1, 3.5 dS/m; EC2, 7 dS/m), spent engine oil stress (SEO1, 0.5%; SEO2, 1%) and the combinations (EC1/SEO2, 3.5 dS/m EC/1% SEO; EC2/SEO1, 7 dS/m EC/0.5% SEO).

(7 dS/m EC, 0.5% SEO) was, respectively, dominated by SEO and idiosyncratic. However, both stress combinations gave additive responses for titratable acidity, but idiosyncratic for a*/b* ratio.

Carotenoid Content

Ordinary red tomato fruit contains up to 200 mg of lycopene per kg of fresh weight (fw), together with much lower contents of β-carotene and other carotenoids, though with large genotype-associated variations (1, 5). In the pulp and peels of ripe Rio Grande tomatoes grown in an open field, the concentration of lycopene was approx. In total, 100.9 and 423.7 mg/kg fw, respectively (39), while the β-carotene level in the entire fruit homogenate was below 7 mg/kg fw (5). In this study, the level of lycopene in the control fruits was halved with respect to our previous report, while β-carotene remained almost unchanged (46.2 and 8.0 mg/kg fw, respectively) possibly due to the different growing conditions. EC and SEO treatments, either applied individually or combined, prompted significant ($P < 0.05$) variations in the contents of both lycopene and β-carotene, which reached concentrations up to 115.1 and 17.0 mg/kg fw, respectively (**Figure 3**). In particular, compared with the control, the lycopene concentration was increased by 48–149% and 37–123% by individual EC and SEO treatments, respectively, and 29–43% by the stress combinations. Similarly, β-carotene levels were increased by 31–49% and 47–90% by the individual EC and SEO treatments, respectively. However, a 17% increase was observed under EC1/SEO2 conditions, while EC2/SEO1 treatment determined a 30% decrease in β-carotene, suggesting idiosyncratic responses. In agreement, a general improvement of tomato fruit quality attributes (lycopene, phenols, ascorbic acid, hydrophilic, and lipophilic antioxidant activities) was reported by Sellitto et al. (40) under 6.0 mS/cm soil EC, as well as in pepper (cv Friariello) fruits grown under 4.4 mS/cm EC in a nutrient film technique hydroponic system. Moles et al. (41) and Borghesi et al. (42) reported that tomato carotenoids exhibited a genotype-dependent trend in response to salinity. However, Serio et al. (43) found that salinity did not affect the lycopene content. Similarly, in the fruits of the tomato cv Boludo F1 grown under hydroponic conditions, phytoene, phytofluene, lycopene, and lutein contents were unaffected by salinity (7.8 dS/m), unlike β-carotene which was increased (8). Sumalan et al. (9) found that tomatoes grown under soil ECs over 6.5 dS/m exhibited moderate-to-high concentrations of various antioxidants including lycopene, phenolics, and ascorbic acid, and also higher total antioxidant activity, highlighting the important role of secondary metabolites in the process of stress adaptation. The discrepancy among the reported studies might be related to genotypic differences in the resistance/tolerance to different stresses, soil type, and several other environmental and agronomic factors. A decline in the content of chlorophyll and leaf carotenoids was observed following irrigation of tomatoes with industrial wastewater highly contaminated with heavy metals (44) and also in the presence of toxic levels of Cd, probably due to inhibition of the carotenoid biosynthetic pathway and severe oxidative stress induction (45–47). It has been also reported that cadmium inhibited the photosynthetic activity of photosystem II by 60% but was ineffective on photosystem I. Muzolf-Panek et al. (48) reported that the levels of lycopene and vitamin C decreased in tomato cvs Emoticon F1 and Alboney F1 fruits grown in hydroponics under increasing Mn concentrations (0–19.2 mg/dm^3).

Total Phenolic and Flavonoid Contents

Phenolics are secondary metabolites widely distributed in plants and characterized by the presence of mono- or poly-hydroxylated aromatic rings. Plants contain tens of thousands of different phenolic compounds, and the number is still increasing, ranging from hydrophilic, lipophilic, to insoluble structures. Phenolics are categorized into groups and sub-groups based on the number of C-atoms and the fundamental arrangement of carbon skeletons in their chemical structure. They may act as antioxidants, structural polymers, coloring pigments, pollinator or pest chemo-attractants/repellents, UV screens, signaling molecules in symbiosis initiation, and

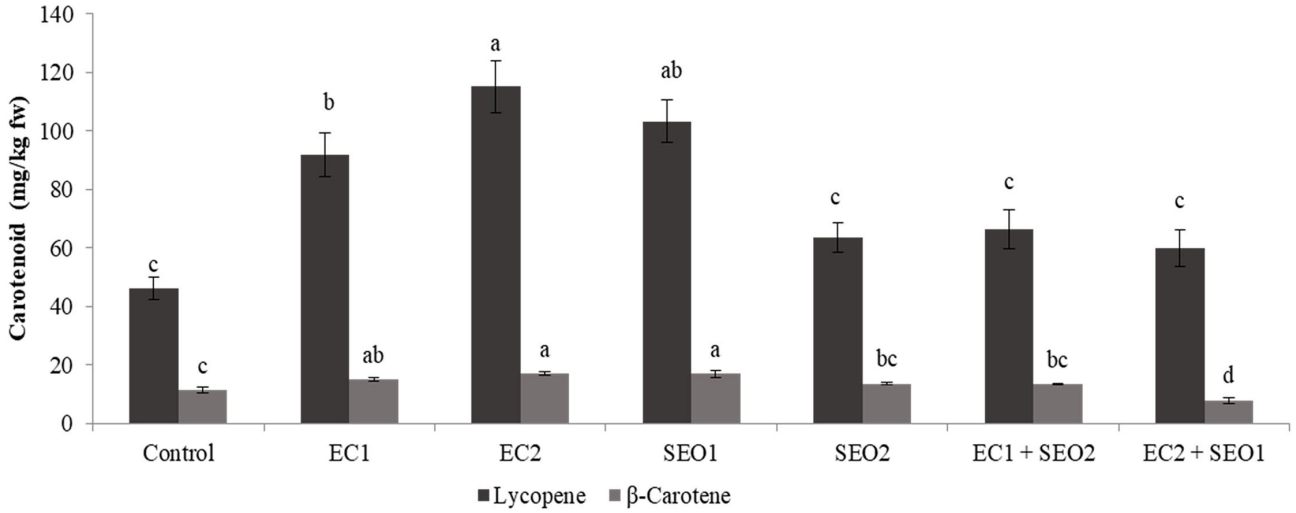


FIGURE 3 | Lycopene and β-carotene content (mg/kg fw) of tomato fruits under control (C, 0.4 dS/m), saline (EC1, 3.5 dS/m; EC2, 7 dS/m), spent engine oil stress (SEO1, 0.5%; SEO2, 1%) and the combinations (EC1/SEO2, 3.5 dS/m EC/1% SEO; EC2/SEO1, 7 dS/m EC/0.5% SEO). Values are means ($n = 3$).

plant-microbe interactions, and defensive weapons against aggressors (49). In this study, total phenolic and flavonoid contents varied significantly between the applied treatments ($P < 0.05$) within ranges of 96.14–140.23 mg GAE/kg fw and 73.05–93.33 mg RE/kg fw (**Figure 4**). Compared to the control, SEO exposure, individually and in combination with EC stress, resulted, respectively, in a 7–8% decrease and a 6–21% increase in the content of total phenolics. However, contradictory effects of EC treatments were observed for the total phenolic content as fruits subjected to EC1 and EC2 treatments exhibited a 17% decrease and 20% increase compared to the control, respectively. ECs and SEO, individually and combined, negatively affected the flavonoid concentration, resulting in a 0.3–21% reduction. Phenolic compounds are important free radical scavengers, whose biosynthesis is significantly stimulated by various environmental stresses (50). Contrasting reports are available on the effect of salinity on phenolics. According to Akladious and Mohamed (51), a moderate increase (from 91.5 to 98.0 mg GAE/100 g fw) in the levels of total phenolics was observed following irrigation of peppers with a 100 mmol/L saline solution. Botella et al. (8) noticed a chemical-class dependent-response of tomato phenolics under salinity stress, with flavanones not affected but flavonols significantly increased, suggesting a specific role of the latter in scavenging salt-stress generated ROS. Moles et al. (41) noticed higher tolerance/resistance to increasing concentration (0–120 mm NaCl) of salt in tomato landraces (Ciettaicale, Linosa, and Corleone) grown in hydroponics, with respect to the commercial cv. UC-82B, which correlated with a differential accumulation of glycoalkaloids, phenolic acids, flavonoids, and their derivatives in the fruits. Yildiztugay et al. (52) proposed that exogenously applied flavonoid naringenin protects bean chloroplasts minimizing salinity-induced stress and increasing the activities of various antioxidant enzymes (ascorbate peroxidase, glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase, and peroxidase).

In the case of SEO stress, the data on fruit quality is scarce, nevertheless, Muzolf-Panek et al. (48) reported a strong genotype-dependent increase in the content of total phenolic compounds (up to 11-fold) in two tomato cvs (Emotion F1 and Alboney F1) exposed to Mn (0.3 and 19.2 mg/dm^3). Aguebor-Ogie et al. (53) noticed an organ-dependent response to spent lubrification oil stress in 2-week-old tomato seedlings. In the stem, total phenolic content was decreased by 0.6% but flavonoids increased by 84%, while in the root, total phenolic content was unaffected and total flavonoids decreased by 35% compared to the control. Molina and Seguna (32) proposed that the stimulation of phenylalanine ammonia-lyase activity and the concentration of the precursor's phenylalanine, tyrosine, and tryptophan increased following heavy-metal treatments highlighting the importance of phenolic compounds in counteracting stress-generated ROS.

Total Vitamin C

Vitamin C has a pivotal role in maintaining redox homeostasis in plants. It acts directly as antioxidants scavenging reactive oxygen species, but can also regenerate glutathione and tocopherol radicals or acts as a cofactor for many enzymes (e.g., ascorbate peroxidases and violaxanthin de-epoxidase in the xanthophyll cycle) (54). In this study, the levels of total vitamin C were significantly ($P < 0.05$) but differentially affected by the applied treatments (**Figure 5**). Compared with the control, EC treatments induced a concentration-dependent increase in total vitamin C levels (47–66%), which was, instead, decreased (up to −31%) when in combination with SEO. Individual SEO applications determined contrasting responses: 95% higher levels of total vitamin C were registered under SEO1, while a 14% decrease resulted from SEO2

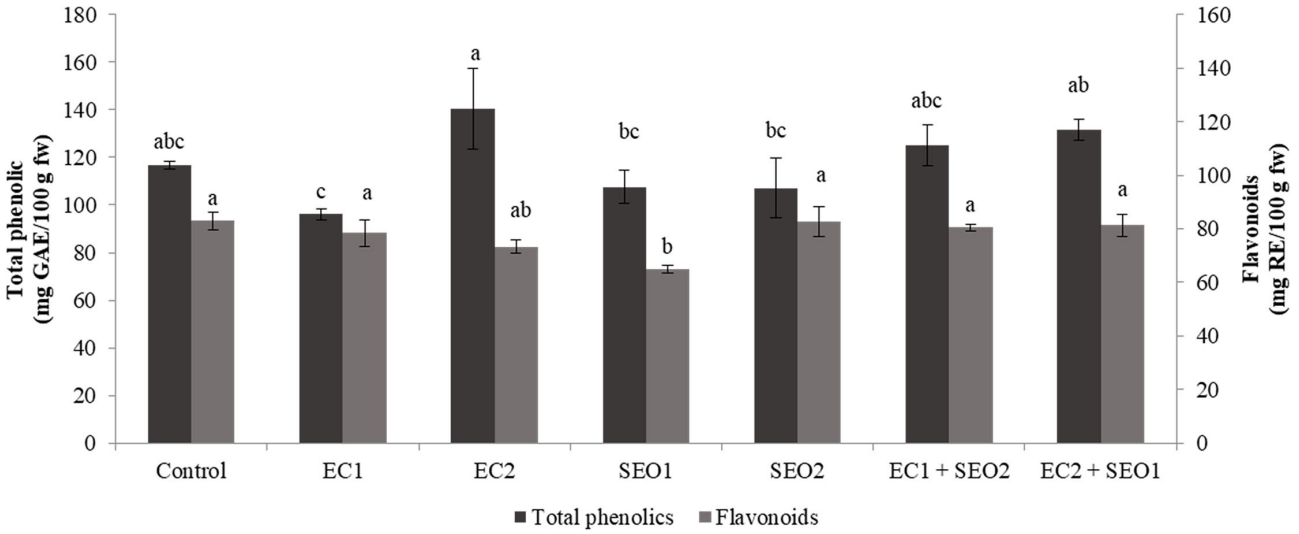


FIGURE 4 | Total phenolics (mg GAE/kg fw) and flavonoids (mg RE/kg fw) content of tomato fruits under control (C, 0.4 dS/m), saline (EC1, 3.5 dS/m; EC2, 7 dS/m), spent engine oil stress (SEO1, 0.5%; SEO2, 1%) and the combinations (EC1/SEO2, 3.5 dS/m EC/1% SEO), (EC2/SEO1, 7 dS/m EC/0.5% SEO). Values are means ($n = 3$).

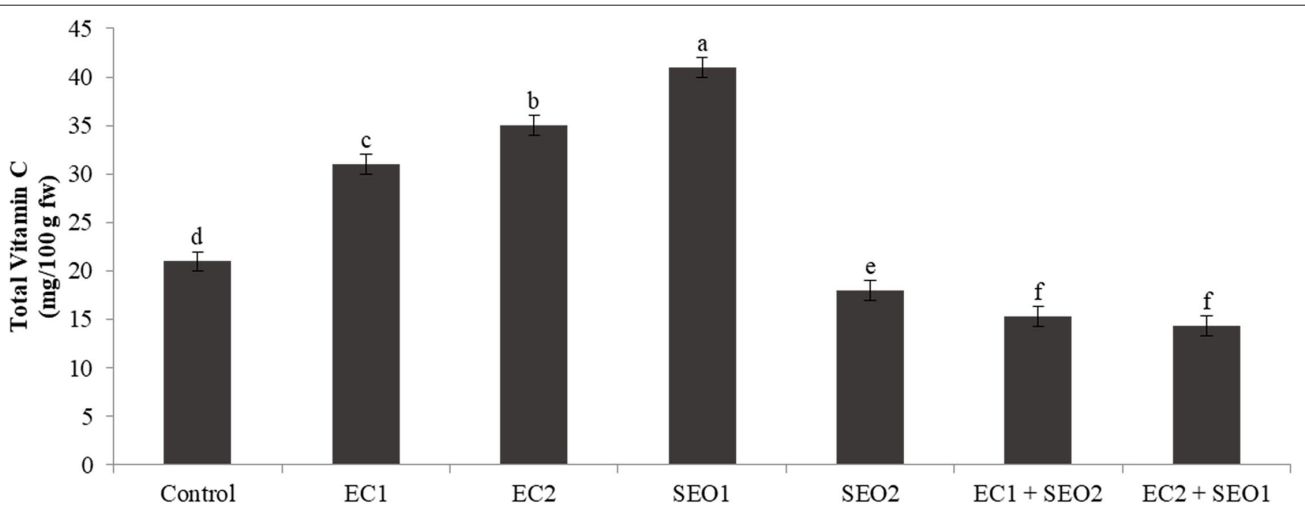


FIGURE 5 | Total vitamin C content (mg/kg fw) of tomato fruits under control (C, 0.4 dS/m), saline (EC1, 3.5 dS/m; EC2, 7 dS/m), spent engine oil stress (SEO1, 0.5%; SEO2, 1%) and the combinations (EC1/SEO2, 3.5 dS/m EC/1% SEO), (EC2/SEO1, 7 dS/m EC/0.5% SEO). Values are means ($n = 3$).

treatment. The content of ascorbic acid was reduced in the stems and roots of 3-week-old tomato seedlings exposed to spent lubrification oil, from 2.28 to 1.52 mg/g and from 1.87 to 1.25 mg/g, respectively (53). Muzolf-Panek et al. (48) reported a significant inverse correlation between Mn concentration and total vitamin C levels in ripe fruits of the tomato cvs Emotion F1 ($R = -0.603$) and Alboney F1 ($R = -0.668$).

Tocopherol Content

Tocopherols play a crucial role in the protection of plants against oxidative stress, with mechanisms that may vary depending upon its severity and duration. Under moderate stress or in the early phase of severe stress, tocopherols act as typical antioxidants, while in the late phase of severe stress they may assist the recovery and recycling of vital compounds for the plant (55). In addition, it is widely recognized that the increase in tocopherol content contributes to plant stress tolerance while a decrease is associated with stress susceptibility (56). In this study, α-, β-, and γ-tocopherol contents varied significantly among the applied treatments ($P < 0.05$) (**Figure 6**) within the ranges 10.23–31.69 mg/kg fw, 0.00–0.56 mg/kg fw, and 0.00–0.39 mg/kg fw, respectively. Compared to the control, EC and SEO individual treatments resulted in a 26–31% increase and a 19–56% decrease in the content of α-tocopherol, respectively. β-Tocopherol biosynthesis seems completely inhibited by SEO

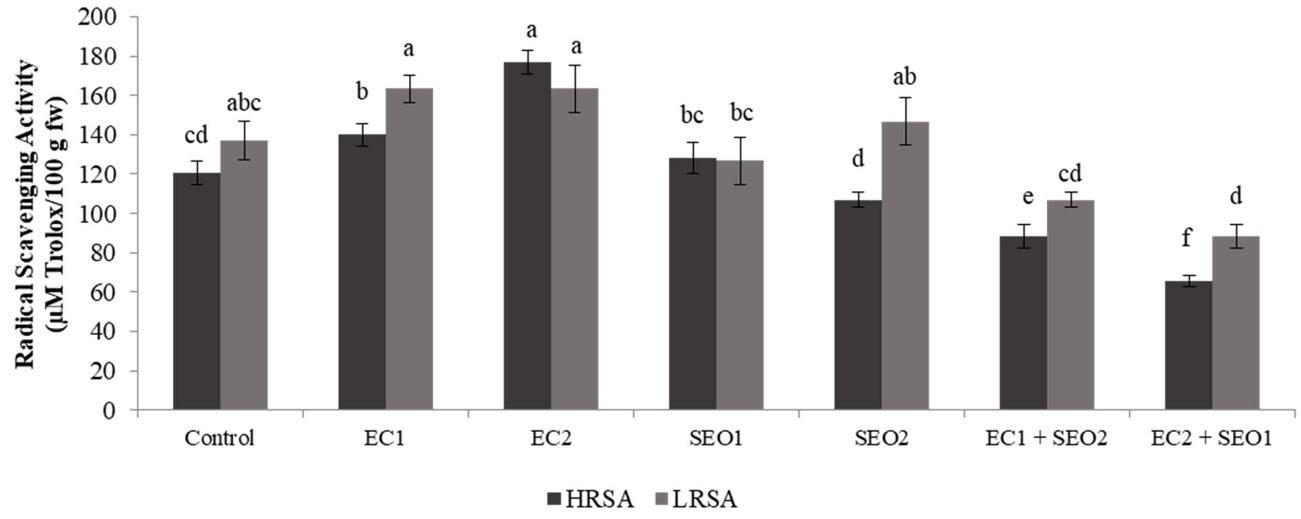


FIGURE 6 | α-, β-, and γ-ocopherol content (mg/kg fw) of tomato fruits under control (C, 0.4 dS/m), saline (EC1, 3.5 dS/m; EC2, 7 dS/m), spent engine oil stress (SEO1, 0.5%; SEO2, 1%) and the combinations (EC1/SEO2, 3.5 dS/m EC/1% SEO), (EC2/SEO1, 7 dS/m EC/0.5% SEO). Values are means ($n = 3$).

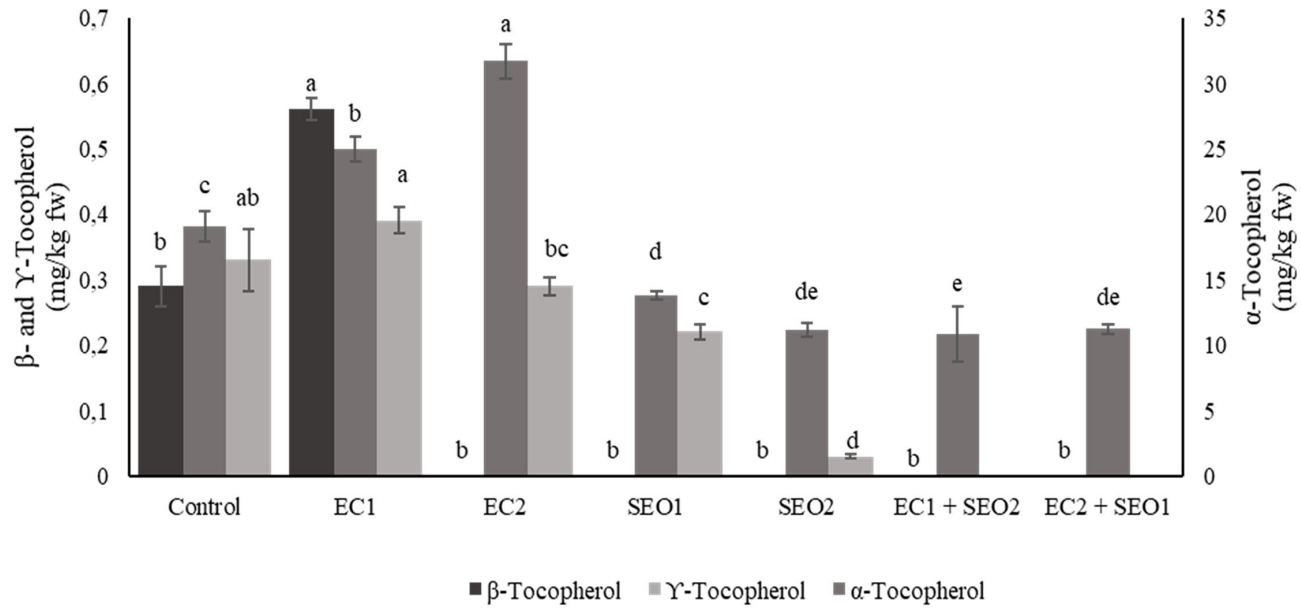


FIGURE 7 | HRSA and LRSA (µM Trlox/100 g fw) of tomato fruits under control (C, 0.4 dS/m), saline (EC1, 3.5 dS/m; EC2, 7 dS/m), spent engine oil stress (SEO1, 0.5%; SEO2, 1%) and the combinations (EC1/SEO2, 3.5 dS/m EC/1% SEO), (EC2/SEO1, 7 dS/m EC/0.5% SEO). Values are means ($n = 3$).

treatments when applied individually or in combination with ECs, and also by the EC2 treatment; however, a 93% increase in β-tocopherol concentration was observed under EC1. Similarly, a 24–86% decrease of γ-tocopherol content was observed in the fruits harvested from SEO-treated tomato plants, while it was not detected under both EC/SEO combined treatments. EC1 induced an 18% increase in γ-tocoperol but EC2 had a 26% reduction compared to the control. Spicher et al. (57) performed a lipidomic study to assess the effect of combined high temperature and light stress on *vte5* transgenic Microtom tomato plants and found that α-tocopherol and plastoquinone/plastoquinol biosynthetic pathways were strongly upregulated, among hundreds of targeted compounds, in response to the applied stresses. The authors concluded that VTE5 protects against combined high-light and high-temperature stress by positively modulating α-tocopherol production.

Hydrophilic and Lipophilic Radical Scavenging Activity

HRSA and LRSA varied significantly between the applied treatments ($P < 0.05$) within the values 65.3–176.7 and 68.3–163.4 µm Trolox/100 g fw (**Figure 7**) EC and SEO treatments

applied individually resulted in 16–26% increase and 16–28% decrease in the HRSA values, respectively, compared with the control, EC treatments applied individually or in combination with SEO resulted in 19.0–19.3% increase and 21–35% decrease in LRSA values, respectively. However, SEO applied individually induced conflicting responses: LRSA was reduced by 7.0% by SEO1 and increased by 7.1% by SEO2. The values obtained in control conditions are similar (116.7 μm trolox/100 g fw to 279.4 μm trolox/100 g fw) to our previous reports for different open-field tomato cvs (5, 39). Sumalan et al. (9), assessing the antioxidant profile of some salt-tolerant tomato landraces, found that the best cultivars in terms of functional quality were those grown under high concentrations of soil salinity ranging from 7.21 dS/m-6.58 dS/m such as landraces CN-254 and L-189b, respectively. Tommonaro et al. (58) assessed the phytochemical and nutritive features of the pulp and seeds from tomato fruits grown in muddy soils revealing high values of antioxidant activity, especially in the lipophilic fraction, with the absence of heavy metals and cytotoxic effect in both fractions. Muzolf-Panek et al. (48) reported that the antioxidant activity significantly increased with increasing Mn concentration (1.2–2.4 mg/dm^3) in tomato cvs Emoticon F1 and Alboney F1 fruits grown in hydroponics. Dursun (59) assessed the activities of ascorbate peroxidase, peroxidase, and superoxide dismutase in the leaves and roots of tomatoes cultivated under heavy metal (Cd, Cu, and Pb at 10, 20, and 50 ppm)-induced stress. The authors reported that ascorbate peroxidase activity in tomato roots changed depending on the heavy metal types and concentrations.

The response to SEO treatments may vary depending not only on treated plant species but also on other aspects including pollutant type, local concentrations, water solubility, plant age at the time of SEO contamination, and soil organic matter content and texture (60, 61).

The effect of combined stresses can be different from those of stresses applied individually, which suggests the importance to focus on how the interaction between stresses can affect the processing attributes and functional quality of tomato fruits.

Correlation Study

Many authors have examined the correlations between and among phytochemicals secondary metabolites and antioxidant activities in several fruits and vegetables, including tomatoes (5, 39). However, little is known concerning tomato cvs subjected to stress. The Pearson's correlation coefficients of all the assayed processing and functional attributes of tomato fruits subjected to EC and SEO individual and combined treatments are reported in **Table 3**. A significant positive correlation was evidenced between HRSA and β-carotene, lycopene, total vitamin C, α-tocopherol, γ-tocopherol, average fruit weight, soluble solids, and a*/b* ratio, as well as between LRSA and β-carotene, lycopene, α-tocopherol, γ-tocopherol, average fruit weight, soluble solids, and a*/b* ratio. Titratable acidity negatively correlated with both HRSA and LRSA. Interestingly, a*/b* ratio was positively and significantly ($P < 0.01$) correlated with β-carotene (R = 0.78), lycopene (R = 0.68), total vitamin C (R = 0.71), α-tocopherol (R = 0.83),

TABLE 3 | Pearson correlation coefficients of β-carotene (β-Crt), lycopene (Lyc), total phenolic compounds (TPC), total flavonoids (TF), total vitamin C (TVC), α-, β-, and γ-Tocopherol (α-, β-, and γ-T), hydrophilic radical scavenging activity (HRSA) and lipophilic radical scavenging activity (LRSA), total soluble solids (TSS), titratable acidity (TA), a*/b* ratio, average fruit weight (Avg. fruit wt), and yield per plant (Y/pl).

Traits[a]	β-Crt	Lyc	TPC	TF	TVC	α-T	β-T	γ-T	HRSA	LRSA	TSS	TA	(a*/b*)	Avg. fruit wt.	Y/pl.
β-Crt	1														
Lyc	0.76**	1													
TPC	−0.08^{ns}	0.014^{ns}	1												
TF	−0.49*	−0.49*	0.13^{ns}	1											
TVC	0.76**	0.79**	−0.16^{ns}	−0.68**	1										
α-T	0.49*	0.58**	0.11^{ns}	−0.19^{ns}	0.57**	1									
β-T	−0.27^{ns}	−0.37^{ns}	−0.084^{ns}	0.16^{ns}	−0.12^{ns}	0.16^{ns}	1								
γ-T	0.41^{ns}	0.39^{ns}	−0.26^{ns}	−0.28^{ns}	0.66**	0.78**	0.50*	1							
HRSA	0.77**	0.70**	−0.04^{ns}	−0.38^{ns}	0.76**	0.86**	0.11^{ns}	0.75**	1						
LRSA	0.63**	0.51*	−0.22^{ns}	0.05^{ns}	0.48*	0.64**	0.13^{ns}	0.63**	0.79**	1					
TSS	0.65**	0.73**	−0.02^{ns}	−0.52*	0.87**	0.83**	−0.02^{ns}	0.76**	0.87**	0.61**	1				
TA	−0.41^{ns}	−0.1^{ns}	0.36^{ns}	0.12^{ns}	−0.45*	−0.50*	−0.56**	−0.82**	−0.60**	−0.63**	−0.47*	1			
a*/b*	0.78**	0.68**	−0.07^{ns}	−0.33^{ns}	0.71**	0.83**	−0.05^{ns}	0.66**	0.93**	0.80**	0.84**	−0.55*	1		
Avg. fruit wt.	0.30^{ns}	0.09^{ns}	−0.25^{ns}	−0.03^{ns}	0.39^{ns}	0.66**	0.66**	0.88**	0.64**	0.62**	0.54*	−0.92**	0.56**	1	
Y/pl.	0.19 ^{ns}	−0.71 ^{ns}	−0.19 ^{ns}	0.70 ^{ns}	0.29	0.51*	0.66**	0.78**	0.51*	0.51*	0.36 ^{ns}	−0.88**	0.40 ^{ns}	0.91**	1

*ns = non-significant; *, ** = significant at P < 0.05 or 0.01, respectively.*

γ-tocopherol (R = 0.66), HRSA (R = 0.93), LRSA (R = 0.80), and soluble solids (R = 0.84), and can thus represent a good indicator of fruit quality even in fruits subjected to soil salt and SEO pollution.

CONCLUSION

In summary, EC treatments applied individually reduced average fruit weight but increased soluble solids, titratable acidity, a*/b* ratio, β-carotene, lycopene, total vitamin C, α-tocopherol, HRSA, and LRSA. SEO stress decreased average fruit weight, HRSA, α-tocopherol, γ-tocopherol, total phenolics, and flavonoids, but increased titratable acidity, a*/b* ratio, β-carotene, and lycopene. Similarly, to the processing attributes, our findings point out that the response to EC1/SEO2 and EC2/SEO1 combined treatments was idiosyncratic for β-carotene and dominated by SEO for lycopene, respectively; dominated by SEO and EC, respectively, for total phenolic, dominated by EC for flavonoids, and dominated by SEO and idiosyncratic for total vitamin C. An idiosyncrasy was observed for α- and γ-tocopherols under EC1/SEO2 and EC2/SEO1, respectively. However, the response was dominated by SEO for β-tocopherol. Regarding the RSA, the response was dominated by SEO and idiosyncratic for HRSA and totally idiosyncratic for LRSA. Although the alteration affecting the processing and functional quality of tomato fruits grown under EC and/or SEO, the produced fruits exhibited increased levels in various metabolites under moderate salinity stress, including β-carotene, lycopene, total phenolics, total vitamin C, tocopherols as well as the HRSA and LRSA. Under (EC2/SEO1, HRSA, and LRSA were severely affected and decreased by more than 25%. In addition, the a*/b* ratio was positively and significantly correlated with most assayed functional attributes metabolites and total soluble solids suggesting that this stress-induced fingerprint may be used to detect early soil contamination to avoid hazard compounds contamination in the food chain.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

RI, IT, and TR'h conceptualized the study and were in charge of tomatoes cultivation, fruit sampling, and reagent preparation. HD, RI, ZP, and LH contributed to the study conception and design, optimization of HPLC analysis, and made valuable recommendations and suggestions for HPLC peaks integration and the calculation of different bioactive compounds contents. ML, MS, MA, ZP, MD, and AM did statistical analysis, interpretation of data made recommendations, and suggestions regarding the article. All the authors contributed directly or indirectly to the study conception and design and interacted positively during the preparation of this article.

FUNDING

This research was supported by the Ministry of Innovation and Technology within the framework of the Thematic Excellence Programme 2020, Institutional Excellence Sub-programme (TKP2020-IKA-12), and the EFOP-3.6.3-VEKOP-16-2017-00008 project.

REFERENCES

1. Siddiqui MW, Lara I, Ilahy R, Tlili I, Ali A, Homa F, et al. Dynamic changes in health-promoting properties and eating quality during off-vine ripening of tomatoes. *Compr Rev Food Sci Food Saf.* (2018) 17:1540–60. doi: 10.1111/1541-4337.12395
2. Tan BL, Norhaizan ME. Carotenoids: how effective are they to prevent age-related diseases? *Molecules*. (2019) 24:1801. doi: 10.3390/molecules24091801
3. Dai XY, Li XW, Zhu SY, Li MZ, Zhao Y, Talukder M, et al. Lycopene ameliorates di (2-ethylhexyl) phthalate-Induced pyroptosis in spleen *via* suppression of classic caspase-1/NLRP3 pathway. *J Agric Food Chem*. (2021) 69:1291–9. doi: 10.1021/acs.jafc.0c06534
4. Liu H, Liu J, Liu Z, Wang Q, Liu J, Feng D, et al. Lycopene reduces cholesterol absorption and prevents atherosclerosis in ApoE–/–Mice by Downregulating HNF-1α and NPC1L1 Expression. *J Agric Food Chem.* (2021) 69:10114–20. doi: 10.1021/acs.jafc.1c03160
5. Ilahy R, Siddiqui MW, Tlili I, Montefusco A, Piro G, Hdider C, et al. When color really matters: horticultural performance and functional quality of high-lycopene tomatoes. *Crit Rev Plant Sci.* (2018) 37:15–53. doi: 10.1080/07352689.2018.1465631
6. Machado RMA, Serralheiro RP. Soil salinity: effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. *Horticulturae.* (2017) 3:30. doi: 10.3390/horticulturae3020030
7. Ben Ali K, Kanzari S, Lamloum S, Ilahy R, Ben Mariem S, Ben Nouna B. Salinity effect on the crop height, root depth and leaf index area of tomato crop in Tunisia Asia. *J Biol Sci.* (2019) 12:604–9. doi: 10.3923/ajbs.2019.604.609
8. Botella MÁ, Hernández V, Mestre T, Hellín P, García-Legaz MF, Rivero RM, et al. Bioactive compounds of tomato fruit in response to salinity, heat and their combination. *Agriculture.* (2021) 11:534. doi: 10.3390/agriculture11060534
9. Sumalan RM, Ciulca SI, Poiana MA, Moigradean D, Radulov I, Negrea M, et al. The antioxidant profile evaluation of some tomato landraces with soil salinity tolerance correlated with high nutraceuticaland functional value. *Agronomy*. (2020) 10:500. doi: 10.3390/agronomy10040500
10. Falciglia PP, De Guidi G, Catalfo A, Vagliasindi FG. Remediation of soils contaminated with PAHs and nitro-PAHs using microwave irradiation. *Chem Eng J*. (2016) 296:162–72. doi: 10.1016/j.cej.2016.03.099
11. Ogunlaja A, Ogunlaja OO, Okewole DM, Morenikeji OA. Risk assessment and source identification of heavy metal contamination by multivariate and hazard index analyses of a pipeline vandalised area in Lagos State, Nigeria. *Sci Total Environ*. (2019) 651:2943–52. doi: 10.1016/j.scitotenv.2018.09.386

12. *European Environment Agency*. (2021). Available online at: https://www.eea.europa.eu/about-us/eea-eionet-strategy-2021-2030-1
13. Belvederesi C, Thompson MS, Komers PE. Statistical analysis of environmental consequences of hazardous liquid pipeline accidents. *Heliyon*. (2018) 4 e00901. doi: 10.1016/j.heliyon.2018.e00901
14. Salam LB, Obayori SO, Nwaokorie FO, Suleiman A, Mustapha R. Metagenomic insights into effects of spent engine oil perturbation on the microbial community composition and function in a tropical agricultural soil. *Environ Sci Pollut Res*. (2017) 24:7139–59. doi: 10.1007/s11356-017-8364-3
15. Adenipekun CO, Oyetunji OJ, Kassim LQ. Screening of Abelmoschus esculentus L. Moench for tolerance to spent engine oil. *J Appl Biosci*. (2009) 20:1131–7.
16. Onwusiri KC, Aguoru CU, Akomolafe GF. Effect of spent engine oil on germination and growth parameters of fluted pumpkin (Telfairia occidentalis Hook F.) in Makurdi, Benue State, Nigeria. *J Res For Wildl Environ*. (2017) 9:1–8.
17. Osuagwu ES, Olaifa E. Effects of oil spills on fish production in the Niger Delta. *PLoS ONE*. (2018) 13:e0205114. doi: 10.1371/journal.pone.0205114
18. Ani E, Adekunle AA, Kadiri AB, Njoku KL. Effect of macrophomina phaseolina, organic manure and spent engine oil on Luffa aegyptica (Mill). *Bayero J Pure Appl Sci*. (2018) 11:138–42. doi: 10.4314/bajopas.v11i1.24
19. Nwachukwu MO, Azorji JN, Adjero LA, Green MC, Igwe CE, Nnadozie RIA. Influence of spent engine oil pollution and organic amendment on soil physicochemical properties, microbial population and growth of capsicum annuum (L.). *Asian Soil Res J*. (2020) 17–25. doi: 10.9734/asrj/2020/v3i130064
20. Odjegba VJ, Idowu FS. Effect of spent engine oil on the germination and growth of Amaranthus hybridus L. *Biosci Res Commun*. (2002) 14:101–5.
21. Daood HG, Bencze G, Palotas G, Pék, Z, Sidikov A, et al. HPLC analysis of carotenoids from tomatoes using cross-linked C18 column and MS detection. *J Chromatogr Sci*. (2014) 52:985–91. doi: 10.1093/chromsci/bmt139
22. Martínez-Valverde I, Periago MJ, Provan G, Chesson A. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (Lycopersicum esculentum). *J Sci Food Agric*. (2002) 82:323–30. doi: 10.1002/jsfa.1035
23. Asami DK, Hong YJ, Barrett DM, Mitchell AE. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. *J Agric Food Chem*. (2003) 51:1237–41. doi: 10.1021/jf020635c
24. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*. (1999) 64:555–9. doi: 10.1016/S0308-8146(98)00102-2
25. Kampfenkel K, Van Montagu M, Inzé D. Effects of iron excess on Nicotiana plumbaginifolia plants (implications to oxidative stress). *Plant Physiol*. (1995) 107:725–35. doi: 10.1104/pp.107.3.725
26. Abushita AA, Hebshi EA, Daood HG, Biacs PA. Determination of antioxidant vitamins in tomatoes. *Food Chem*. (1997) 60:207–12. doi: 10.1016/S0308-8146(96)00321-4
27. Duah SA, e Souza CS, Daood HG, Pék Z, Neményi A, Helyes L. Content and response to γ-irradiation before over-ripening of capsaicinoid, carotenoid, and tocopherol in new hybrids of spice chili peppers. *LWT*. (2021) 147:111555. doi: 10.1016/j.lwt.2021.111555
28. Miller NJ, Rice-Evans CA. Factors influencing the antioxidant activity determined by the ABTS•+ radical cation assay. *Free Radic Res*. (1997) 26:195–9. doi: 10.3109/10715769709097799
29. Sivaram AK, Subashchandrabose SR, Logeshwaran P, Lockington R, Naidu R, Megharaj M. Metabolomics reveals defensive mechanisms adapted by maize on exposure to high molecular weight polycyclic aromatic hydrocarbons. *Chemosphere*. (2019) 214:771–80. doi: 10.1016/j.chemosphere.2018.09.170
30. Xia L, Xiaodong M, Yunhe C, Junxiang L, Junzhu Z, Feifei Z, et al. Transcriptomic and metabolomic insights into the adaptive response of salix viminalis to phenanthrene. *Chemosphere*. (2021) 262:127573. doi: 10.1016/j.chemosphere.2020.127573
31. Yurtseven E, Kesmez GD, Ünlükara A. The effects of water salinity and potassium levels on yield, fruit quality and water consumption of a native central anatolian tomato species (Lycopersicon esculantum). *Agric Water Manag*. (2005) 78:128–35. doi: 10.1016/j.agwat.2005.04.018
32. Molina L, Segura A. Biochemical and metabolic plant responses toward polycyclic aromatic hydrocarbons and heavy metals present in atmospheric pollution. *Plants*. (2021) 10:2305. doi: 10.3390/plants10112305
33. Tadesse T, Nichols MA, Fisher KJ. Nutrient conductivity effects on sweet pepper plants grown using a nutrient film technique: 1. Yield and fruit quality. *NZJ Crop Hortic Sci*. (1999) 27:229–37. doi: 10.1080/01140671.1999.9514101
34. Savvas D, Stamati E, Tsirogiannis IL, Mantzos N, Barouchas PE, Katsoulas N, et al. Interactions between salinity and irrigation frequency in greenhouse pepper grown in closed-cycle hydroponic systems. *Agric Water Manag*. (2007) 91:102–11. doi: 10.1016/j.agwat.2007.05.001
35. Sharma RK, Agrawal M. Biological effects of heavy metals: an overview. *J Environ Biol*. (2005) 26:301–13.
36. Nagajyoti PC, Lee KD, Sreekanth TVM. Heavy metals, occurrence and toxicity for plants: a review. *Environ Chem Lett*. (2010) 8:199–216. doi: 10.1007/s10311-010-0297-8
37. Helyes L, Pék Z, Lugasi A. Tomato fruit quality and content depend on stage of maturity. *HortScience*. (2006) 41:1400–01. doi: 10.21273/HORTSCI.41.6.1400
38. Prasch CM, Sonnewald U. Signaling events in plants: stress factors in combination change the picture. *Environ Exp Bot*. (2015) 114:4–14. doi: 10.1016/j.envexpbot.2014.06.020
39. Ilahy R, Piro G, Tlili I, Riahi A, Sihem R, Ouerghi I, et al. Fractionate analysis of the phytochemical composition and antioxidant activities in advanced breeding lines of high-lycopene tomatoes. *Food Funct*. (2016) 7:574–83. doi: 10.1039/C5FO00553A
40. Sellitto VM, Golubkina NA, Pietrantonio L, Cozzolino E, Cuciniello A, Cenvinzo V, et al. Tomato yield, quality, mineral composition and antioxidants as affected by beneficial microorganisms under soil salinity induced by balanced nutrient solutions. *Agriculture*. (2019) 9:110. doi: 10.3390/agriculture9050110
41. Moles TM, de Brito Francisco R, Mariotti L, Pompeiano A, Lupini A, Incrocci L, et al. Salinity in autumn-winter season and fruit quality of tomato landraces. *Front Plant Sci*. (2019) 10:1078. doi: 10.3389/fpls.2019.01078
42. Borghesi E, Ferrante A, Gordillo B, Rodríguez-Pulido FJ, Cocetta G, Trivellini A, et al. Comparative physiology during ripening in tomato rich-anthocyanins fruits. *Plant Growth Regul*. (2016) 80:207–14. doi: 10.1007/s10725-016-0158-y
43. Serio F, Gara LD, Caretto S, Leo L, Santamaria P. Influence of an increased NaCl concentration on yield and quality of cherry tomato grown in posidonia (Posidonia oceanica (L) Delile). *J Sci Food Agric*. (2004) 84:1885–90. doi: 10.1002/jsfa.1883
44. Hashem HA, Hassanein RA, El-Deep MH, Shouman AI. Irrigation with industrial wastewater activates antioxidant system and osmoprotectant accumulation in lettuce, turnip and tomato plants. *Ecotoxicol Environ Saf*. (2013) 95:144–52. doi: 10.1016/j.ecoenv.2013.05.030
45. Baszyński T, Wajda L, Krol M, Wolińska D, Krupa Z, Tukendorf A. Photosynthetic activities of cadmium-treated tomato plants. *Physiol Plant*. (1980) 48:365–70. doi: 10.1111/j.1399-3054.1980.tb03269.x
46. Hatata MM, Abdel AE. Oxidative stress and antioxidant defense mechanisms in response to cadmium treatments. *American-Eurasian J Agric Environ Sci*. (2008) 4:655–69.
47. Cherif J, Mediouni C, Ammar WB, Jemal F. Interactions of zinc and cadmium toxicity in their effects on growth and in antioxidative systems in tomato plants (Solarium lycopersicum). *J Environ Sci*. (2011) 23:837–44. doi: 10.1016/S1001-0742(10)60415-9
48. Muzolf-Panek M, Kleiber T, Kaczmarek A. Effect of increasing manganese concentration in nutrient solution on the antioxidant activity, vitamin C, lycopene and polyphenol contents of tomato fruit. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*. (2017) 34:379–89. doi: 10.1080/19440049.2016.1277037
49. Ilahy R, Tlili I, Siddiqui MW, Hdider C, Lenucci MS. Inside and beyond color: comparative overview of functional quality of tomato and watermelon fruits. *Front Plant Sci*. (2019) 10:769. doi: 10.3389/fpls.2019.00769
50. Toscano S, Trivellini A, Cocetta G, Bulgari R, Francini A, Romano D, et al. Effect of preharvest abiotic stresses on the accumulation of bioactive compounds in horticultural produce. *Front Plant Sci*. (2019) 10:1212. doi: 10.3389/fpls.2019.01212

51. Akladious SA, Mohamed HI. Ameliorative effects of calcium nitrate and humic acid on the growth, yield component and biochemical attribute of pepper (Capsicum annuum) plants grown under salt stress. *Sci Hortic.* (2018) 236:244–50. doi: 10.1016/j.scienta.2018.03.047
52. Yildiztugay E, Ozfidan-Konakci C, Kucukoduk M, Turkan I. Flavonoid naringenin alleviates short-term osmotic and salinity stresses through regulating photosynthetic machinery and chloroplastic antioxidant metabolism in Phaseolus vulgaris. *Front Plant Sci.* (2020) 11:682. doi: 10.3389/fpls.2020.00682
53. Aguebor-Ogie BN, Osagie OA, Oriaki K, Ezeugwu N, Aguebor O, Nuntah LC. Effect of different fractions of spent lubricating oil on some anti-oxidant properties and anti-oxidant enzymes of radicle and stem of germinating tomato (Solanum lycopersicum). *NISEB J.* (2012) 12:97–102.
54. Gest N, Gautier H, Stevens R. Ascorbate as seen through plant evolution: the rise of a successful molecule? *J Exp Bot.* (2013) 64:33–53. doi: 10.1093/jxb/ers297
55. Skłodowska M, Gapińska M, Gajewska E, Gabara B. Tocopherol content and enzymatic antioxidant activities in chloroplasts from NaCl-stressed tomato plants. *Acta Physiol Plant*. (2009) 31:393–400. doi: 10.1007/s11738-008-0248-1
56. Gossett DR, Banks SW, Millhollon EP, Lucas MC. Antioxidant response to NaCl stress in a control and a NaCl-tolerant cotton cell line grown in the presence of paraquat, buthionine sulfoximine, and exogenous glutathione. *Plant Physiol.* (1996) 112:803–9. doi: 10.1104/pp.112.2.803
57. Spicher L, Almeida J, Gutbrod K, Pipitone R, Dörmann P, Glauser G, et al. Essential role for phytol kinase and tocopherol in tolerance to combined light and temperature stress in tomato. *J Exp Bot*. (2017) 68:5845–56. doi: 10.1093/jxb/erx356
58. Tommonaro G, Nicolaus B, De Prisco R, Pergamo R, Marra N, Caporale A, et al. Evaluation of heavy metals, cytotoxicity, and antioxidant activity of tomatoes grown in toxic muddy soils. *Environ Sci Pollut Res*. (2015) 22:5756–61. doi: 10.1007/s11356-014-3861-0
59. Dursun KISA. The responses of antioxidant system against the heavy metal-induced stress in tomato. *Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi*. (2018) 22:1–6. doi: 10.19113/sdufbed.52379
60. Czerniawska-Kusza I, Ciesielczuk T, Kusza G, Cichoń A. Comparison of the Phytotoxkit microbiotest and chemical variables for toxicity evaluation of sediments. *Environ Toxicol*. (2006) 21:367–72. doi: 10.1002/tox.20189
61. Korade DL, Fulekar MH. Effect of organic contaminants on seed germination of Lolium multiflorum in soil. *Biol Med*. (2009) 1:28–34.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Ilahy, Tlili, Pék, Montefusco, Daood, Azam, Siddiqui, R'him, Durante, Lenucci and Helyes. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 27 May 2022
doi: 10.3389/fnut.2022.902208

Biocompatible Polyelectrolyte Complex Nanoparticles for Lycopene Encapsulation Attenuate Oxidative Stress-Induced Cell Damage

Dongjing Zhang[1,2†], Yun Jiang[1†], Ming Xiang[1], Fen Wu[1], Min Sun[1], XianFeng Du[3] and Lei Chen[1*]*

[1] Anhui Key Laboratory of Eco-Engineering and Biotechnology, School of Life Sciences, Anhui University, Hefei, China, [2] School of Biological and Food Engineering, Suzhou University, Suzhou, China, [3] State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei, China

OPEN ACCESS

Edited by:
Spyridon Alexandros Petropoulos, University of Thessaly, Greece

Reviewed by:
Andrey Cherstvy, University of Potsdam, Germany
Luigi Castaldo, University of Naples Federico II, Italy

***Correspondence:**
XianFeng Du
dxf@ahau.edu.cn
Lei Chen
sunang.32@163.com

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: 22 March 2022
Accepted: 25 April 2022
Published: 27 May 2022

Citation:
Zhang D, Jiang Y, Xiang M, Wu F, Sun M, Du X and Chen L (2022) Biocompatible Polyelectrolyte Complex Nanoparticles for Lycopene Encapsulation Attenuate Oxidative Stress-Induced Cell Damage. Front. Nutr. 9:902208. doi: 10.3389/fnut.2022.902208

In this study, lycopene was successfully encapsulated in polyelectrolyte complex nanoparticles (PEC NPs) fabricated with a negatively charged polysaccharide, TLH-3, and a positively charged sodium caseinate (SC) *via* electrostatic interactions. Results showed that the lycopene-loaded PEC NPs were spherical in shape, have a particle size of 241 nm, have a zeta potential of −23.6 mV, and have encapsulation efficiency of 93.6%. Thus, lycopene-loaded PEC NPs could serve as effective lycopene carriers which affected the physicochemical characteristics of the encapsulated lycopene and improved its water dispersibility, storage stability, antioxidant capacity, and sustained release ability in aqueous environments when compared with the free lycopene. Moreover, encapsulated lycopene could enhance the cells' viability, prevent cell apoptosis, and protect cells from oxidative damage through the Nrf2/*HO-1*/AKT signalling pathway, *via* upregulation of antioxidase activities and downregulation of MDA and ROS levels. Therefore, the biocompatible lycopene-loaded PEC NPs have considerable potential use for the encapsulation of hydrophobic nutraceuticals in the food and pharmaceutical industries.

Keywords: lycopene, PEC NPs, storage stability, sustained release, antioxidant capacity

INTRODUCTION

Lycopene is a carotenoid primarily found in ripe tomatoes and carrots (1), and it has been widely used as a colourant, an antioxidant, and a flavouring agent in the food industry. Lycopene, as a functional ingredient, has attracted considerable academic attention because of its antioxidant, anti-inflammatory, and antitumor effects on humans, and it can reduce the risk of prostate cancer and cardiovascular disease (2). However, lycopene is a hydrophobic compound with low water-solubility, gastrointestinal instability, high photosensitivity, thermal sensitivity, and inferior bioavailability, which limits its practical application in functional foods and health care products (3). Thus, an effective delivery system is necessary to improve lycopene absorption and bioavailability. Researchers have made substantial attempts to enhance the bioavailability and functionality of lycopene by using various delivery carriers or vehicles such as nanocomplexes, emulsions, nanoemulsions, polymeric micelles, liposomes, and nanostructured

lipid carriers (NLCs) (4–9). Polyelectrolyte complexes that are based on oppositely charged polysaccharides and proteins seem to be ideal microcarriers, which can improve the water solubility, stability, compatibility, and bioavailability of lycopene (10). In recent years, the usage of PE complexation and biomolecular delivery via liposomes and lipid nanoparticles has received attention, particularly from the viewpoint of ES-driven complexation. Cherstvy (11) presented the exact solution of the linear Poisson–Boltzmann equation for several problems relevant to electrostatics of DNA complexes with cationic lipids. Caetano (12) investigated the adsorption properties of hen egg-white lysozyme into a negatively charged silica pore by using a coarse-grained model and using constant-pH Monte Carlo simulations.

Casein is an easily digestible protein derived from milk, and it has been extensively utilised in the food industry as a flavouring agenet, a colourant, and a preservative. The properties, including remarkable surface activity, stability, emulsification, and self-assembly, promote the combining capacity with ions and small molecules and facilitate their functionality in drug delivery systems (13, 14). In addition, casein can improve the bioavailability of bioactive substances because of its shielding capabilities, which are essential for protecting sensitive payloads. Casein can polymerise with polyanions through electrostatic interaction to form nanoparticle polymers, which can be used as carrier materials for the transport of bioactive substances (10). We used sodium caseinate (SC) which exists in the form of protein particles through self-assembly in an aqueous solution with a diameter of 10–20 nm, which has good emulsification, thermal stability, film formation, and rheological properties (15). Compared with other food proteins, SC can form a thick sterically stabilising layer on the emulsion droplet interface that protects newly formed droplets against flocculation and coalescence (16). Thus, encapsulating bioactive compounds into SC nanoparticles can improve water dispersibility and physicochemical stability. Tricholoma lobayense, a delicious edible mushroom, has been commonly utilised as a functional food in China and other Asian nations for its taste and health values (17). Polysaccharide TLH-3 isolated from Tricholoma lobayense is an acidic polysaccharide with a molecular weight of 4.22×10^3 Da, which is primarily composed of 1,3-linked-α-d-glucopyranosyl branched at C-6 and 1,3-linked-β-d-galactopyranosyl (18). Our previous studies have proven that polysaccharide TLH-3 exerts excellent antioxidant, anti-ageing, tumour-suppressing, and immunoregulatory abilities (17, 19). TLH-3 exists in an aqueous solution in the form of negatively charged polyanions and forming natural polymer polysaccharide hydrosol, which has good water solubility, emulsification, and stability (18). Thus, TLH-3 can form stable polyelectrolyte complex nanoparticles (PEC NPs) with polyanions such as SC in aqueous media for lycopene encapsulation. However, to our best knowledge, no studies have been conducted on the construction and utilisation of PEC via polyanion SC and polysaccharide TLH-3 for lycopene to improve solubility, bioactivity, and bioavailability of lycopene.

At present, considerable evidence suggests that, when oxidation and antioxidants are uneven in the body, the elevated production of reactive oxygen species (ROS) causes disruptions in the normal mechanism involved in cellular signalling pathways, leading to cellular dysfunction and apoptotic cell death (20, 21). The pivotal signal transduction pathway, such as the PI3K/AKT pathway *in vivo*, is involved in multiple cellular processes such as cell growth and survival induced by oxidative stress, and it regulates the expression of various inflammatory mediators and cytokines (22, 23). Studies have found that chronic diseases such as cancer, diabetes, cardiovascular disease, and neurodegenerative diseases (24) are related to oxidative stress, and ameliorating oxidative stress has become an effective way to alleviate these chronic diseases. Therefore, finding drugs with strong antioxidant activity is important to relieve various diseases caused by oxidative stress.

In this study, we aimed to construct stable PEC NPs with negatively charged TLH-3 and positively charged SC in an aqueous solution. Lycopene was incorporated into PEC NPs, and the storage stability, controlled release, and antioxidant activity of lycopene in PEC NPs were evaluated. Moreover, the protective effect of lycopene-loaded PEC NPs on H_2O_2-induced cellular oxidative damage and the underlying mechanism were also investigated for the first time. Thus, lycopene-loaded PEC NPs might constitute a potential health supplement or pharmaceutical product to improve human health and well-being in the food and pharmaceutical industries.

MATERIALS AND METHODS

Materials and Chemicals

Lycopene (with a purity of ≥98%, product codes: 820354) and sodium caseinate (with a purity of ≥98.07%, product codes: Z3625) were purchased from Sigma Corporation (St Louis, MO, USA). Polysaccharide TLH-3 was prepared according to the method described in our previous study (16). The degree of branching (DB) value and weight-average molecular weight (Mw) were 0.74 and 4.22 × 103 g/mol, respectively. Human normal hepatocytes (L02 cells) were purchased from the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). 2, 4, 6-tris (2-pyridyl)-s-triazine, 2-diphenyl-1-picrylhydrazyl (DPPH), and 2, 2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All primary and secondary antibodies were purchased from Abcam (Abcam, Cambridge, UK). All other chemicals and solvents were of an analytical grade or higher and were obtained from commercial sources.

Abbreviations: PEC NPs, Polyelectrolyte complex nanoparticles; SC, sodium caseinate; ROS, Reactive oxygen species; DPPH, 2-diphenyl-1-picrylhydrazyl; ABTS, 2, 2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid); PDI, Polydispersity index; DLS, Zetasizer dynamic light scattering detector; FTIR, Fourier transform infrared; DSC, Differential scanning calorimetry; XRD, X-ray diffraction; TEM, A transmission electron microscope; SGF, Simulated gastric fluid; SIF, Simulated intestinal fluid; Vc, Vitamin C; DCFH-DA, 2,7-Dichlorodi-hydrofluorescein diacetate; Nrf2, Nuclear factor erythroid 2-related factor 2.

Preparation of PEC NPs and Lycopene-Loaded PEC NPs

Sodium caseinate and TLH-3 were dissolved in deionised water and magnetically stirred overnight to completely hydrate SC and TLH-3 powders. Then, the obtained solutions were passed through a 0.45 μm filtration membrane to prepare stock solutions of SC and TLH-3 (10 mg/ml). The TLH-3 solution (10 mg/ml) was added to the SC solution (10 mg/ml) and magnetically stirred at various mass ratios (TLH-3:SC = 1:10, 2:10, 3:10, 4:10 w/w). The pH was adjusted to the desired value (2, 3, 4, 5, 6, and 7) by adding an aqueous solution containing 0.1 mol/L of HCl and/or 1 mol/L of NaOH. The obtained TLH-3/SC mixture was lyophilised to yield the final PEC NPs after the solution was finally subjected to ultrasonic treatment for 1 h. The effects of the TLH-3/SC mass ratio and pH on PEC NP formation were studied and recorded.

Lycopene was added to the SC solution in different proportions (4, 8, 12, and 16% W/W). After stirring and mixing, the solution was ultrasonically treated for 1 h. The pH value was adjusted to 3 with 1 mol/L of HCl. Then, TLH-3 solution (10 mg/ml) was added to a mixed solution at various mass ratios (1:10, 2:10, 3:10, and 4:10 w/w) and then magnetically stirred. Free lycopene was removed by centrifugation (5,000 rpm, 15 min), and the final lycopene-loaded PEC NPs were obtained by freeze-drying. The detailed schematic for the production of lycopene-loaded PEC NPs is shown in **Figure 1.**

Characterisation of PEC NPs and Lycopene-Loaded PEC NPs

Polyelectrolyte NPs and lycopene-loaded PEC NPs were diluted with deionised water. Then, the zeta potential, Z-average size, and polydispersity index of PEC NPs and lycopene-loaded PEC NPs were measured by using a Zetasizer dynamic light scattering detector and Zeta Siser Nano Series (Malvern, United Kingdom). All measurements were performed at least three times at 25°C, and the results were averaged. Fourier transform infrared (FT-IR) spectroscopy was used to characterise the chemical structure of lycopene, SC, TLH-3, PEC NPs, and lycopene-loaded PEC NP samples. The dried samples were ground with potassium bromide powder and pressed into a pellet for spectrometric measurement. FT-IR spectroscopy was performed using a VERTEX 80 FT-IR spectrometer (Bruker Co., Ettlingen, Germany) within the wavelength range of 400–4,000 cm^{-1}. Differential scanning calorimetry (DSC) thermograms of lycopene, PEC NPs, and lycopene-loaded PEC NPs were measured using a DSC Q2000 thermal analyser (TA Co., USA). Five milligrammes of samples were placed in aluminium capsules and sealed with aluminium lids. Subsequently, thermal analysis was performed in a dry

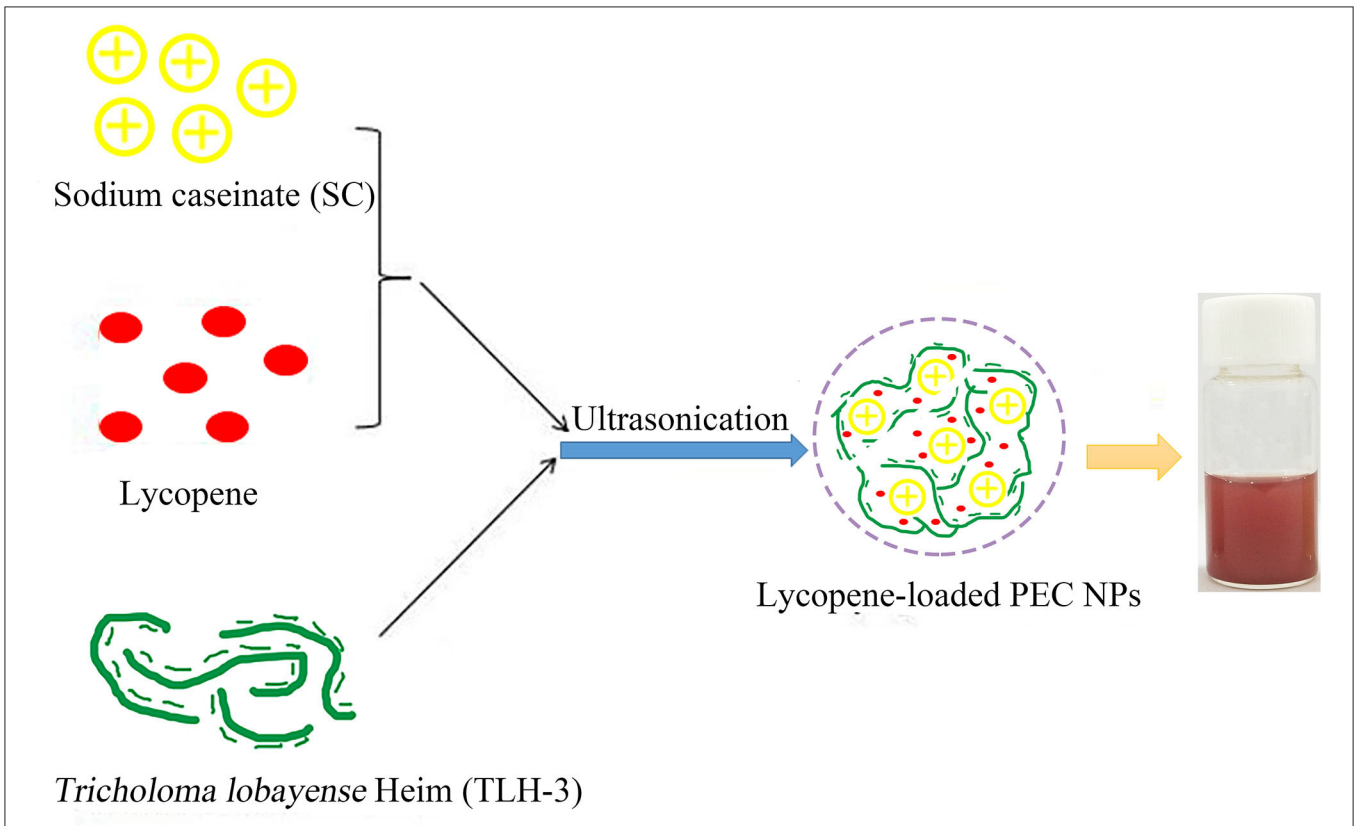


FIGURE 1 | The detailed schematic for the production of lycopene-loaded polyelectrolyte complex nanoparticles (PEC NPs).

nitrogen atmosphere with a flow rate of 50 ml·min^{-1}, and the temperature was increased from 25 to 300°C at a heating rate of 10°C·min^{-1}. The crystalline and amorphous nature of lycopene, PEC NPs, and lycopene-loaded PEC NPs were analysed by X-ray diffraction (XRD) (SmartLab 9KW, JEOL, Co., Japan). XRD patterns were recorded at 45 kV and 200 mA within the 2θ range of 5–50° at a scanning rate of 4°·min^{-1}. Thermogravimetric analysis of lycopene, PEC NPs, and lycopene-loaded PEC NP samples (5 mg) was performed on a thermogravimetric analyser (TG 209F3, Netzsch, Germany) at a heating rate

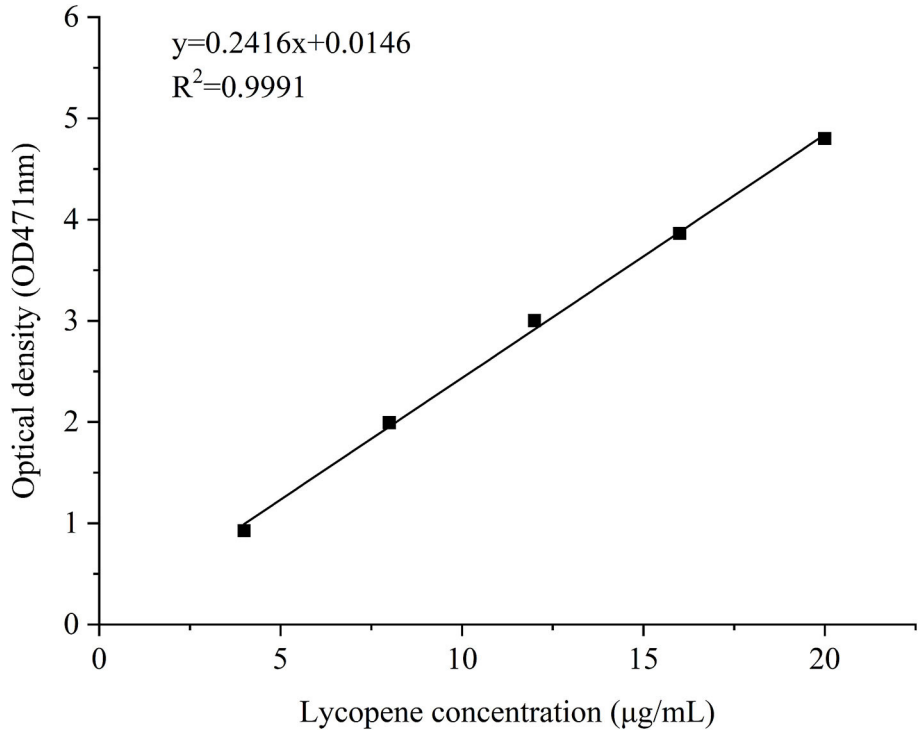


FIGURE 2 | A standard curve of free lycopene.

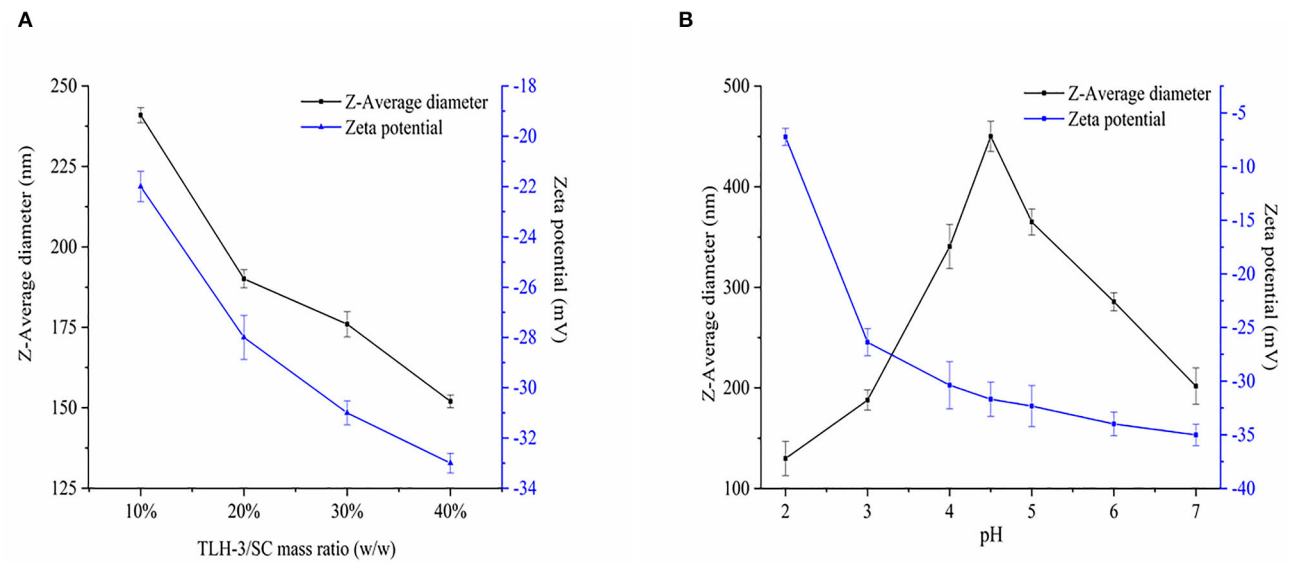


FIGURE 3 | Effects of TLH-3/SC mass ratio **(A)** and pH **(B)** on the Z-average diameter, and Zeta potential of TLH-3/SC PEC NPs. Data represent the mean ± standard deviation (SD, $n = 3$).

of 20°C·min^{-1} under nitrogen with a mass flow rate of 40 mL·min^{-1} at 40 to 800°C. A transmission electron microscope (TEM; JEM-1400flash, JEOL, 120 kV) was used to observe the morphology and particle size of PEC NPs and lycopene-loaded PEC NPs. For TEM detection, the samples were diluted with ultra-pure water and then dripped onto copper wires to dry before testing.

Encapsulation Efficiency (EE) and Loading Content (LC) of Lycopene

Polyelectrolyte NP samples were prepared in accordance with Method 2.3, and freshly prepared lycopene-loaded PEC NPs were centrifuged at 10,000 rpm for 10 min to precipitate unencapsulated lycopene. The supernatant was collected, and ethyl acetate was added to the precipitate until it was completely dissolved. The concentration of lycopene in the collected supernatant was determined by spectrophotometry at a wavelength of 471 nm using a standard curve of free lycopene ($y = 0.2461x + 0.0146$, $R^2 = 0.9991$) (**Figure 2**). The EE and LC of lycopene were determined as described previously with minor modifications (25).

All experiments were repeated three times at room temperature. Calculations were performed in accordance with the following equations:

$$EE\,(\%) = \frac{Lycopene\ encapsulated\ in\ PEC\ NPs}{Total\ weight\ of\ lycopene} \times 100 \quad (1)$$

$$LC\,(\%) = \frac{Lycopene\ encapsulated\ in\ PEC\ NPs}{Total\ weight\ of\ lycopene - loaded\ PEC\ NPs} \times 100 \quad (2)$$

Storage Stability

The storage stability of lycopene and lycopene-loaded PEC NPs at different temperatures and the light was estimated. The thermal stability of lycopene and lycopene-loaded PEC NPs was estimated in accordance with previous studies with slight modifications (25, 26). In brief, the solutions of lycopene and lycopene-loaded PEC NPs were stored in a sealed glass bottle under dark conditions and then incubated at 0, 25, 45, 65, and 85°C for 0.5 h in a

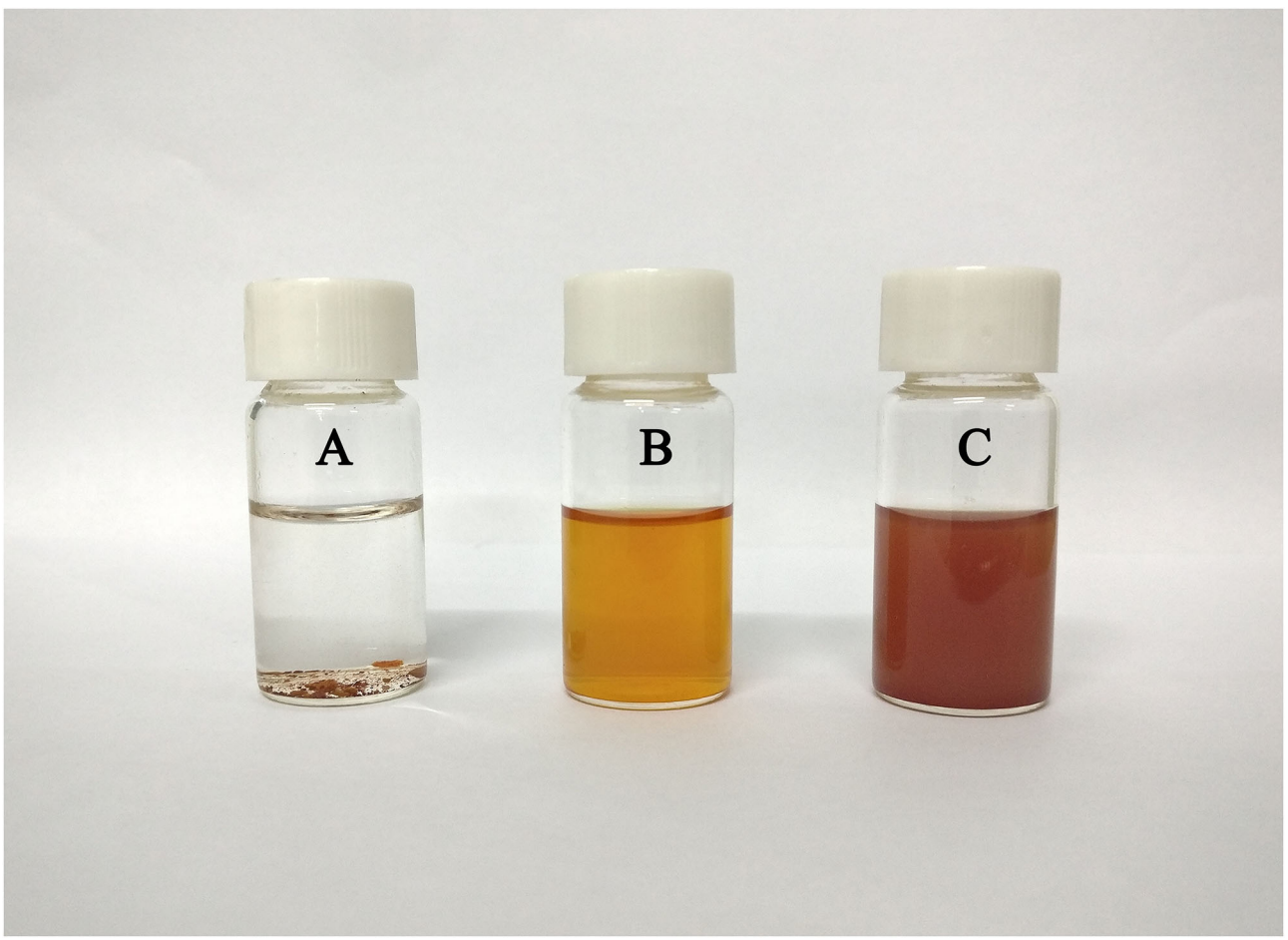


FIGURE 4 | Solubility of lycopene: **(A)** lycopene in distilled water, **(B)** lycopene in acetone, and **(C)** lycopene-loaded PEC NPs in distilled water.

thermostatic water bath. All samples were equilibrated at room temperature for 10 min before analysis. The light stability of lycopene and lycopene-loaded PEC NPs was estimated using a light incubator in accordance with a previous study (27). In brief, the solutions of lycopene and lycopene-loaded PEC NPs were placed in a light incubator and exposed to a lamp (10 W) for different durations (0, 5, 10, 15, and 20 days) at room temperature. Afterwards, the resultant solutions of all tests were centrifuged at 5,000 × g for 10 min. The collected supernatants were diluted and spectrophotometrically analysed at 471 nm. The residual concentration of lycopene in PEC NPs was derived from the standard curve of lycopene (**Figure 2**). The retention rate of lycopene was measured using the following equation in accordance with a previously published report (28):

$$Retention\ rate\ of\ lycopene\ (\%) = \frac{the\ content\ of\ lycopene\ after\ test}{the\ initial\ content\ of\ lycopene} \times 100 \quad (3)$$

In vitro Drug Release Studies

The drug release profiles of lycopene-loaded PEC NPs and free lycopene in the simulated gastrointestinal fluid were assessed in accordance with a published report with minor modifications (29). In brief, 10 ml of freshly prepared lycopene-loaded PEC NPs (10 mg/ml) and free lycopene were mixed with 10 ml of simulated gastric fluid (SGF, pH 1.5, containing 0.5 g of NaCl, 2 ml of HCl and 0.8 g of pepsin added in 250 ml of deionised water), and the resulting pH was adjusted to three by adding 0.1 mol/L of HCl. The mixed dispersions were then conducted in a thermostatic shaker (37°C, 100 rpm/min) under gentle shaking for 2 h. Samples were removed at 0.5, 1, 1.5, and 2 h for analysis. Afterwards, gastric digestion was terminated by adjusting the pH of mixed dispersions to 7. Subsequently, the resulting gastric digestion dispersions were mixed with simulated intestinal fluid (SIF, pH 7.5, containing 1.7 g of KH_2PO_4, 0.38 g of NaOH, 2.5 g of pancreatin, and 1.25 g of bile salts added in 250 ml of deionised water). After intestinal digestion, the samples were incubated for 4 h at 37°C in a thermostatic shaker at 100 rpm. Samples were collected at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, and 6 h for analysis. The released lycopene of collected samples at SGF and SIF digestion stages was determined spectrophotometrically at 471 nm, as described in Section 2.4. Lycopene release (%) was quantified using the following equation:

$$Lycopene\ release\ (\%) = \frac{Released\ lycopene}{Total\ lycopene} \times 100 \quad (4)$$

In vitro Antioxidant Activity Analysis

In vitro antioxidant activities of lycopene in acetone, lycopene in water, and lycopene-loaded PEC NPs was evaluated by DPPH radical scavenging ability, hydroxyl radical scavenging ability,

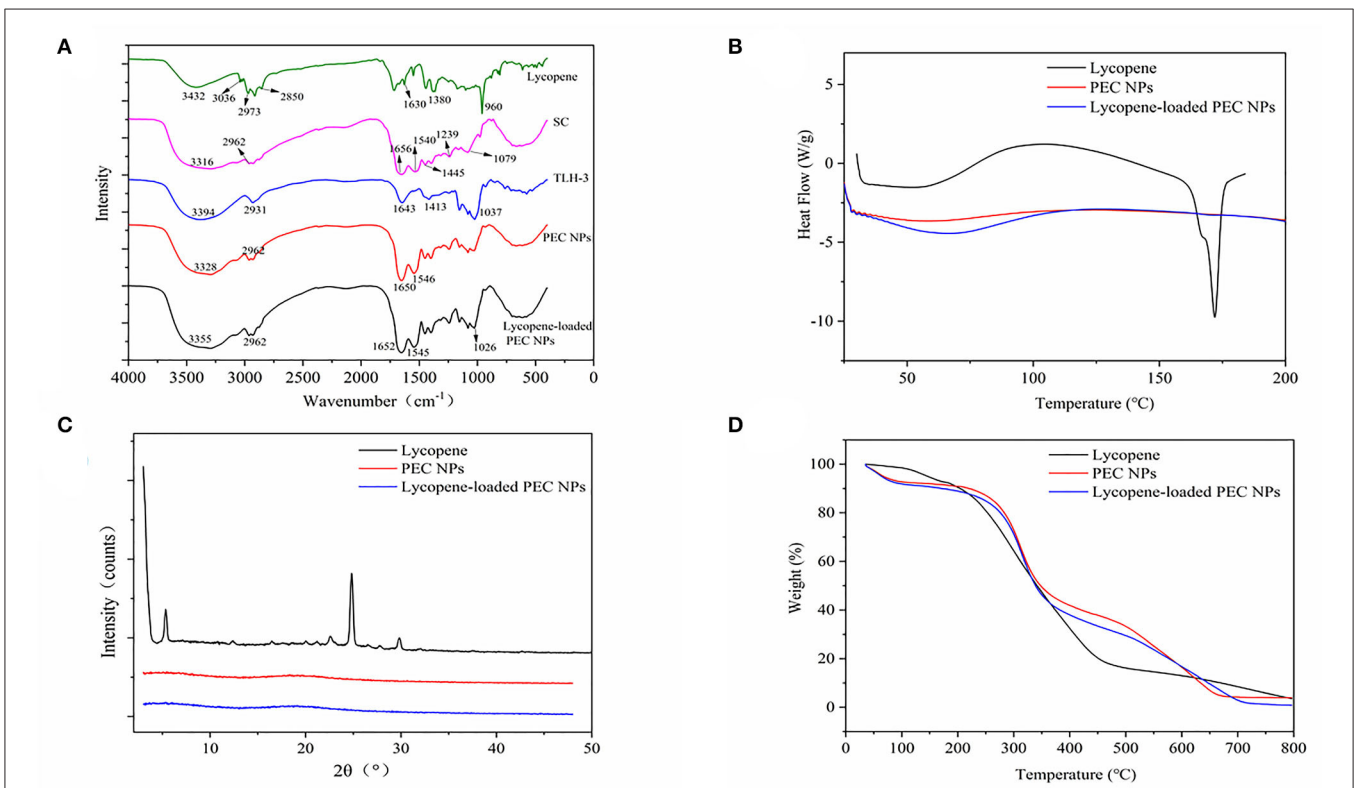


FIGURE 5 | (A) Fourier transform infrared spectroscopy spectrum. **(B)** The differential scanning calorimetry. **(C)** X-ray diffraction spectra and **(D)** Thermogravimetric analysis.

and ABTS radical scavenging ability. Solutions of lycopene in acetone, lycopene in water, and lycopene-loaded PEC NPs at different concentrations (0–100 μg/ml) were added with the same volume of DPPH reagent separately. Then, DPPH radical scavenging activities were evaluated as described previously with minor modifications (30). Hydroxyl radical scavenging activities were measured in accordance with a previously published report (31). In addition, ABTS was determined based on a previously described method with modifications (30). The absorbance of the resulting solution was determined using a TU-190 spectrophotometer (Beijing Puxi General Analytical Instrument Co., Ltd., China). For all assays, vitamin C (Vc) was used as the positive control, and distilled water was used as the blank control.

The scavenging effects were all calculated in accordance with the following equation:

$$Scavenging\ activity\ (\%) = \frac{A_0 - A_1}{A_0} \times 100 \quad (5)$$

where A_0 denotes the absorbance value of a blank control in the system, and A_1 denotes the absorbance value of different samples.

Protective Effects of Lycopene-Loaded PEC NPs Against Cellular Stress Damage

Cell Culture and Treatment

L02 cells were cultured in DMEM in accordance with a previously described method (32). Cells at the sub-confluence level were subjected to various concentrations of H_2O_2 (0, 100, 200, 400, 600, and 800 μM) in fresh and DMEM high-glucose medium for 4 h to induce oxidative damage until cell apoptosis with condensed and fragmented nuclei. Various concentrations of H_2O_2 treatments were selected to determine the appropriate H_2O_2 concentration for cell apoptosis induction by detecting cell viability.

Cell Viability Assay

Cell viability was measured using MTT assays as described by Wang et al. (33). L02 cells were seeded into 96-well-plates at

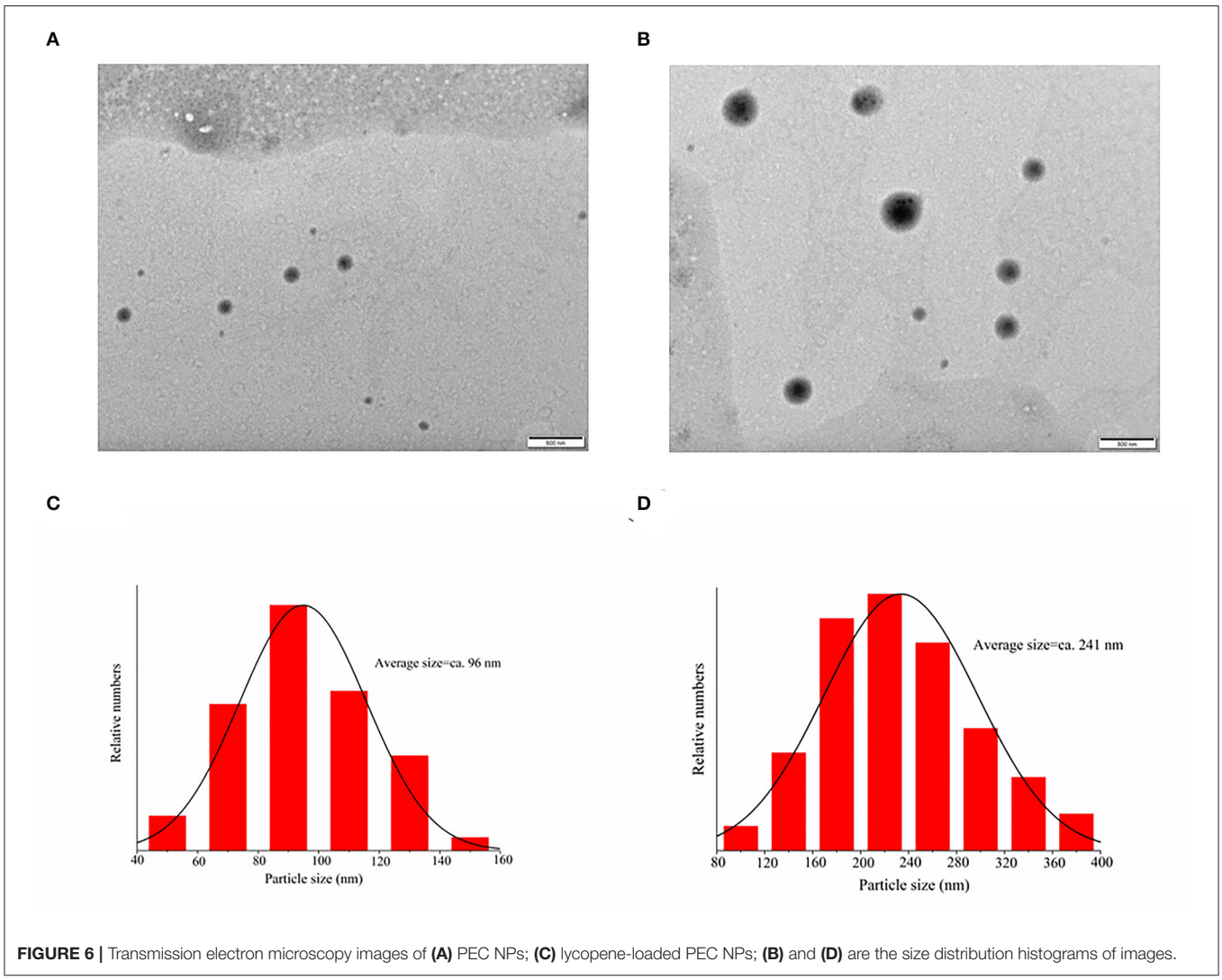


FIGURE 6 | Transmission electron microscopy images of **(A)** PEC NPs; **(C)** lycopene-loaded PEC NPs; **(B)** and **(D)** are the size distribution histograms of images.

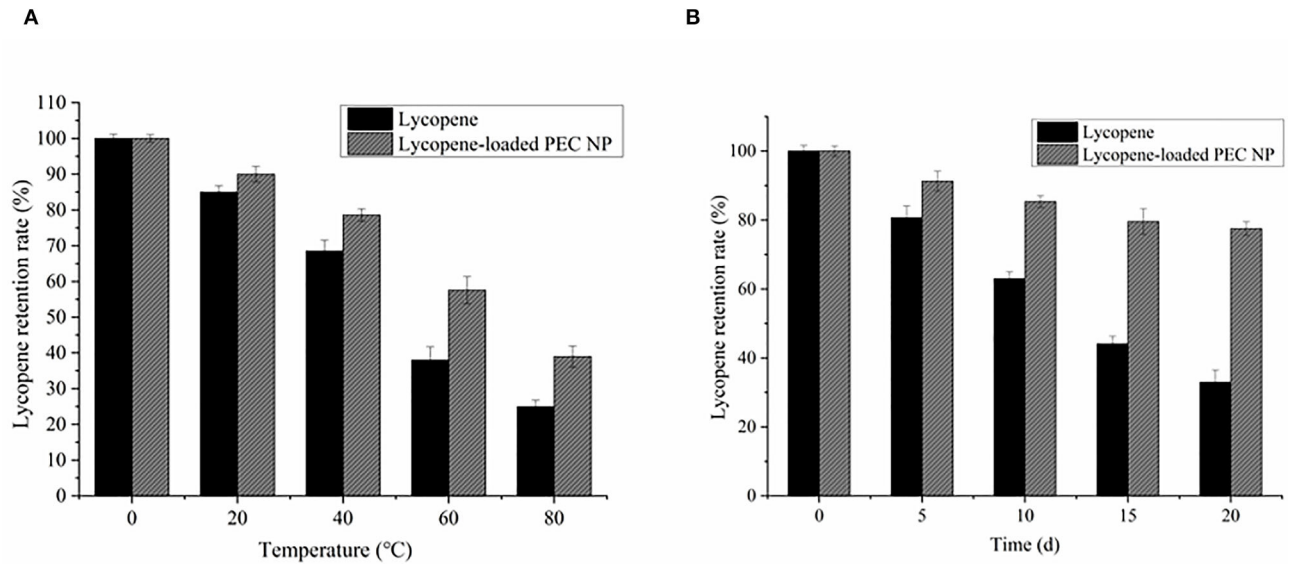


FIGURE 7 | Effects of **(A)** thermal treatment **(B)** light radiation on the retention rate of lycopene and lycopene-loaded PEC NPs. Data represent the mean ± standard deviation (SD, $n = 3$).

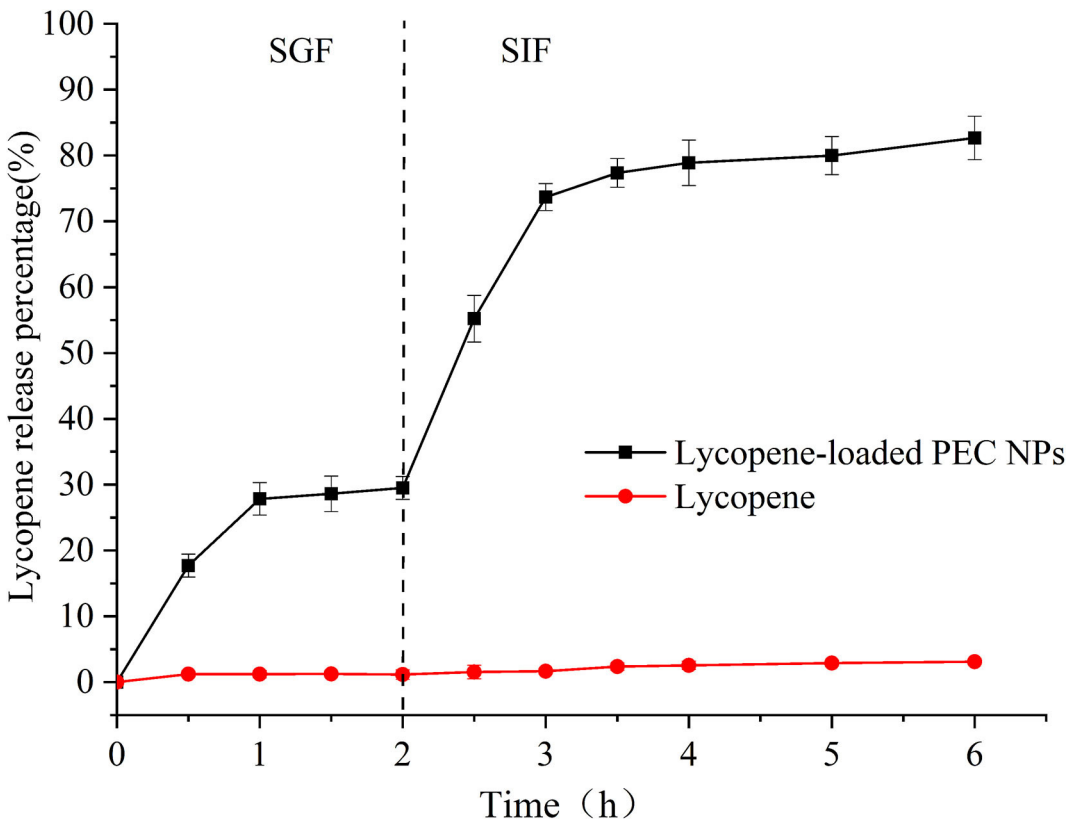


FIGURE 8 | Release of lycopene in lycopene-loaded PEC NPs and free lycopene in SGF and SIF.

a density of 1 × 10^5/mL in a culture medium overnight and then treated with different concentrations of lycopene-loaded PEC NP solution (1, 5, 10, and 25 μmol/L) for 24 h. MTT solution was subsequently added to each well, and absorbance was measured at 570 nm using a microplate reader to determine whether lycopene-loaded PEC NPs have a cytotoxic effect on L02 cells. Next, L02 cells were treated with the most appropriate concentration of H_2O_2 solution for 4 h to set up a cell oxidative damage model to evaluate the protection of lycopene-loaded PEC NP solution against H_2O_2-induced cell apoptosis. Then, cells were washed two times with PBS (pH 7.4) and treated with various concentrations of lycopene-loaded PEC NP solution (1, 5, and 10 μmol/L) for 24 h. MTT solution (.5 mg/ml, 100 μL) was added to each well and incubated at 37°C for an additional 4 h. The absorbance value was detected at 570 nm using a microtitre plate reader (Bio-Rad, California, USA). Cell viability was calculated in accordance with the following equation:

$$Cell\ viability\ (\%) = \frac{A_{sample} - A_{blank}}{A_{control} - A_{blank}} \times 100 \qquad (6)$$

where A_{sample} is the average absorbance value of the solution with different concentrations of lycopene-loaded PEC NP samples at 570 nm. A_{blank} is the average absorbance value of the solution without cell samples at 570 nm. $A_{control}$ is the average absorbance value of the solution without lycopene-loaded PEC NP samples at 570 nm.

Hoechst 33342 Staining

The protective effects of lycopene-loaded PEC NPs were confirmed by performing microscopic analysis through Hoechst 33342 staining for nuclei in L02 cells in accordance with a previously published method (34).

Intracellular ROS Detection

As previously described by Dong et al. (34), 2,7-dichlorodihydrofluorescein diacetate was used to evaluate intracellular ROS generation in L02 cells, and a quadrant investigation was performed utilising WinMDI.

Determination of Biochemical Parameters

The activities of SOD, GSH-px, and the level of MDA were measured using commercially available kits according to the manufacturer's instructions. In brief, the L02 cells were treated with lycopene-loaded PEC NP solution for 24 h after treatment with H_2O_2 for 4 h. The cells were lysed; cell supernatants were

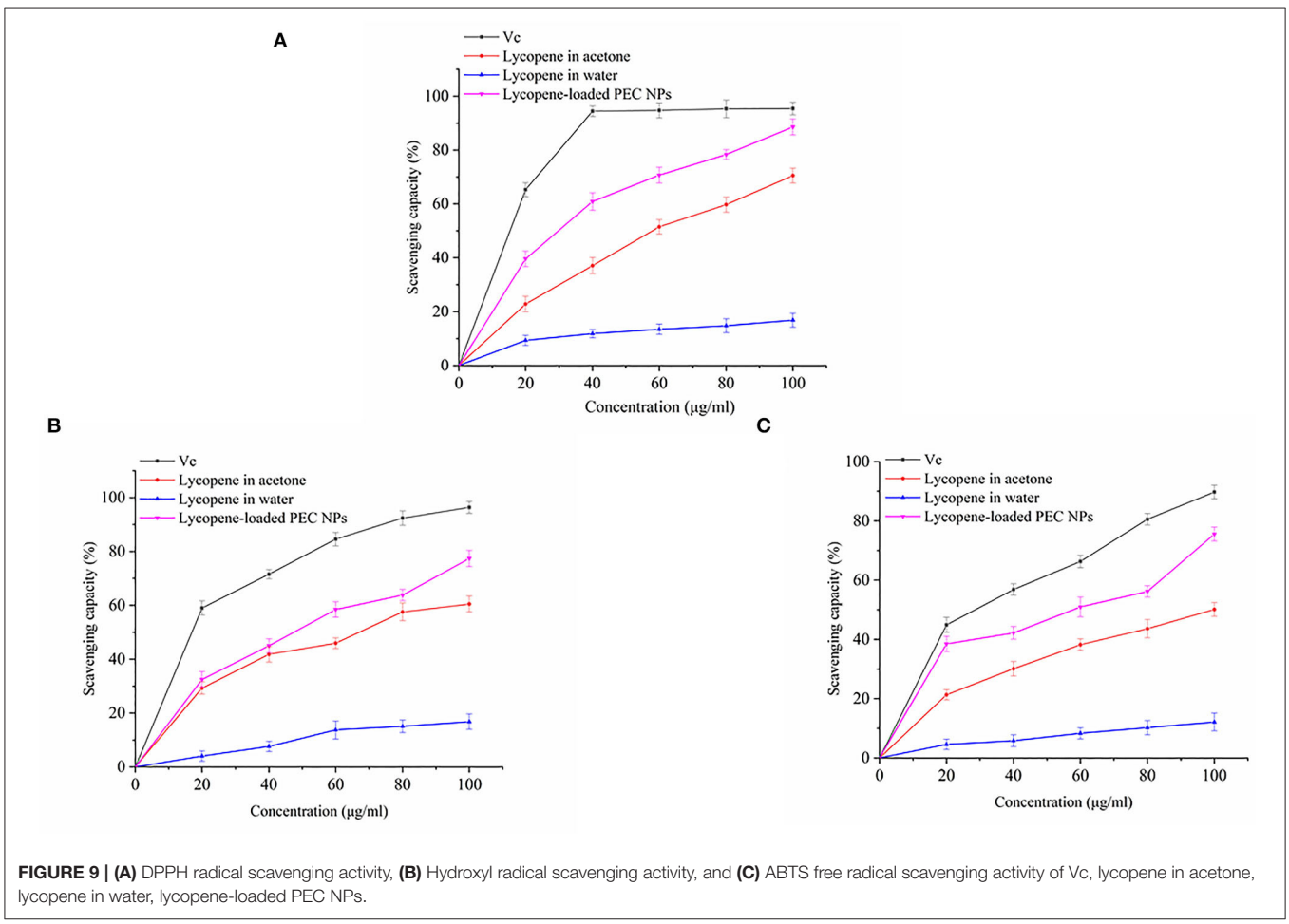


FIGURE 9 | (A) DPPH radical scavenging activity, **(B)** Hydroxyl radical scavenging activity, and **(C)** ABTS free radical scavenging activity of Vc, lycopene in acetone, lycopene in water, lycopene-loaded PEC NPs.

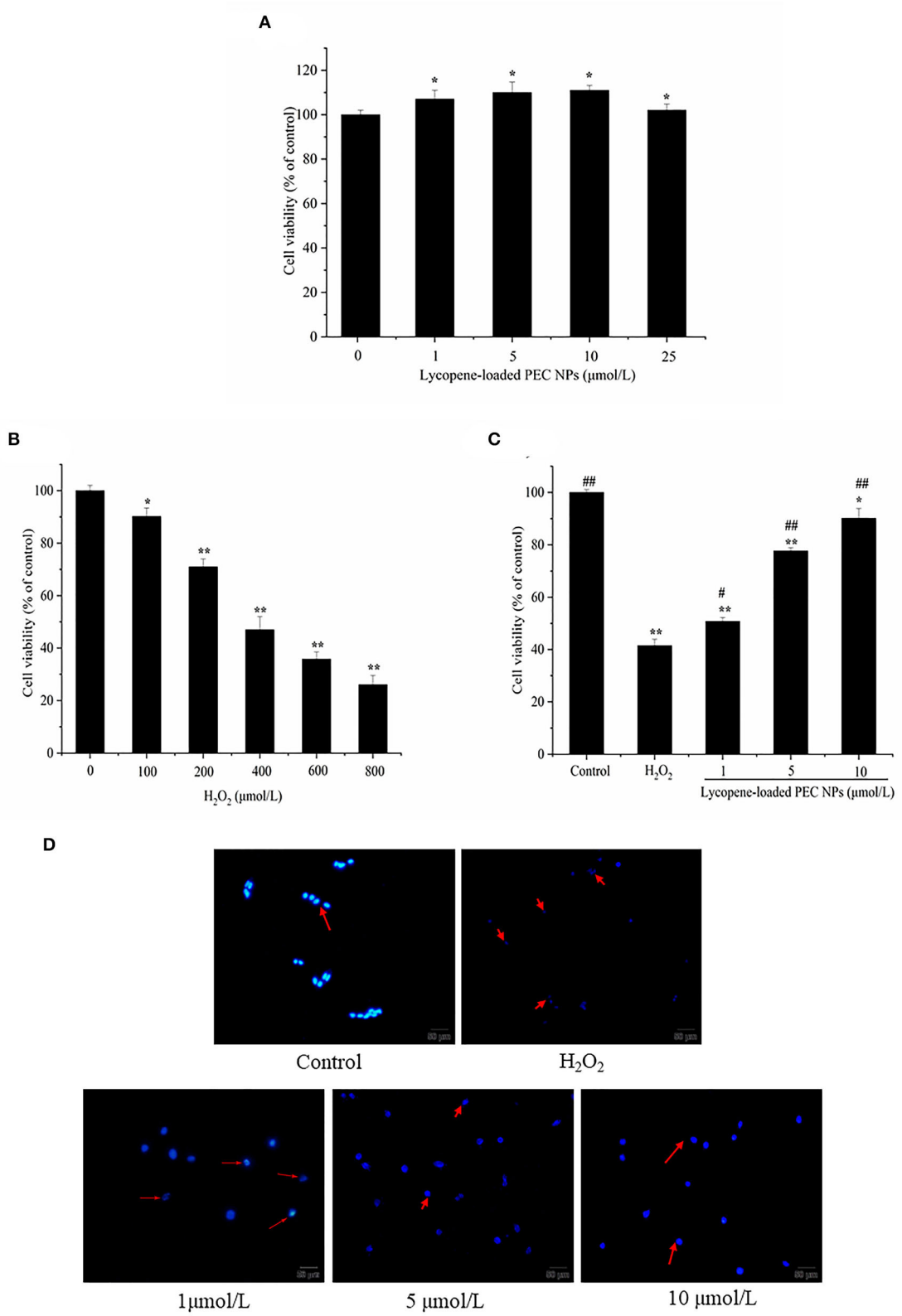


FIGURE 10 | Lycopene-loaded PEC NPs inhibit H_2O_2-induced cell apoptosis. **(A)** Cytotoxicity of lycopene-loaded PEC NPs in cells at different concentrations. **(B)** The viability of H_2O_2-induced cells. **(C)** Effects of lycopene-loaded PEC NPs on the viability of L02 cells. **(D)** Morphological analysis of the nucleus in cells. Data (mean ± SD) represent three experimental replicates. * $p < 0.05$, ** $p < 0.01$, vs. control group. # $p < 0.05$, ## $p < 0.01$, vs. H_2O_2-treated group.

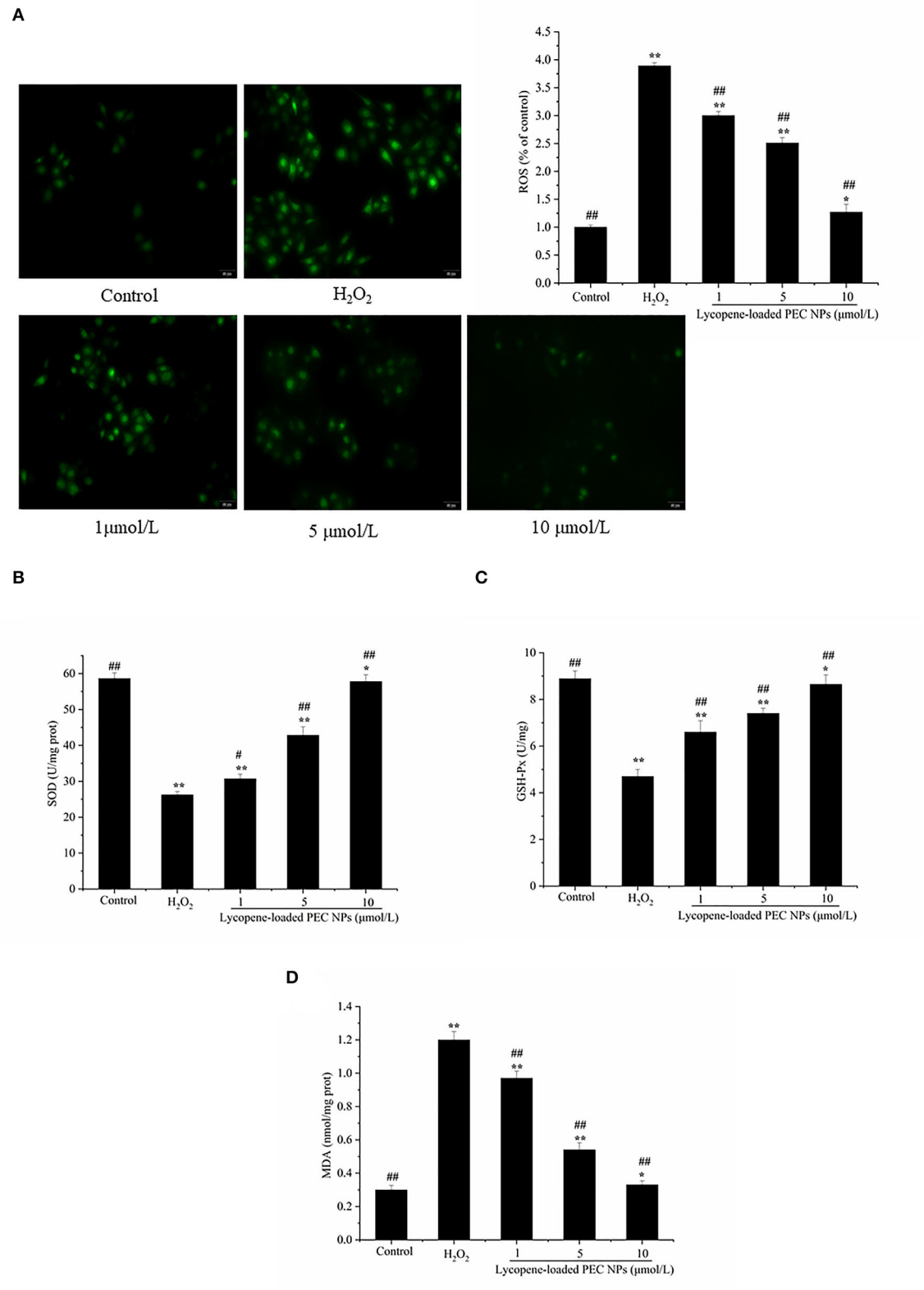


FIGURE 11 | Lycopene-loaded PEC NPs alleviate H_2O_2-induced oxidative stress. **(A)** Effect of lycopene-loaded PEC NPs on H_2O_2-induced ROS generation in cells. The fluorescence increase meant that DCF was indicative of enhanced ROS generation. **(B)** Effect of lycopene-loaded PEC NPs on SOD activity. **(C)** Effect of lycopene-loaded PEC NPs on GSH-Px level. **(D)** Effect of lycopene-loaded PEC NPs on MDA level. Data (mean ± SD) represent three experimental replicates. * $p < 0.05$, ** $p < 0.01$, vs. control group. # $p < 0.05$, ## $p < 0.01$, vs. H_2O_2-treated group.

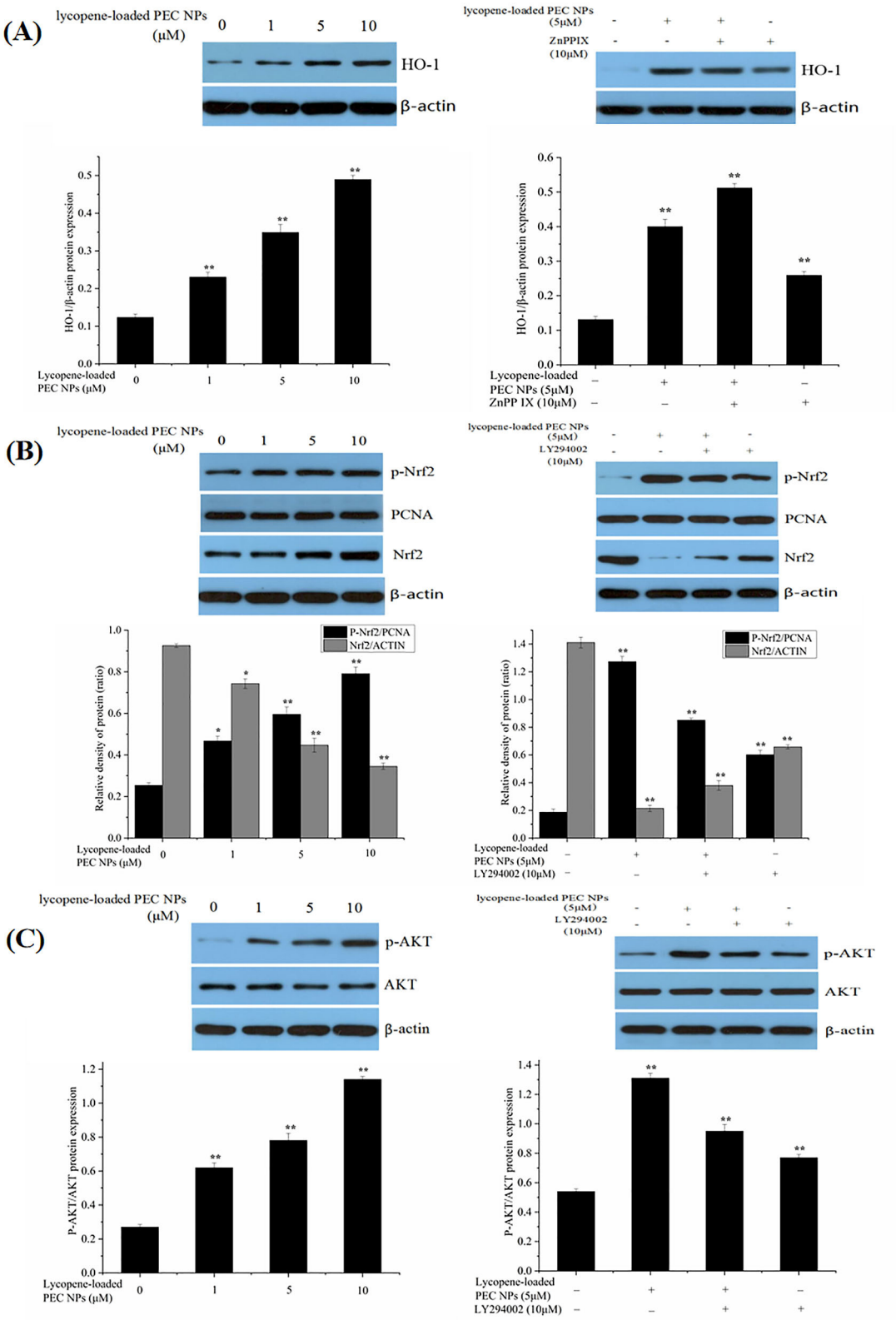


FIGURE 12 | Effect of lycopene-loaded PEC NPs on protein expression of HO-1, p-Nrf2, Nrf2, p-AKT, and AKT. **(A)** Cells were treated with lycopene-loaded PEC NPs (0, 1, 5, and 10 μmol/L) for 24 h, and then protein expression of *HO-1* was determined by western blot analysis (Left); Cells were pretreated with 10 μM ZnPP-IX for 1 h prior to incubation with or without 5 μmol/L lycopene-loaded PEC NPs for 24 h and then protein expression of *HO-1* was determined by western blot

(Continued)

FIGURE 12 | analysis (Right). **(B)** Nrf2 and p-Nrf2 protein levels were measured by western blots after treatment with lycopene-loaded PEC NPs (0, 1, 5, and 10 μmol/L) for 24 h (Left); Cells were pretreated with 10 μM LY294002 for 1 h and then treated with or without 5 μmol/L lycopene-loaded PEC NPs for 24 h, and Nrf2 and p-Nrf2 were determined by western blot analysis (Right). **(C)** Cells were treated with lycopene-loaded PEC NPs (0, 1, 5, and 10 μmol/L) for 24 h, and AKT and p-AKT were determined by western blot analysis (Left); Cells were pretreated with 10 μM LY294002 for 1 h prior to incubation with or without 5 μmol/L lycopene-loaded PEC NPs for 24 h, and AKT and p-AKT were determined by western blot analysis (Right). Data (mean ± SD) represent three experimental replicates. *$p < 0.05$, ** $p < 0.01$, vs. control group.

collected, and MDA, GSH-px, and SOD were determined using a commercial detection kit.

Western Blot Analysis

Western blot analysis was performed in accordance with a previously described method with minor modification (34). Cells were treated with lycopene-loaded PEC NPs (0, 1, 5, and 10 μmol/L) for 24 h, and then, the protein expression of heme oxygenase-1 (*HO-1*), nuclear factor erythroid 2-related factor 2 (Nrf2), phospho-Nrf2 (p-Nrf2), AKT, and p-AKT were determined by Western blot analysis. Meanwhile, cells were pre-treated with 10 μM of ZnPP-IX or LY294002 for 1 h prior to incubation with or without 5 μmol/L of lycopene-loaded PEC NPs for 24 h, and then, the protein expression was determined by Western blot analysis.

Statistical Analysis

Data were presented as the mean ± standard derivations (SDs) of three replicates. Statistical analysis was performed by analysis of variance using the OriginPro Software Version 8 (OriginLab Corp., Northhampton, MA, USA). Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Formation and Characterisation of PEC NPs

The polysaccharide TLH-3 is an acidic one that has a molecular weight of 4.22×10^3 Da, exists as a negatively charged polyanion in an aqueous solution, and forms a natural polysaccharide hydrosol. Given that SC is positively charged, TLH-3/SC PEC NPs can be formed by electrostatic interactions when TLH-3 and SC are fully hydrated. **Figure 3A** indicates the effect of TLH-3/SC mass ratio (10, 20, 30, and 40%) on the Z-average diameter and zeta potential of PEC NPs. TLH-3 is highly soluble in water because it contains a mass of hydrophilic carboxylic and hydroxyl groups. Therefore, the large amount of TLH-3 on the PEC NP surface is beneficial to improving the water solubility of TLH-3/SC PEC NPs. The Z-average diameter decreased from 237 nm to 150 nm when the TLH-3/SC mass ratio was increased from 10 to 40%, suggesting that the PEC NPs with a high TLH-3/SC mass ratio exhibited a small Z-average diameter. At low concentrations (such as when the TLH-3/SC mass ratio was 10%), TLH-3 cannot completely cover the SC surface. One TLH-3 molecule will be adsorbed on multiple SC molecules, thus destroying the repulsion stability of the original SC system and leading to the bridging flocculation of SC. Therefore, the resulting PEC NPs showed the largest Z-average diameter (237 nm). With further increase in the TLH-3 content, sufficient TLH-3 will be adsorbed on the SC surface and cover the SC, preventing the interconnection of multiple SC molecules and forming water-soluble TLH-3/SC PEC NPs, thus enhancing electrostatic repulsion and steric hindrance, inhibiting molecular aggregation, reducing *Z*-average diameter, and improving the stability of PEC NPs (35, 36). In an aqueous medium, the negative charge of TLH-3/SC PEC NPs significantly reduced with the increasing SC content due to the self-association of SC and the charge neutralisation of PEC NPs. However, when the mass ratio was changed from 10 to 40%, the zeta potential of PEC NPs increased from −21 mV to −33 mV because the increase in TLH-3 content also increased the negative charges on the PEC NP surface. Therefore, the obtained PEC NPs had a high net negative charge and were stable to flocculation, which was in accordance with previous studies (37, 38).

Figure 3B shows the effect of pH on *Z*-average diameter and zeta potential. When the solution pH value increased, the *Z*-average diameter of PEC NPs first increased and then decreased. When the pH was around 4.5, the *Z*-average diameter reached the maximum value. Given that this pH was close to the isoelectric point of SC, the intermolecular repulsive force decreased or even disappeared, and the *Z*-average diameter increased owing to SC coalescence or flocculation SC under the influence of van der Waals force and other interactions (39, 40). When the pH value was far from the isoelectric point of SC, the SC returned to a soluble state and the *Z*-average diameter decreased. With the increase in pH, the negative zeta potential of PEC NPs continued to increase from −7.23 to −35 mV, suggesting the electrostatic adsorption of anion polysaccharide (TLH-3) and cationic protein (SC) (16, 39). SC was positively charged when the pH was lower than the isoelectric point of SC ($IP = 4.5$) but was negatively charged when the pH was above 4.5. The zeta potential of PEC NPs rapidly increased from −7.23 to −31.68 mV within a low pH range (pH = 2–4.5) due to the increased negative charges on the PEC NP surface. At high pH (pH = 4.5–7), the change in the zeta potential value of PEC NPs was relatively small because SC still had a partial positive charge on its surface despite being negatively charged at this time. Therefore, anionic polysaccharides (TLH-3) could be absorbed onto the SC surface by local electrostatic attraction to form a weak reversible electrostatic complex, thus partially reducing the zeta potential of PEC NPs. These behaviours were consistent with the results from previous studies (16, 41).

Characterisation of Lycopene-Loaded PEC NPs

Lycopene was encapsulated inside the PEC NPs to form lycopene-loaded PEC NPs with improved stability and bioavailability. The results of the DLS test showed that the

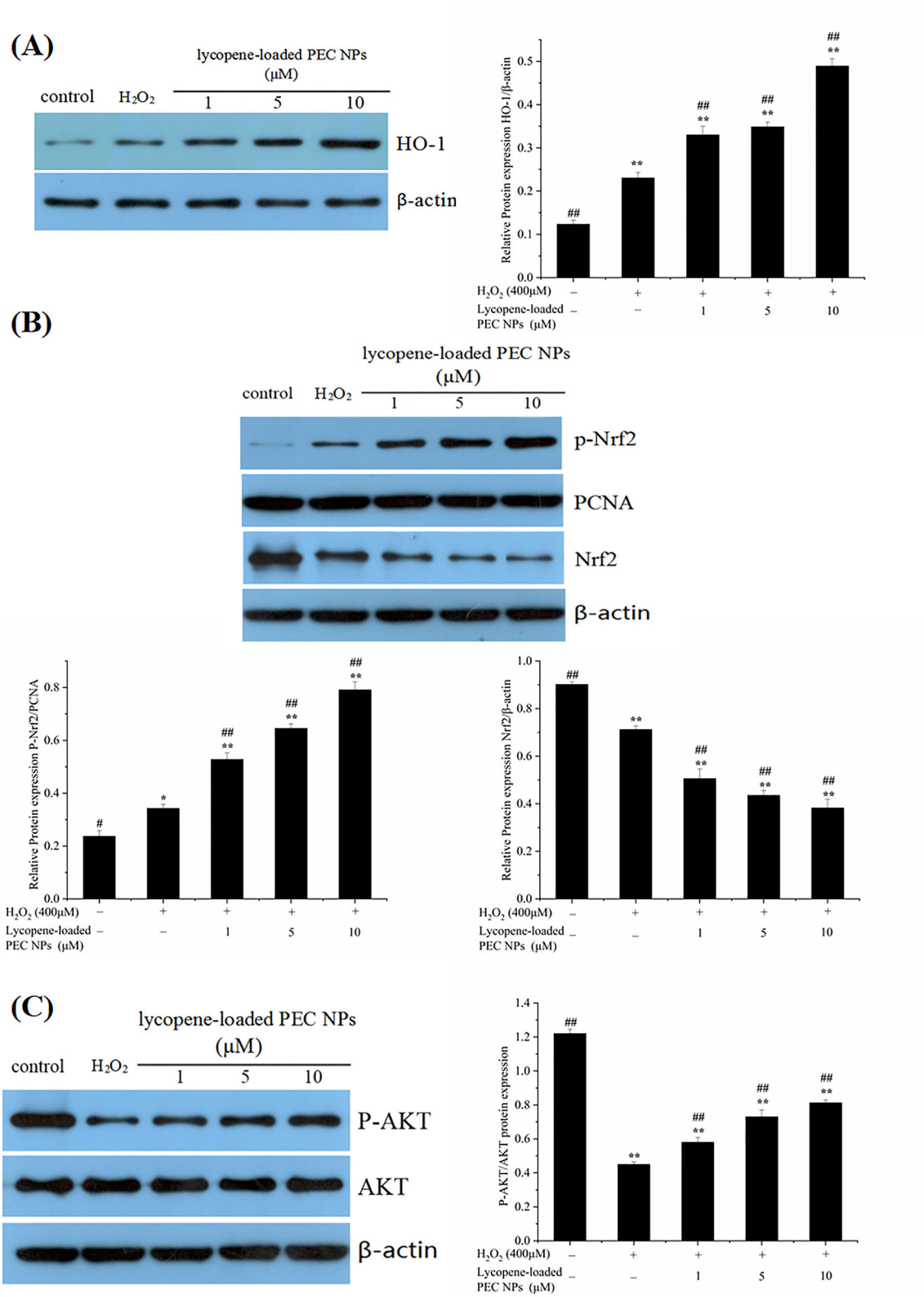


FIGURE 13 | Mechanism of protective effects on H_2O_2-treated L02 cells. **(A)** Western blot assay of HO-1 expression; Quantification of relative protein expression quantity of *HO-1*. **(B)** Western blot assay of p-Nrf2 and Nrf2 expression. Quantification of relative protein expression quantity of p-Nrf2 and Nrf2. PCNA was used as a nuclear loading control. **(C)** Western blot assay of p-AKT and AKT; Quantification of relative protein expression quantity of p-AKT/AKT. Data (mean ± SD) represent three experimental replicates. $^{*}p < 0.05$, $^{**}p < 0.01$, vs. control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, vs. H_2O_2-treated group.

Z-average diameter increased significantly from 190 nm to 350 nm after encapsulation, indicating that lycopene was successfully encapsulated inside the PEC NPs. The zeta potential of PEC NPs and lycopene-loaded PEC NPs were about −27.8 and −23.6 mV, respectively, indicating their stability in an aqueous solution. Furthermore, 12% lycopene was loaded into PEC NPs with a 20% TLH-3 and SC mass ratio. The final EE and LC of lycopene-loaded PEC NPs were 93.6 and 10.03%, respectively. Previous studies had reported that the encapsulation efficiency of lycopene by whey protein isolate was 64.7% (4), and the maximum encapsulation efficiency was 63.73% when plant and dairy protein blends were used as delivery vehicles for lycopene (8). Therefore, the TLH-3/SC PEC NP delivery system is superior to other similar delivery systems because of its relatively high encapsulation efficiency for lycopene, thus meeting the requirements of the food industry for nutrient delivery.

Free lycopene is almost insoluble in an aqueous solution due to its strong hydrophobicity and thus is deposited at the bottom (**Figure 4A**). In this study, lycopene was completely dissolved in acetone, and the colour of the solution was light yellow (**Figure 4B**). Lycopene-loaded PEC NPs were almost completely dissolved in an aqueous solution and formed a red emulsion solution (**Figure 4C**). These results indicated that the water solubility of lycopene was significantly enhanced after being encapsulated inside the PEC NPs.

Figure 5A shows the FT–IR spectra of lycopene, SC, TLH-3, PEC NPs, and lycopene-loaded PEC NPs. A relatively broad and strong peak (1,500–1,700 cm^{-1}) was formed, indicating that the positively charged amino group of SC, namely, –NH3+ bending vibrations (1,537 cm^{-1}) and C=O stretching vibrations (1,656 cm^{-1}), interacted with the negatively charged COO– of TLH-3 (1,645 cm^{-1}). The FT–IR spectrum also indicated that SC and TLH-3 could form relatively stable PEC NPs through

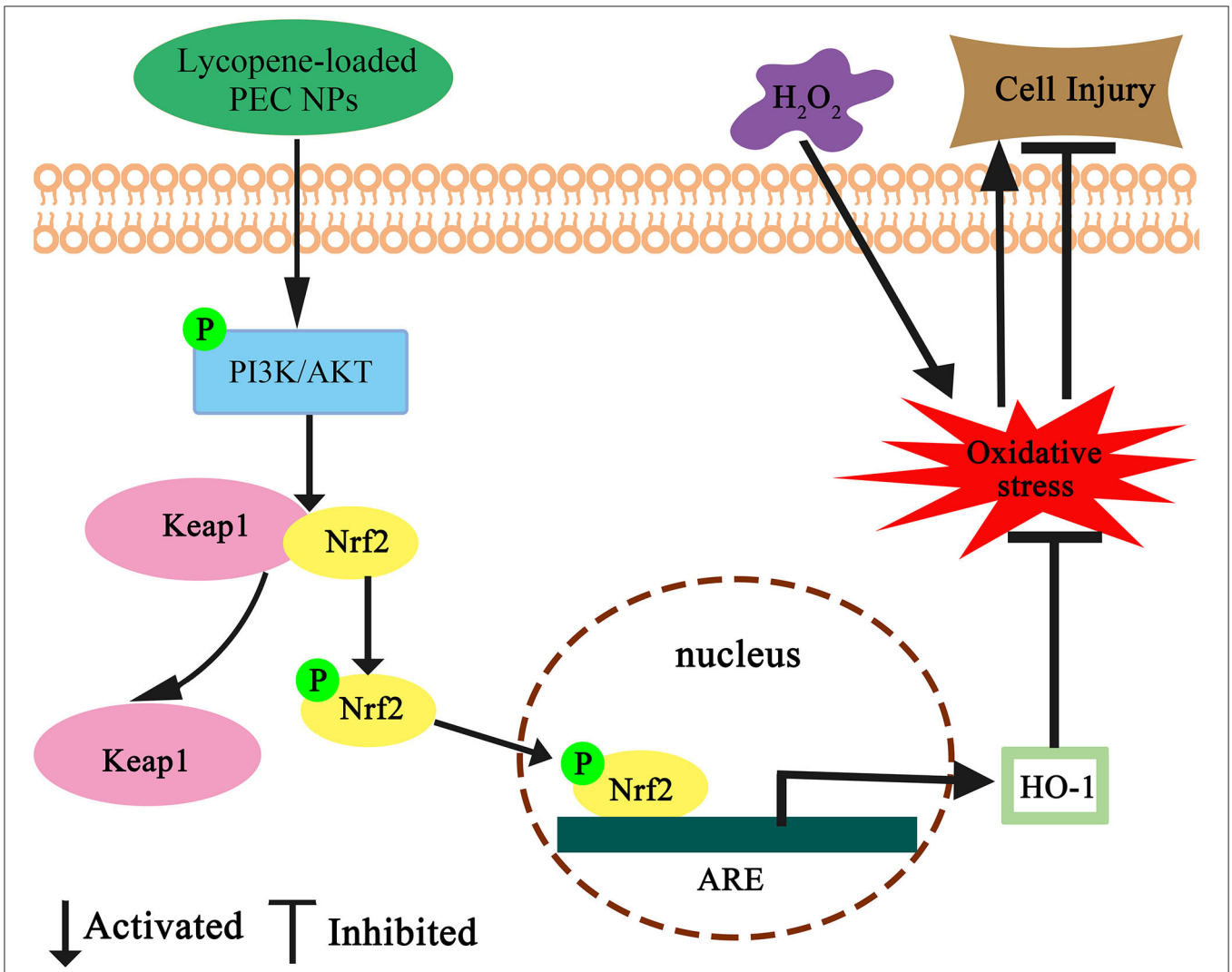


FIGURE 14 | Schematic diagram for the protective effect of lycopene-loaded PEC NPs against oxidative stress in L02 cells. Where ↑ indicates increase, ↓ indicates a decrease.

electrostatic interactions rather than simple physical mixing. The characteristic absorption peaks in the lycopene spectrum at around 3036, 2973, and 2,850 cm^{-1} corresponded to C–H stretching vibrations, asymmetric methyl vibrations, and stretching vibration peaks of methyl and methylene, respectively. In addition, the absorption bands at about 1,630, 1,380, and 960 cm^{-1} were due to the C=C stretching vibrations, methyl group bending vibrations, and R1HC=CR2H (trans) rocking vibrations, respectively, which were discharged from the trans-monoenes. By contrast, some characteristic absorption peaks of lycopene vanished in the spectrum of lycopene-loaded PEC NPs, suggesting that lycopene was encapsulated inside the PEC NPs through electrostatic interaction (42). **Figure 5B** shows the DSC thermograms of lycopene, PEC NPs, and lycopene-loaded PEC NPs. The thermograms of lycopene showed a strong endothermic peak at about 174°C due to lycopene crystal melting, thus further illustrating the high crystallinity of lycopene. However, no endothermic peak was shown by lycopene-loaded PEC NPs before 200°C and after encapsulation. This finding confirmed the loss of the lycopene crystalline structure, suggesting that the lycopene was successfully encapsulated and formed an amorphous state. **Figure 5C** illustrates the XRD diffractograms of lycopene, PEC NPs, and lycopene-loaded PEC NPs. Lycopene exhibited multiple characteristic peaks (5.3, 12.4, 22.5, 24.8, and 29.8°) at 2θ from 5to 50° due to its crystalline nature. By contrast, lycopene-loaded PEC NPs displayed no characteristic peaks, suggesting that lycopene was successfully encapsulated inside the PEC NPs. A thermogravimetric analyzer was used to evaluate the TGA properties of free lycopene, PEC NPs, and lycopene-loaded PEC NPs (**Figure 5D**). In the second weightlessness region, the thermal decomposition temperature increased from 174°C in lycopene to 250°C in lycopene-loaded PEC NPs, suggesting that the degradation degree of lycopene-loaded PEC NPs was substantially delayed when lycopene was successfully encapsulated inside the PEC NPs. These results indicated that the thermal stability of lycopene was significantly improved after encapsulation.

Figure 6 shows the TEM images of PEC NPs and lycopene-loaded PEC NPs. The lycopene-loaded PEC NPs (**Figure 6C**) exhibited a larger nanoscale spherical morphology with a smooth, uniform, and compact surface compared with the PEC NPs (**Figure 6A**). TEM analysis revealed that the average particle size increased from 96 nm in PEC NPs (**Figure 6B**) to 241 nm in lycopene-loaded PEC NPs (**Figure 6D**), suggesting that lycopene was successfully loaded inside the PEC NPs on a nanometre scale. This finding coincided with the greater Z-average size of lycopene-loaded PEC NPs than that of PEC NPs. In addition, the average diameter of PEC NPs (96 nm) and lycopene-loaded PEC NPs (241 nm) observed by TEM was significantly smaller than the average particle size measured by DLS (about 190 and 350 nm). This difference can be attributed to the method used: TEM describes the actual size of the sample (dry sample state), and DLS shows the hydrodynamic diameter (hydration state) of the sample. In addition, the nanoparticles display a large hydrodynamic volume in the hydration state due to solvent action (43, 44).

Storage Stability

The effects of thermal and light treatments on the lycopene encapsulated inside the PEC NPs were examined by evaluating the changes in the lycopene retention rate during the storage period (**Figure 7**). The thermal stabilities of lycopene encapsulated inside the PEC NPs and free lycopene were examined after incubation at different storage temperatures (0, 25, 45, 65, and 85°C) and are shown in **Figure 7A**. The free lycopene and encapsulated lycopene exhibited no significant differences in lycopene retention rate at low temperatures (0 °C and 25°C). However, the retention rates of free lycopene and encapsulated lycopene were approximately 37.98 and 57.55%, respectively, after heat treatment at 65°C for 0.5 h and 24.93 and 38.99%, respectively, after being heated at 85°C for 0.5 h. These results confirmed that the lycopene encapsulated inside the PEC NPs had higher retention rates than the non-encapsulated lycopene at different storage temperatures (25, 45, 65, and 85°C), thus revealing the significant improved thermal stability of lycopene. This improvement can be attributed to the spherical structure of PEC NPs being promoted and compacted by TLH-3 and SC through electrostatic interaction, thus protecting lycopene against thermal treatment. DSC, XRD, and TGA analysis results also indicated that the thermal stability of lycopene was significantly improved after encapsulation.

As illustrated in **Figure 7B**, the lycopene retention rate decreased with the increase in storage days. The degradation rate of encapsulated lycopene was significantly slower than that of free lycopene. The lycopene-loaded PEC NPs had a lycopene retention rate of 77.55% after 20 days of light radiation. These results suggested that PEC NPs have a good protective effect against the degradation of encapsulated lycopene under prolonged light radiation exposure from 0 to 20 days. The compact spherical structure of PEC NPs formed by TLH-3 and SC through electrostatic interaction may have protected lycopene from light radiation. Similar findings have been previously reported in previous studies, such as the improved photostability of hydrophobic nutraceuticals encapsulated in PEC NPs or ZNPs coated with biopolymers (30, 45), which revealed that encapsulating lycopene inside PEC NPs enhances its thermostability and light stability. Moreover, it was reported that the lycopene-loaded W/O emulsions were prepared by orange oil, tributyrin, and corn oil, and the corn oil lycopene emulsions were physically more stable than orange oil and tributyrin lycopene emulsions (46). Because the addition of corn oil enhanced the physical stability of the beverage during chilled storage by inhibiting Ostwald ripening, lycopene nanoemulsions were fabricated using high-pressure homogenization and using medium-chain triglycerides (MCT) as carrier oils (47). It was found that the molecules stretched into the O/W interface as the lycopene loading increased, strengthening the lateral packing of OSA molecules on the interfacial membranes and then decreasing the mean particle diameters and improving the stability of nanoemulsions.

In vitro Release and Antioxidation Activities

Figure 8 shows the *in vitro* release profiles of free lycopene and lycopene encapsulated inside PEC NPs under simulated

gastrointestinal conditions. Owing to its poor water solubility, free lycopene showed negligible release rates during the simulated gastrointestinal digestion, including SGF and SIF. The lycopene encapsulated inside the PEC NPs was discharged into the SGF environment and exhibited burst release in the first 30 min of gastric digestion due to either the entrapment of lycopene near the interface or the absorption of lycopene on the PEC NP surface. Afterwards, a slight and steady release of lycopene occurred between 30 and 120 min from the start of SGF digestion. The cumulative release rates of lycopene from PEC NPs were approximately 30.15% after 2 h of SGF digestion and 85.67% after 4 h of SIF digestion, indicating that the release of lycopene was faster in SIF digestion than in SGF digestion. As indicated in 3.1, TLH-3 wrapped the hydrophobic amino acids of SC into a hydrophobic core, leading to a significant steric hindrance effect. These results suggested that the PEC NPs formed by the electrostatic adsorption of SC and TLH-3 possess a close and heavy protective layer that can increase the charge density on the PEC NP surface and the electrostatic repulsion among PEC NPs. Owing to the physical barrier formed by strong electrostatic interactions, SGF cannot directly interact with SC, which in turn could not be degraded by pepsin. Therefore, the amount of lycopene released in simulated gastric juice between 30 and 120 min was relatively small. In the simulated intestinal fluid, the electrostatic attraction of SC and TLH-3 and the TLH-3 protective layer wrapping the SC outer layer were weakened. Afterwards, SC was broken down by trypsin in SIF, and lycopene was then released from PEC NPs in large quantities. Therefore, TLH-3/SC PEC NPs significantly improved the *in vitro* control release of lycopene in the simulated gastrointestinal tract, especially burst releases in SIF. Gan et al. also reported that the bioaccessibility of phytosterol encapsulated sodium caseinate (NaCas)/pectin-based phytosterols (NCP-PSs) nanoparticles was increased by at least 43.8% compared to free phytosterols, indicating that the presence of pectin could be adsorbed on the casein micelles by electrostatic interaction and form coating, protecting the nanoparticles from degradation in the gastric environment (48). A type of lycopene nanoscale liposome carriers (NLCs) was prepared and the adsorption of NLCs in the gastrointestinal wall can prolong the contact time of lycopene with intestinal epithelial cells, thereby increasing the bioavailability of lycopene (49). Other research illustrated that sodium caseinate and pectin might protect phytosterols from degradation in the gastric environment (50). Dai et al. also reported the great improvement of curcumin release in the gastrointestinal tract environment caused by zein and rhamnolipid complex nanoparticles (44).

The *in vitro* antioxidant activities of lycopene-loaded PEC NPs, lycopene in acetone, and lycopene in water were determined via DPPH scavenging, hydroxyl radical scavenging, and ABTS radical scavenging assays. Vc was used as the positive control. The stable DPPH radical model is a widely used method for evaluating the free radical scavenging ability of antioxidants. **Figure 9A** shows that, when the lycopene concentration was 0–100 μg/mL, the DPPH radical scavenging activity of the lycopene-loaded PEC NPs was higher than that of lycopene in acetone and was significantly stronger than that of lycopene in water in a concentration-dependent manner. At 100 μg/mL, lycopene-loaded PEC NPs exhibited a scavenging ability of 88.57%, which was close to that of Vc (95.41%). As the most reactive oxygen species, hydroxyl radicals can react with biological molecules and induce severe damage in living cells (51). **Figure 9B** shows that, after lycopene was encapsulated by SC/TLH-3 PEC NPs, the scavenging ability for the hydroxyl radical increased in a dose-dependent manner. At 0–100 μg/mL, the scavenging ability was close to that of lycopene in acetone and higher than that of lycopene in water. At 100 μg/mL, the highest scavenging ability of lycopene-loaded PEC NPs on hydroxyl radical was 77.38%, which was weaker than that of Vc (96.37%). **Figure 9C** indicates that the ABTS radical scavenging activity of lycopene-loaded PEC NPs at 0–20 μg/mL was close to that of Vc and at 0–100 μg/mL was always higher than that of lycopene in acetone and lycopene in water. At 100 μg/mL, the lycopene-loaded PEC NPs exhibited the highest scavenging ability of 75.53%, which was close to that of Vc (89.77%). The results showed that lycopene-loaded PEC NPs exhibited strong antioxidant activities *in vitro* that were positively correlated with their concentration. At 0–100 μg/mL, the activities of lycopene-loaded PEC NPs were higher than those of lycopene in acetone and water, indicating that the water solubility and dispersibility of lycopene were improved after being encapsulated inside the PEC NPs. As a result, its antioxidant activities were promoted. Our previous study also proved that TLH-3 exerts excellent antioxidant activities (18). The enhanced radical scavenging ability of encapsulated lycopene may be attributed to the large surface areas of PEC NPs that are beneficial to the adequate diffusion of encapsulated lycopene in the reaction medium. It can be inferred from the results that the higher the retention of lycopene in PEC NPs, the better is the antioxidant capacity of PEC NPs when compared with free lycopene. Other researchers have also reported that there is a positive correlation between the antioxidant ability and the lycopene retention of the different delivery systems (25, 52). Sodium caseinate (NaCas) is one of the milk protein components with hydrophilic and lipophilic properties that facilitate rapid absorption at the oil-water interface (53). Furthermore, proteins such as SC may have contributed to the oxidative stability of PEC NPs by forming an interfacial physical and electrostatic barrier to pro-oxidants that are common to the aqueous phase. Consequently, lycopene-loaded PEC NPs have a promising prospect in the effective prevention and treatment of diseases related to oxidative damage. Similar results have also been reported in another study, for example, curcumin encapsulated inside protein-polysaccharide nanoparticles exhibited stronger antioxidant and radical scavenging activities than curcumin solubilised in ethanol solutions (39). Lycopene was encapsulated in whey protein isolate-xylo-oligosaccharide conjugates prepared by Maillard reaction, which enhanced the emulsification performance and antioxidant capacity. Therefore, whey protein isolates glycosylated with xylo-oligosaccharides by Maillard reaction can be used to encapsulate lycopene or other bioactives and improve their properties (54). Previous research indicated that the type of carrier oil impacts the reducing power of the beverage emulsions as lycopene was more susceptible to

chemical degradation in the presence of unsaturated, long-chain triglycerides, and the antioxidant capacity was reduced (46).

Effects on Cell Viability of L02 Cells

As shown in **Figure 10A**, lycopene-loaded PEC NPs did not cause any critical toxicity for 24 h, even at 25 μmol/L concentration. As illustrated in **Figure 10B**, the viability of H_2O_2-induced L02 cells decreased in a dose-dependent manner. Nearly 46.99% of the cells had survived when treated with H_2O_2 at 400 μmol/L. Hence, this concentration was used in the subsequent experiments. As shown in **Figure 10C**, the percentage of living cells in lycopene-loaded PEC NPs treated groups at 1, 5 and 10 μmol/L increased in a dose- and concentration-dependent manner. The H_2O_2-induced cells treated with lycopene-loaded PEC NPs at 10 μmol/L showed the highest percentage of living cells (90.16%). The results suggested that exposure to H_2O_2 resulted in an increase in cell death, but lycopene-loaded PEC NPs alleviated the oxidative damage in a dose-dependent manner and significantly protected L02 cells against H_2O_2-induced cell apoptosis. As a well-known antioxidant, lycopene has a protective effect on oxidative stress cell damage. For example, it was reported that lycopene decreased the apoptosis rate of H_2O_2-induced bovine mammary epithelial cells (bMECs) and the accumulation of intracellular reactive oxygen species (ROS) when lycopene was delivered to cells using THF as a solvent which contained 0.025% butylated hydroxytoluene (55). The hepatic cell viability decreased significantly after hypoxia/reaeration treatment but increased in a dose-dependent manner after cells were treated with different concentrations of lycopene by MTT assay (56). Previous studies revealed that nanoliposomes of lycopene (L-LYC) remarkably inhibited JNK-MAPK-induced cell apoptosis, and the total number of apoptotic cells in the ischemic cortex was significantly reduced in L-LYC pre-treatment groups when compared with the vehicle group (57). However, to our best knowledge, the protective effect of lycopene delivery systems such as PEC NPs on H_2O_2-induced oxidative stress cell damage has not been previously reported. The low water-solubility and gastrointestinal instability led to the low bioavailability of lycopene, thereby the higher the retention of lycopene in PEC NPs, the better the protective effect on oxidative stress cell damage when compared with free lycopene.

Hoechst 33342 Staining

Morphological changes in L02 cells were examined with Hoechst 33342 staining to further understand the protective effects of lycopene-loaded PEC NPs (**Figure 10D**). The L02 cell nucleus was round and was stained homogeneously with Hoechst 33342. After H_2O_2 treatment, a considerable proportion of the cells displayed apoptotic characteristics with condensed and fragmented nuclei, an important marker of apoptosis. Meanwhile, the number of apoptotic cells with nuclear fragmentation was significantly reduced after treatment with lycopene-loaded PEC NPs. This finding indicated that lycopene-loaded PEC NPs can inhibit the nucleic morphological changes in H_2O_2-induced L02 cells. Lycopene could protect AML12 hepatic cells from apoptosis as the cells' apoptosis rate was downregulated compared with the hypoxia/reaeration injury group (56). Lycopene also ameliorated H_2O_2-induced SH-SY5Y cell damage and reduced the expression of apoptotic markers, such as Bcl-2, Bax, and cleaved caspase 3 (58).

Effects on H_2O_2-Induced Oxidative Stress

As shown in **Figure 11A**, exposure to H_2O_2 resulted in a 3.8-fold increase in intracellular ROS generation. Meanwhile, the L02 cells treated with lycopene-loaded PEC NPs exhibited significantly reduced ROS production, as indicated by their weak fluorescence intensity for ROS. The lycopene-loaded PEC NPs at 1, 5, and 10 μmol/L decreased the ROS generation by 21.08, 34.21, and 60.53%, respectively, compared with that in the H_2O_2-treated group.

As shown in **Figures 11B,C**, SOD and GSH-px activities were greatly reduced by H_2O_2 exposure but dose-dependently enhanced by treatment with lycopene-loaded PEC NPs. On the contrary, the MDA level increased after treatment with H_2O_2 but decreased after treatment with lycopene-loaded PEC NPs by 20.8 to 34.4% in **Figure 11D**. The above results suggested that lycopene-loaded PEC NPs protect against oxidative damage by stimulating antioxidative enzymes and decreasing the MDA level. Yusuf et al. deal with the encapsulation of lycopene (LYC) as polysorbate-80 (P-80) coated phosphatidylserine-chitosan self-assembled nanoparticles (P-80-LYC-PSCNP), with an approach to reduce oxidative stress and improve the antioxidant enzymatic functioning of CAT, SOD, and GPx (59). Nanoliposomes of lycopene (L-LYC) pre-treatment suppressed oxidative stress in ischemic brains, significantly elevated the total SOD, GSH, and CAT levels, and reduced the level of MDA (57).

Protective Effects on H_2O_2-Treated L02 Cells

The expression levels of some oxidative stress-related major genes [e.g., Nrf2, *HO-1,* and AKT] were evaluated by Western blot to determine whether lycopene-loaded PEC NPs attenuate oxidative damage in cells by inducing various signalling pathways. As indicated in **Figure 12A**, lycopene-loaded PEC NPs dramatically and dose-dependently enhanced the level of *HO-1* protein. However, *HO-1* expression induced by lycopene-loaded PEC NPs was suppressed after treatment with ZnPPIX, a specific *HO-1* inhibitor. As shown in **Figure 12B**, Western blot results indicated that the phosphorylated Nrf2 expression was remarkably increased in the groups treated with lycopene-loaded PEC NPs, suggesting that the PEC NPs significantly upregulated the phosphorylated Nrf2. Moreover, Nrf2 phosphorylation was inhibited upon pre-treatment with the special PI3K/AKT inhibitor (LY294002). These results confirmed that LY294002 could inhibit Nrf2 phosphorylation and decrease *HO-1* transcription and expression, suggesting that AKT phosphorylation is associated with Nrf2/*HO-1* signalling pathway activation. **Figure 12C** shows that, compared with that of the cells under normal conditions, phosphorylated AKT was significantly and dose-dependently upregulated after the cells were treated with lycopene-loaded PEC NPs. This finding suggested that pre-treatment with lycopene-loaded PEC NPs could induce AKT phosphorylation. However, a special

PI3K/AKT inhibitor (LY294002) can significantly inhibit the AKT phosphorylation promoted by lycopene-loaded PEC NPs.

As shown in **Figure 13A**, *HO-1* expression levels were significantly elevated after the H_2O_2-induced cells were treated with lycopene-loaded PEC NPs. As illustrated in **Figure 13B**, phosphorylated Nrf2 was significantly and dose-dependently upregulated in the PEC NP-treated group compared with that in the H_2O_2 treated group, thus supporting the idea that lycopene-loaded PEC NPs could activate Nrf2 by upregulating its phosphorylation. As indicated in **Figure 13C**, AKT phosphorylation decreased upon exposure to H_2O_2 but was upregulated after treatment with lycopene-loaded PEC NPs.

Nuclear factor erythroid 2-related factor 2 is a key regulatory factor that maintains the redox balance, especially under the continuous stimulation of activated AKT (60, 61). Previous studies have confirmed that *HO-1* regulates cell apoptosis by protecting cells from oxidative damage, and its upregulation is involved in the cellular defence mechanism against oxidation (62, 63). In our study, the PI3K/AKT signalling pathway was activated by lycopene-loaded PEC NPs, thus leading to increased p-AKT levels in cells. Upon stimulation by activated AKT, Nrf2 plays an indispensable role against oxidative stress by rapidly detaching from cytoplasmic chaperone protein Keapl and transferring into the nucleus to combine with the antioxidant responsive element in the nucleus, thus transcriptionally activating phase II enzymes/antioxidant genes, such as *HO-1*, NQO1, and GCLC, and increasing the expression levels of GSH-px and SOD to maintain intracellular redox balance and remove the excess oxygen free radicals (23, 64). The above results confirmed that lycopene-loaded PEC NPs could protect L02 cells against H_2O_2-induced oxidative stress by activating the PI3K/AKT/Nrf2 signalling pathway and upregulating the downstream protein *HO-1*. Lycopene supplementation improves the mRNA expressions of the NQO-1 and *HO-1* as antioxidant enzymes, and lycopene decreased neuronal oxidative damage by activating Nrf2 and by inactivating NF-κB translocation in a H_2O_2-related SH-SY5Y cell antioxidant model (59). Lycopene also could promote the transfer of Nrf2 from the cytoplasm into the nucleus and the Nrf2/*HO-1* pathway activation, protecting hepatic cells (AML12 Cells) against hypoxia/Reaeration injury (56). Previous studies revealed that Nanoliposomes of lycopene (L-LYC) significantly reduced cerebral infarction and improved neurobehavior of the rats more efficiently than "naked" lycopene. In addition, L-LYC reduced protein levels of nitric oxide synthase and NOX2, increased the level of Bcl-2, lowered caspase-3, and suppressed apoptosis by inhibiting MAPK-JNK (56). In recent years, lycopene delivery systems have received increasing academic attention, owing to their actions in improving bioavailability (49) and treating tumours (65). However, until this moment, there are no data available about the protective effect of lycopene delivery systems, such as PEC NPs on oxidative stress-induced cell damage and the underlying molecular mechanism. Lycopene-loaded solid lipid nanoparticles (LYC-SLNs) enhanced cytotoxicity in MCF-7 breast cancer cells compared to the free lycopene, which combined with methotrexate (MTX) could be a promising approach to improve the therapeutic benefits of anticancer agents (65).

CONCLUSION

Nowadays, an effective delivery system is needed to improve the absorption and bioavailability of liposoluble nutrients such as lycopene. In this study, the biocompatible TLH-3/SC PEC NPs for lycopene encapsulation were successfully constructed by electrostatic complexation. The lycopene-loaded PEC NPs with high EC and LC exhibited enhanced water-solubility, storage stability, excellent antioxidant capacity, and controlled release ability during a simulated gastrointestinal environment when compared with those of free lycopene. Furthermore, encapsulated lycopene could protect L02 cells from H_2O_2-induced cellular oxidative damage by reducing MDA and ROS levels, and the molecular mechanism of its antioxidation activity was summarised as the Nrf2/*HO-1*/AKT signalling pathway (**Figure 14**). The results indicated that the TLH-3/SC PEC NPs can be developed as effective nanocarriers for delivering liposoluble nutrients and drugs for controlled release, which has a potential application prospect in the food and pharmaceutical industries. Furthermore, this study provides a safe, green, and effective delivery system and a new idea for the development and utilisation of biocompatible PEC NPs.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

XD and LC administrated the project and acquired the funding. LC and YJ conducted experiments and wrote the manuscript. DZ, MS, and MX executed the experiments and analysed the data. LC and FW reviewed and edited this manuscript. All authors contributed to the article and agreed to the published version of the manuscript.

FUNDING

This work was supported by the Open Fund of State Key Laboratory of Tea Plant Biology and Utilisation (SKLTOF20190121); the Provincial Program of Natural Science of Anhui Higher Education (KJ2021A0052); Suzhou University Scientific Research Platform Open Project (2020yzd02); Anhui Quality Engineering Project (2019zyrc107); and Innovation and entrepreneurship training program for college students (202110357047).

REFERENCES

1. Kim MJ, Kim H. Anticancer effect of lycopene in gastric carcinogenesis. *J Cancer Preven.* (2015) 20:92–6. doi: 10.15430/JCP.2015.20.2.92
2. Muller L, Caris-Veyrat C, Lowe G, Böhm V. Lycopene and its antioxidant role in the prevention of cardiovascular diseases-a critical review. *Crit Rev Food Sci Nutr.* (2016) 17:1868–79. doi: 10.1080/10408398.2013.801827
3. Liang XP, Ma CC, Yan XJ, Liu XB, Liu FG. Advances in research on bioactivity, metabolism, stability and delivery systems of lycopene. *Trends Food Sci Technol.* (2019) 93:185–196. doi: 10.1016/j.tifs.2019.08.019
4. Jain A, Sharma G, Ghoshal G, Kesharwani P, Singh B. U.S Shivhare, et al. Lycopene loaded whey protein isolate nanoparticles: an innovative endeavor for enhanced bioavailability of lycopene and anti-cancer activity. *Int J Pharmaceut.* (2018) 546:97–105. doi: 10.1016/j.ijpharm.2018.04.061
5. Aredo V, Passalacqua ES, Prataviera S, Oliveira AD. Formation of lycopene-loaded hydrolysed collagen particles by supercritical impregnation. *Lwt.* (2019) 110:158–67. doi: 10.1016/j.lwt.2019.04.055
6. Zardini AA, Mohebbi M, Farhoosh R. Bolurian S.Production and characterization of nanostructured lipid carriers and solid lipid nanoparticles containing lycopene for food fortification. *J Food Sci Technol.* (2018) 55:287–98. doi: 10.1007/s13197-017-2937-5
7. Bortnowska G. Multilayer oil-in-water emulsions: formation, characteristics and application as the carriers for lipophilic bioactive food components–a review. *Polish J Food Nutri Sci.* (2015) 65:157–66. doi: 10.2478/v10222-012-0094-0
8. Ho KKH, SchroH, K, MartSchroracteristicBerton-Carabin CC. Synergistic and antagonistic effects of plant and dairy protein blends on the physicochemical stability of lycopene-loaded emulsions. *Food Hydrocoll.* (2018) 81:180–90. doi: 10.1016/j.foodhyd.2018.02.033
9. Li W, Yalcin M, Lin Q, Ardawi M, Mousa SA. Self-assembly of green tea catechin derivatives in nanoparticles for oral lycopene delivery. *J Control Rel.* (2017) 248:117–24. doi: 10.1016/j.jconrel.2017.01.009
10. Jain A. D Thakur, Ghoshal G, Katare OP, Singh B, Shivhare US. Formation and functional attributes of electrostatic complexes involving casein and anionic polysaccharides: an approach to enhance oral absorption of lycopene in rats *in vivo*. *Int J Biol Macromol.* (2016) 93:46–756. doi: 10.1016/j.ijbiomac.2016.08.071
11. Cherstvy AG. Electrostatics of DNA complexes with cationic lipid membranes. *J Physical Chem B.* (2007) 111:7914–27. doi: 10.1021/jp0700175
12. Caetano DLZ, Metzler R, Cherstvy A G, De Carvalho S J. Adsorption of lysozyme into a charged confining Pore. *Phy Chem Chem Phy.* (2021) 23:27195. doi: 10.1039/D1CP03185F
13. Elzoghby AO, El-Fotoh W, Elgindy NA. Casein-based formulations as promising controlled release drug delivery systems. *Journal of controlled release.* (2011) 153:206–16. doi: 10.1016/j.jconrel.2011.02.010
14. Livney YD. Milk proteins as vehicles for bioactives. *Curr Opin Colloid Interface Sci.* (2010) 15:73–83. doi: 10.1016/j.cocis.2009.11.002
15. Yi J, Yue L, Fang Z, Yokoyama W. The physicochemical stability and *in vitro* bioaccessibility of beta-carotene in oil-in-water sodium caseinate emulsions. *Food Hydrocoll.* (2014) 35:19–27. doi: 10.1016/j.foodhyd.2013.07.025
16. Cho H, Jung H, Lee H, Kwak HK, Hwang KT. Formation of electrostatic complexes using sodium caseinate with high-methoxyl pectin and carboxymethyl cellulose and their application in stabilisation of curcumin. *Int J Food Sci Technol.* (2016) 51:1655–65. doi: 10.1111/ijfs.13137
17. Ding Q, Yang D, Zhang W, Lu Y, Zhang M, Wang L, et al. Antioxidant and anti-aging activities of the polysaccharide TLH-3 from Tricholoma lobayense. *Int J Biol Macromol.* (2016) 85:133–40. doi: 10.1016/j.ijbiomac.2015.12.058
18. Chen Y, Li XH, Zhou LY, Lu YM. Structural elucidation of three antioxidative polysaccharides from Tricholoma lobayense. *Carbohydr Polym.* (2017) 157:484–92. doi: 10.1016/j.carbpol.2016.10.011
19. Pan WJ, Ding QY, Wang Y, Wang DD, Yu YM, Yang WW, et al. A bioactive polysaccharide TLH-3 isolated from Tricholoma lobayense. protects against oxidative stress-induced premature senescence in cells and mice. *J Func Foods.* (2018) 42:159–70. doi: 10.1016/j.jff.2017.12.070
20. Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiol Rev.* (2014) 94:329–54. doi: 10.1152/physrev.00040.2012
21. Nathan C, Cunningham-Bussel A. Beyond oxidative stress: an immunologist's guide to reactive oxygen species. *Nature Reviews Immunology.* (2013) 13:349–61. doi: 10.1038/nri3423
22. Mozaffari MS, Liu JY, Schaffer SW. Effect of pressure overload on cardioprotection via PI3K-Akt: comparison of postconditioning, insulin, and pressure unloading. *Am J Hypertens.* (2010) 23:668–74. doi: 10.1038/ajh.2010.43
23. Li H, Duan LR, Shwng JJ, Xie YH, Yang Q, Chen Y, et al. Paeonol and danshensu combination attenuates apoptosis in myocardial infarcted rats by inhibiting oxidative stress: roles of Nrf2/HO-1 and PI3K/Akt pathway. *Sci Rep.* (2016) 6:23693. doi: 10.1038/srep23693
24. Kim J, Cha YN, Surh YJ. A protective role of nuclear factor-erythroid related factor-2 (Nrf2) in inflammatory disorders. *Mutat Res.* (2010) 690:12–23. doi: 10.1016/j.mrfmmm.2009.09.007
25. Jain S, Winuprasith T, Suphantharika M. Encapsulation of lycopene inemulsions and hydrogel beads using dual modified rice starch: characterization, stability analysis and release behaviour during *in-vitro* digestion. *Food Hydrocoll.* (2020) 104:105730. doi: 10.1016/j.foodhyd.2020.105730
26. Xu W, Jin W, Zhang C, Li Z, Huang Q, Ye S, et al. Curcumin loaded and protective system based on complex of chsing dual modified rice st*Food Res Int.* (2014) 59:61–6. doi: 10.1016/j.foodres.2014.01.059
27. Zhao C, Wei L, Yin B, Li J. Liu X,Wang J, et al. Encapsulation of lycopene within oil-in-water nanoemulsions using lactoferrin: Impact of carrier oils on physicochemical stability and bioaccessibility. *Int J Biol Macromol.* (2020) 153:912–20. doi: 10.1016/j.ijbiomac.2020.03.063
28. Yu YB, Wu MY, Wang C, Wang ZW, Chen TT, Yan JK. Constructing biocompatible carboxylic curdlan-coated zein nanoparticles for curcumin encapsulation. *Food Hydrocoll.* (2020) 108:106028. doi: 10.1016/j.foodhyd.2020.106028
29. Zhang H, Fu Y, Xu Y, Niu F, Li Z, Ba C, et al. One-step assembly of zein/caseinate/alginate nanoparticles for encapsulation and improved bioaccessibility of propolis. *Food Funct.* (2019) 10:635–45. doi: 10.1039/C8FO01614C
30. Inanc Horuz T, Belibagli KB. Nanoencapsulation by electrospinning to improve stability and water solubility of carotenoids extracted from tomato peels. *Food Chem.* (2018) 268:86–93. doi: 10.1016/j.foodchem.2018.06.017
31. Wang ZB, Pei JJ, Ma HL, Cai PF, Yan JK. Effect of extraction media on preliminary characterizations and antioxidant activities of Phellinus linteus polysaccharides. *Carbohydr Polym.* (2014) 109:49–55. doi: 10.1016/j.carbpol.2014.03.057
32. Wang W, Deng Z, Feng Y, Liao F, Zhou F, Feng S. PM25 induced apoptosis in endothelial cell through the activation of the p53-bax-caspase pathway. *Chemosphere.* (2017) 177:135–43. doi: 10.1016/j.chemosphere.2017.02.144
33. Kim YS, Lee SJ, Hwang JW, Kim EK, Kim SE, Kim EH, et al. *In vitro* protective effects of Thymus quinquecostatus Celak extracts on t-BHP-induced cell damage through antioxidant activity. *Food Chem Toxicol.* (2012) 50:4191–8. doi: 10.1016/j.fct.2012.08.015
34. Yang J, Dong H, Wang Y, Jiang Y, Zhang W, Lu Y, et al. Cordyceps cicadae polysaccharides ameliorated renal interstitial fibrosis in diabetic nephropathy rats by repressing inflammation and modulating gut microbiota dysbiosis. *Int J Biol Macromol.* (2020) 163:442–56. doi: 10.1016/j.ijbiomac.2020.06.153
35. McClements DJ. Theoretical analysis of factors affecting formation and stability of multilayered colloidal dispersions. *Langmuir.* (2005) 21:9777–85. doi: 10.1021/la0512603
36. Tan J, Tan X, Zhong J, Zeng F, Cui M. Effect of sodium alginate on the stability of whey protein solution. *Food Ferment Indust.* (2018) 44:107–13. doi: 10.13995/j.cnki.11-1802/ts.016007
37. Yan JK, Qiu WY, Wang YY, Wu JY. Biocompatible polyelectrolyte complex nanoparticles from lactoferrin and pectin as potential vehicles for antioxidative curcumin. *J Agric Food Chem.* (2017) 65:5720–30. doi: 10.1021/acs.jafc.7b01848
38. Sarika PR, Pavithran A, James NR. Cationized gelatin/gum arabic polyelectrolyte: study of electrostatic interactions. *Food Hydrocoll.* (2015) 49:176–82. doi: 10.1016/j.foodhyd.2015.02.039
39. Huang X, Huang X, Gong Y, Xiao H, McClements DJ. Enhancement of curcumin water dispersibility and antioxidant activity using

coreusing corend antioxidant activity using corel*Food Res Int*. (2016) 87:1–9. doi: 10.1016/j.foodres.2016.06.009
40. Zhao Q, Liu D, Long Z, Yang B, Fang M, Kuang W. Effect of sucrose ester concentration on the interfacial characteristics and physical properties of sodium caseinate-stabilized oil-in-water emulsions. *Food Chem*. (2014) 151:506–13. doi: 10.1016/j.foodchem.2013.11.113
41. Eghbal N, Yarmand MS, Mousavi M, Degraeve P, Oulahal N, Gharsallaoui A. Complex coacervation for the development of composite edible films based on LM pectin and sodium caseinate. *Carbohydr Polym*. (2016) 151:947–56. doi: 10.1016/j.carbpol.2016.06.052
42. Baskar D, Nallathambi G. Dual functional property of lycopene as a reducing agent to synthesis TiO2 nanoparticles and as a ligand to form lycopene-TiO2 nanoparticles complex. *Mater Lett*. (2017) 209:303–6. doi: 10.1016/j.matlet.2017.08.038
43. She W, Luo K, Zhang C, Wang G, Geng Y, Li L, et al. The potential of self-assembled, pH-responsive nanoparticles of mPEGylated peptide dendron-doxorubicin conjugates for cancer therapy. *Biomaterials*. (2013) 34:1613–23. doi: 10.1016/j.biomaterials.2012.11.007
44. Dai L, Li R, Wei Y, Sun C, Mao L, Gao Y. Fabrication of zein and rhamnolipid complex nanoparticles to enhance the stability and *in vitro* release of curcumin. *Food Hydrocoll*. (2018) 77:617–28. doi: 10.1016/j.foodhyd.2017.11.003
45. Xie H, Xiang C, Li Y, Wang L, Zhang Y, Song Z, et al. Fabrication of ovalbumin/kappa-carrageenan complex nanoparticles as a novel carrier for curcumin delivery. *Food Hydrocoll*. (2019) 89:111–21. doi: 10.1016/j.foodhyd.2018.10.027
46. Meroni E, Raikos V. Formulating orange oil-in-water beverage emulsions for effective delivery of bioactives:Improvements in chemical stability, antioxidant activity and gastrointestinal fate of lycopene using carrier oils. *Food Res Int*. (2018) 106:439–45. doi: 10.1016/j.foodres.2018.01.013
47. Li DH, Li L, Xiao N, Li MY,Xie XN. Physical Properties of Oil-in-Water Nanoemulsions Stabilized by OSA-modified Starch for the Encapsulation of Lycopene. *Colloids Surfs A:Physicochem Enginee Aspects*. (2018) 552:59–66. doi: 10.1016/j.colsurfa.2018.04.055
48. Gan CF, Liu Q, Zhang Y, Shi TY, He WS, Jia CS. A novel phytosterols delivery system based on sodium caseinate-pectin soluble complexes: improving stability and bioaccessibility. *Food Hydrocoll*. (2022) 124:107295. doi: 10.1016/j.foodhyd.2021.107295
49. Singh A, Neupane YR, Panda BP, Kohli K. Lipid based nanoformulation of lycopene improves oral delivery: formulation optimization, *ex vivo* assessment and its efficacy against breast cancer. *J Microencapsul*. (2017) 34:416–29. doi: 10.1080/02652048.2017.1340355
50. Yuan YK, Li H, Zhu JX, Liu CZ, Sun X, Wang DF. Fabrication and characterization of zein nanoparticles by dextran sulfate coating as vehicles for delivery of curcumin. *Int J Biol Macromol*. (2020) 151:1074–83. doi: 10.1016/j.ijbiomac.2019.10.149
51. Chen R, Liu Z, Zhao J, Chen R, Meng F, Zhang M. Antioxidant and immunobiological activity of water-soluble polysaccharide fractions purified from Acanthopanax senticosu. *Food Chem*. (2011) 127:434–40. doi: 10.1016/j.foodchem.2010.12.143
52. Souza AL, Hidalgo-Chávez, DW, Pontes SM,Gomes FS, Cabral LM, Tonon RV. Microencapsulation by spray drying of a lycopene-rich tomato concentrate: characterization and stability. *LWT-Food Sci Technol*. (2018) 91:286–92. doi: 10.1016/j.lwt.2018.01.053
53. Sabouri S,Wright A J, Corredig M. *In vitro* digestion of sodium caseinate emulsions loaded With epigallocatechin gallate. *Food Hydrocoll*. (2017) 69:350–8. doi: 10.1016/j.foodhyd.2017.02.008
54. Jia CS, Cao DD, Ji SP, Lin WT, Zhang XM, Muhoza B. Whey protein isolate conjugated with xylo-oligosaccharides via maillard reaction: characterization, antioxidant capacity, and application for lycopene microencapsulation. *LWT-Food Sci Technol*. (2020) 118:108837. doi: 10.1016/j.lwt.2019.108837
55. Sun X D, Jia H D, Xu Q S, Zhao C X, Xu C. Lycopene alleviates H2O2-induced oxidative stress, inflammation and apoptosis in bovine mammary epithelial cells via the NFE2L2 signaling pathway. *Food & Func*. (2019) 10:6276–85. doi: 10.1039/C9FO01922G
56. Liu B, Yan LH, Jiao XF, Sun XZ, Zhao ZG, Yan JW, et al. Lycopene alleviates hepatic hypoxia/reoxygenation injury through Nrf2/HO-1 pathway in AML12 cell. *J Inter Cytokine Res*. (2020) 40:406–17. doi: 10.1089/jir.2020.0038
57. Zhao YS, Xin Z, Li NN, Chang SY, Chen YD, Geng LN, et al. Nanoliposomes of lycopene reduces ischemic brain damage in rodents by regulating iron metabolism. *Free Rad Biol Med*. (2018)124:1–11. doi: 10.1016/j.freeradbiomed.2018.05.082
58. Zhao B, Ren B, Guo R, Zhang W, Ma S, Yao Y, et al. Supplementation of lycopene attenuates oxidative stress induced neuroinflammation and cognitive impairment via Nrf2/NF-κN transcriptional pathway. *Food Chem Toxicol*. (2017) 109:505–16. doi: 10.1016/j.fct.2017.09.050
59. Yusuf M. Formulation and cognitive evaluation of self-assembled phosphatidylserine-chitosan nanoparticles of lycopene, an innovative technique to lessen STZ-induced oxidative stress: a vital persuader of major neurological diseases. *J Drug Del Sci Technol*. (2021) 63:102534. doi: 10.1016/j.jddst.2021.102534
60. Xu X, Li H, Hou X, Li D, He S, Wan C, et al. Punicalagin induces Nrf2/HO-1 expression via upregulation of PI3K/AKT pathway and inhibits LPS-induced oxidative stress in RAW2647. *Macrophag Mediat Inflamm*. (2015) 2015:380218. doi: 10.1155/2015/380218
61. Qi H, Han Y, Rong J. Potential roles of PI3K/Akt and Nrf2-Keap1 pathways in regulating hormesis of Z-ligustilide in PC12 cells against oxygen and glucose deprivation. *Neuropharmacology*. (2012) 62:1659–659. doi: 10.1016/j.neuropharm.2011.11.012
62. Han D, Chen W, Gu X, Shan R, Zou J, Liu G, et al. Cytoprotective effect of chlorogenic acid against hydrogen peroxide-induced oxidative stress in MC3T3-E1 cells through PI3K/Akt-mediated Nrf2/HO-1 signaling pathway. *Oncotarget*. (2017) 8:14680-4680. doi: 10.18632/oncotarget.14747
63. Nitti M, Piras S, Furfaro AL, Brondolo L, Marinari UM, Pronzato MA. Neuroblastoma cell response to oxidative stress is impaired by retinoic acid-induced differentiation: role of HO-1. *Free Rad Biol and Med*. (2016) 100:S106100. doi: 10.1016/j.freeradbiomed.2016.10.272
64. Liu SX, Zhang Y, Wang YF Li XC, Xiang MX, Bian C, et al. Upregulation of heme oxygenase-1 expression by hydroxysafflor yellow a conferring protection from anoxia/reoxygenation-induced apoptosis in H9c2 cardiomyocytes. *Int J Cardiol*. (2012) 160:95–101. doi: 10.1016/j.ijcard.2011.03.033
65. Jain A, Sharma G, Kushwah V, Thakur K, Gargi G, Singh B, Jain S, Shivhare US, Katare OP. Fabrication and functional attributes of lipidic nanoconstructs of lycopene: an innovative endeavour for enhanced cytotoxicity in MCF-7 breast cancer cells. *Coll Surf B: Biointer*. (2017) 152:482–91. doi: 10.1016/j.Colsurfb.2017.01.050

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Zhang, Jiang, Xiang, Wu, Sun, Du and Chen. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 28 June 2022
doi: 10.3389/fnut.2022.845317

Prediction of Soluble Solids and Lycopene Content of Processing Tomato Cultivars by Vis-NIR Spectroscopy

*Márton Égei[1], Sándor Takács[1], Gábor Palotás[2], Gabriella Palotás[2], Péter Szuvandzsiev[2], Hussein Gehad Daood[3], Lajos Helyes[1] and Zoltán Pék[1]**

[1] Institute of Horticultural Science, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary, [2] Univer Product PLC, Kecskemét, Hungary, [3] Regional Knowledge Center, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary

Tomato-based products are significant components of vegetable consumption. The processing tomato industry is unquestionably in need of a rapid definition method for measuring soluble solids content (SSC) and lycopene content. The objective was to find the best chemometric method for the estimation of SSC and lycopene content from visible and near-infrared (Vis-NIR) absorbance and reflectance data so that they could be determined without the use of chemicals in the process. A total of 326 Vis-NIR absorbance and reflectance spectra and reference measurements were available to calibrate and validate prediction models. The obtained spectra can be manipulated using different preprocessing methods and multivariate data analysis techniques to develop prediction models for these two main quality attributes of tomato fruits. Eight different method combinations were compared in homogenized and intact fruit samples. For SSC prediction, the results showed that the best root mean squared error of cross-validation (RMSECV) originated from raw absorbance (0.58) data and with multiplicative scatter correction (MSC) (0.59) of intact fruit in Vis-NIR, and first derivatives of reflectance (R^2 = 0.41) for homogenate in the short-wave infrared (SWIR) region. The best predictive ability for lycopene content of homogenate in the SWIR range (R^2 = 0.47; RMSECV = 17.95 mg kg^{-1}) was slightly lower than that of Vis-NIR (R^2 = 0.68; 15.07 mg kg^{-1}). This study reports the suitability of two Vis-NIR spectrometers, absorbance/reflectance spectra, preprocessing methods, and partial least square (PLS) regression to predict SSC and lycopene content of intact tomato fruit and its homogenate.

Keywords: tomato, Vis-NIR, spectroscopy, SSC, lycopene, absorbance, reflectance, preprocessing

OPEN ACCESS

Edited by:
Spyridon Alexandros Petropoulos, University of Thessaly, Greece

Reviewed by:
Rajko Vidrih, University of Ljubljana, Slovenia
Ahmed Rady, University of Nottingham, Food, Water, Waste Research Group, United Kingdom

****Correspondence:***
Zoltán Pék
pek.zoltan@uni-mate.hu

Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *29 December 2021*
Accepted: *02 June 2022*
Published: *28 June 2022*

Citation:
Égei M, Takács S, Palotás G, Palotás G, Szuvandzsiev P, Daood HG, Helyes L and Pék Z (2022) Prediction of Soluble Solids and Lycopene Content of Processing Tomato Cultivars by Vis-NIR Spectroscopy. Front. Nutr. 9:845317. doi: 10.3389/fnut.2022.845317

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) and tomato-based products are significant components of vegetable consumption. The volume of processed tomatoes in 2020 exceeded 38 million tons in the world (1). From a processing point of view, the two most important quality attributes of tomato fruits are soluble solids content (SSC) and lycopene content (2, 3).

The highest cost of compaction is the energy used to evaporate water from the raw material to concentrate it to 28–38°Brix, which results in a product that can be transported more easily in this form and is eligible for further processing. Thus, when it comes to the SSC level of raw tomato, the higher SSC, the less water has to be evaporated from it, reducing the cost and energy consumption of this operation (4). This means that the processing industry pays extra price for raw tomatoes above a certain level of SSC (5).

In general, the total dry matter (DM) and SSC of the fruit increase as it ripens, in parallel with their pigmentation. The color of red-fruit varieties makes it easy to distinguish whether they are ripe or not (6–8). While SSC can be easily measured by refractometer in°Brix (9), its estimation based only on maturity depends on the cultivar (10). Therefore, it is of great importance to develop a non-destructive method for the accurate estimation of SSC of intact tomato fruits (11–14) or rapid monitoring of their homogenates in analytical laboratory or during the quality check of incoming raw material at the receiving area of processing plant (15, 16).

Lycopene, the main carotenoid component of red ripe tomato fruit, is often considered the main preference of consumers' acceptance and a major factor for cardiovascular protection in addition to its importance in the reduction of oxidative stress active substances (17, 18).

There are examples for the estimation of lycopene content of red-ripe fruits based on their color (19, 20), but accurate values can only be obtained by expensive and time-consuming laboratory analytics (21–24). There is demand for making this procedure quicker, cheaper, and easier. Measuring spectral reflectance can be a good option for this approach (25–29).

The method is based on the near-infrared (NIR) absorption of the overtones and combination bands of water and organic molecules, mainly O–H, C–H, N–H, and C = O groups. NIR spectra are complex and more difficult to interpret as in other spectral regions like visible (Vis) spectra (30, 31).

Recently, visible-near infrared (Vis-NIR) spectroscopy has been increasingly used in studies for non-destructive determination of ingredients of fruits (32–36) and especially of tomato (22, 23, 37, 38). The rapid determination of SSC in an intact fruit or in a sample homogenized from it is a difficult task due to its high water content (39). A number of usable calibrations have already been made for the rapid determination of SSC and lycopene content in paste from processing tomatoes as the material concentrated to a°Brix value of 28–38 already contains less water (40, 41).

The development of a NIR calibration is a complex task that involves spectral collection using a NIR device, chemometrics, spectra pretreatment, calibration model development, and model validation.

The objective of this study was to evaluate the use of Vis-NIR range of spectra for measuring the SSC and lycopene content of tomato fruit and its homogenate. Choosing the best preprocessing and calibration method for the validation of these important parameters can contribute for developing a non-destructive way, which is applicable to measure these parameters quickly and accurately.

MATERIALS AND METHODS

Plant Material

Fruits were produced in open-field experiments of processing tomato in 2019 and 2020. These experiments were carried out at the Experimental Farms of the Hungarian University of Agriculture and Life Sciences (S1), Gödöllő (47°34′N. 19°22′E; elevation 231 m) and Szarvas (S2) (46°53′N. 20°31′E; elevation 81 m) in 2019, and Experimental Farms of the Univer Agro Kft in Szentkirály (S3) (46°54′N. 19°59′E; elevation 91 m) in 2020. Production technology was the same as our previous processing tomato experiments (42–44). There were different processing tomato hybrids from three different seed companies: HeinzSeed (Pomodoro Agro Kft., Mezőberény, Hungary): H1015, H1281, H1307, H1765, H1776, H1879, H1884; BASF Nunhems (Nunhems Hungary Kft., Budapest, Hungary): N6438, NUN283, NUN287, NUN507, NUN812, NUN912, Ussar; and United Genetics (Orosco Kft., Orosháza, Hungary): UG812J, UG1410, UG5202, UG8114, UG13577, UG13579, UG14014, Prestomech. H1015 F1 and UG812J were used in both years only. Samples were formulated from 10 healthy fruits with similar visual appearance in four repetitions of each treatment combinations. The fruits were harvested by hand in red ripe stage in August of both years.

Spectral Acquisition of the Sample's Reflectance

Spectral and analytical measurements were performed with tomato samples right after harvesting. For the intact tomato samples, data are output as reflectance only, by ASD, because Perten is inappropriate for measuring intact fruits as its sample container does not fit intact fruit size. The reflectance and absorbance data were obtained from the laboratory of Regional Knowledge Centre of Hungarian University of Agriculture and Life Sciences (Gödöllő). For the spectral acquisition, the tomato samples were used in two forms, namely, intact tomato fruits (n = 132) and homogenates (n = 192). In the first step, the intact tomato fruits were cleaned before the collection of spectra. Spectral measurements were taken with two instruments, namely, ASD FieldSpec HandHeld 2^{TM} (Analytical Spectral Devices. Inc., Co., United States) Portable Spectroradiometer (spectral range: 325–1,075 nm) and Perten DA7200 (Perten Instruments, Forr-Lab Kft., Budapest, Hungary) NIR analyzer (spectral range: 950–1,650 nm).

A total of 132 spectral samples were directly acquired in the range of 325–1,075 nm from S1 site in 2019 using the ASD spectroradiometer. Fruit samples were derived from irrigation and microbiological treatment combinations of H1015 and UG812J processing tomato hybrids (45).

After measurement of intact fruits, a total of 1,920 tomato fruits were washed, cut, and homogenized for the 192 samples from S2 and S3 sites in 2020. A black Teflon plate (diameter 75 mm) was filled with 26 ± 1 mm of samples, ASD spectroradiometer positioned 2 mm above samples, with Hi-Brite Contact Probe (Analytical Spectral Devices. Inc., Co., United States). Light source of the device was halogen bulb with

color temperature 2,900 K, using Zenith Polymer® reference panel made of sintered polytetrafluoroethylene (SphereOptics GmbH, Uhldingen, Germany) for calibration. The spectral scanning was made in five replicates. The instrument has a spectral resolution of < 3.0 nm at 700 nm and wavelength accuracy of ± 1 nm. The black plate perfectly fit into the Perten DA7200 rotation cup, the instrument was worked in the 950–1,650 nm spectral range, and the spectral resolution was 5 nm. For further spectral analysis, an average of five reflectance and absorbance recordings from each sample was used.

Analytics

Soluble Solid Content

Mettler-Toledo Easy R40 refractometer (Mettler Toledo Kft., Budapest, Hungary) was used to measure the SSC of homogenized tomato samples in each replicate (10 fruits) with temperature control on 20°C (46). Its integrated Peltier temperature control quickly heats up or cools down the measurement cell, maintaining the sample reliably at the desired temperature. The tomato homogenate was filtered with gauze and dripped on the measuring cell. An average of two measurements from each repetition of samples were used for the models.

Lycopene

The sample was made from homogenization of 10 fruits. The sample preparation was conducted according to Daood et al. (47). Hitachi Chromaster HPLC (VWR International Kft., Debrecen, Hungary) using a Model 5110 Pump, a Model 5430 Diode Array Detector, and a Model 5210 auto-sampler. The separation and data processing were operated using the EZChrom Elite software (Agilent Technologies, Inc., Santa Clara, CA, United States). Carotenoids were detected between 190 and 700 nm. Separation of carotenoids was performed on a core C-30, 150 × 4.6 mm, 2.6 μm (Thermo Scientific, Waltham, MA, United States) column with a gradient elution of (A) tert-butyl-methyl ether in (B) 2% water in methanol (48). The gradient started with 3% A in B, changed to 35% A in B in 20 min, steady isocratic for 5 min, and finally turned to 3% A in B in 5 min. The flow rate was 0.6 ml min^{-1}. For quantification, the area of each compound was recorded at the maximum absorbance wavelength. Concentration of carotenoids was calculated as 8-apo-carotenal equivalent. The internal standard was set at a known concentration to the samples. Standard material for lycopene (Sigma-Aldrich, Budapest, Hungary) was also used, as an external standard, for its identification and quantitation.

Spectral Data Analysis

The spectral data were analyzed using the Unscrambler 11.0 software (CAMO Analytics AS., Oslo, Norway). Preprocessing of spectral data is often of vital importance if reasonable results are to be obtained whether the analysis is used for exploratory data mining, classification, or building a good robust prediction model (49). The obtained spectra can be manipulated using different preprocessing methods and multivariate data analysis techniques to develop prediction models for these two main quality attributes of tomato fruits. Three preprocessing methods were used to improve the quality of original spectra, multiplicative scatter correction (MSC), standard normal variate (SNV), Savitzky-Golay based on first derivative (1DER) as in previous studies (32, 50, 51), and their combinations with reflectance and absorbance spectra. Partial least-square regression (PLSR) was used to develop calibration models between spectral data and SSC or lycopene content of tomatoes. Eight different method combinations were compared in fruit and homogenized samples (**Table 1**). The calibration set was 75%, and the validation set was 25% of the total samples. The correct number of regression factors for the PLSR model was judged by root mean square error of cross-validation (RMSECV) obtained by 10-fold cross-validation.

Reflectance data were obtained from intact tomato fruits (n = 132) by ASD only, while from homogenized samples (n = 192) reflectance and absorbance were measured by ASD and Perten, respectively. One of the four replicates of each individual tomato groups was selected to represent the entire population for validation.

RESULTS AND DISCUSSION

Spectral Acquisition

Figure 1 shows the visual representation of reflectance spectra of all intact tomato fruit samples obtained by ASD HH2 device. The profiles present broad but identifiable bands, ascribable to the contributions of the main constituents of the food matrix such as water and sugar. Reflectance value is below 0.1 from 400 to 575 nm as previously detected by ElMasry and Sun (52), including an intense absorption peak between 450 and 475 nm as found by Ciaccheri et al. (53). Above 560 nm, reflectance values rose sharply because of the red coloration of ripened fruits (54). The variability of spectra was the highest in the Vis-NIR region between 650 and 930 nm. The reflectance maximum was measured between 700 and 705 nm, as found by Clément et al. (52). In the NIR, there was a local absorption maximum at 976 nm.

Figure 2 shows average reflectance spectra of all tomato homogenate samples for calibration and validation dataset in the range 375–1,075 nm. The results showed some absorbance peaks due to the vibration of O–H, C–H, and N–H bonds, which are related to inner fruit compositions such as sugars and acids. The absorption in the visible spectra is due to the fruit pigments such as chlorophyll, β-carotene, and lycopene. The highest bands in the VIS region (peaks at 550 and 607 nm) are because of the absorption of the chlorophyll, β-carotene, and lycopene similar to the results described previously (55). Yellow (570–590 nm), orange (590–620 nm), and red (620–750 nm) regions of reflectance spectra correlated well with tomato fruit color (56).

The average absorbance spectra of the homogenized tomato fruit samples in the SWIR region measured by Perten can be seen in **Figure 3**. The highest bands (peaks at 1,095 nm) were in the SWIR region, which are due to the C–H, O–H, and N–H bonds (30, 57). The typical absorption bands related to the high water content of tomato samples can be seen around 950 and 1,450 nm, and a sugar-related peak appears also in the spectrum around

TABLE 1 | Predictive capability of calibration models for SSC and lycopene content of tomato samples by ASD and Perten spectrometers.

		Fruit (n = 132)				Homogenate (n = 192)							
		ASD (Vis-NIR)				ASD (Vis-NIR)				Perten (SWIR)			
		R^2CAL	RMSEC	R^2VAL	RMSECV	R^2CAL	RMSEC	R^2VAL	RMSECV	R^2CAL	RMSEC	R^2VAL	RMSECV
SSC	Reflectance	0.73	0.49	0.62	0.64	0.17	0.70	0.20	0.59	0.65	0.45	0.55	0.44
	Absorbance	**0.87**	**0.35**	**0.68**	**0.58**	0.61	0.48	0.58	0.43	0.67	0.44	0.58	0.43
	REF + MSC	0.67	0.55	0.54	0.70	0.01	0.76	0.00	0.66	0.59	0.49	0.57	0.43
	REF + SNV	0.63	0.58	0.52	0.71	0.38	0.60	0.52	0.46	0.64	0.45	0.60	0.42
	REF + 1DER	0.47	0.69	0.47	0.74	0.30	0.64	0.37	0.52	**0.70**	**0.42**	**0.61**	**0.41**
	ABS + MSC	**0.88**	**0.34**	**0.72**	**0.59**	0.62	0.47	0.54	0.45	0.60	0.49	0.56	0.44
	ABS + SNV	0.85	0.37	0.66	0.60	**0.64**	**0.46**	**0.58**	**0.43**	0.66	0.45	0.59	0.42
	ABS + 1DER	0.77	0.46	0.55	0.69	0.57	0.50	0.51	0.46	**0.70**	**0.42**	**0.60**	**0.42**
LYCOPENE	Reflectance	0.36	41.01	0.42	41.06	0.07	27.38	0.10	23.47	0.46	20.99	0.43	18.70
	Absorbance	0.30	42.94	0.40	41.63	**0.86**	**10.56**	**0.68**	**15.07**	0.48	20.47	0.44	18.41
	REF + MSC	0.54	34.89	0.41	41.19	0.01	28.37	0.00	24.68	0.46	20.94	0.38	19.48
	REF + SNV	0.57	33.70	0.41	41.34	0.52	19.79	0.52	17.07	0.45	21.15	0.37	19.51
	REF + 1DER	0.50	36.37	0.28	45.68	0.26	24.47	0.20	22.10	0.44	21.21	0.42	18.77
	ABS + MSC	**0.64**	**30.62**	**0.46**	**39.64**	0.61	17.66	0.52	17.09	0.49	20.34	0.38	19.50
	ABS + SNV	**0.66**	**30.14**	**0.44**	**40.31**	0.63	17.25	0.52	17.14	0.45	21.05	0.35	19.84
	ABS + 1DER	0.52	35.50	0.34	43.64	0.46	20.98	0.41	18.99	**0.51**	**19.86**	**0.47**	**17.95**

ASD, ASD FieldSpec HandHeld 2™ portable spectroradiometer; PERTEN, Perten DA7200 NIR analysis system; Vis-NIR, visible and near infrared; SWIR, short-wave infrared; CAL, calibration; VAL, validation; RMSEC, root mean square error of calibration; RMSECV, root mean square error of cross-validation; REF, reflectance; ABS, absorbance; MSC, multiplicative scattering correction; SNV, standard normal variate; 1DER, first derivative. Bold numbers mean the best calibration and prediction of models.

1,100 nm. Similar absorption bands were reported in the studies of tomato fruits and nectarines (58).

Reference Values in Tomato Samples

To produce good quality paste of tomatoes, they need to be harvested at red ripe stage of fruits, with the highest possible DM content. Immediately after the spectra were measured, SSC measurements were performed using homogenized fruit samples. The samples were then frozen as the high-performance liquid chromatography (HPLC) capacity for lycopene determination did not allow simultaneous measurement of all samples. To make our models as general as possible, 25 varieties harvested over 2 years from three regions of Hungary were included in the samples.

Tomato fruit SSC is the first and lycopene content is the second most important quality attribute for the processing

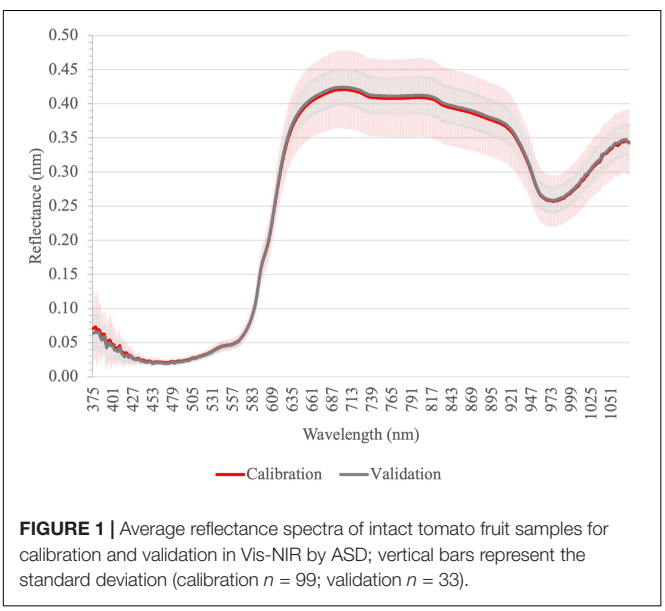


FIGURE 1 | Average reflectance spectra of intact tomato fruit samples for calibration and validation in Vis-NIR by ASD; vertical bars represent the standard deviation (calibration n = 99; validation n = 33).

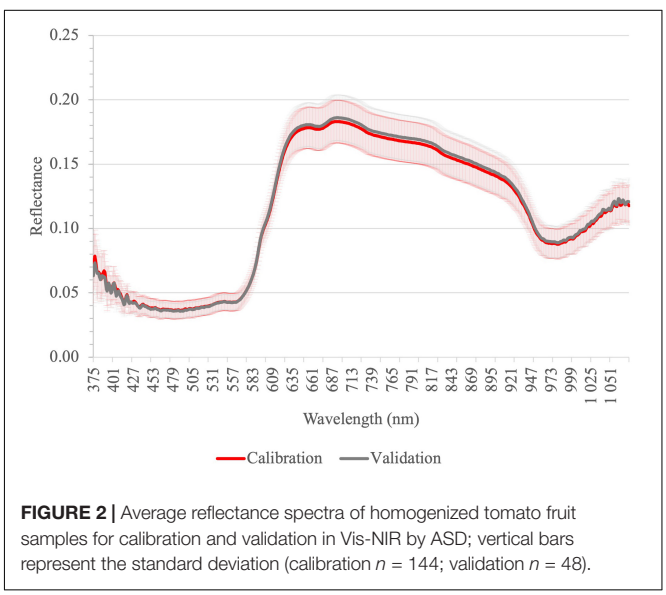


FIGURE 2 | Average reflectance spectra of homogenized tomato fruit samples for calibration and validation in Vis-NIR by ASD; vertical bars represent the standard deviation (calibration n = 144; validation n = 48).

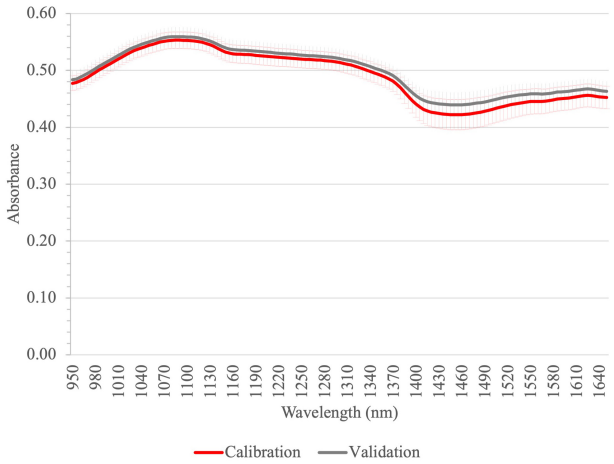


FIGURE 3 | Average absorbance spectra of homogenized tomato fruit samples for calibration and validation in SWIR by Perten; vertical bars represent the standard deviation (calibration n = 144; validation n = 48).

industry. For both properties, the distributions of the reference values in the calibration and validation set were comparable. The sequences of validation samples were designed to represent the characteristics of the calibration sequences. Samples were selected by genotype, by treatment combination, with three from the four replicates for calibration and one for validation (**Figures 4**, **5**). In the figures, the transparent bars indicate the number of validation samples that contained fewer categories than the calibration samples. Values below 4°Brix have been found in the calibration samples, which would limit the opportunities of profitable processing and imply price reduction for the grower when measured at delivery. Processors expect a high SSC because the lower the water content of the raw material, the lower the cost of concentration.

Table 2 represents the average SSC and lycopene content of intact tomato fruits and homogenates used for calibration and validation. The parameters of the sample population selected for calibration and validation only slightly differed. Since the samples of intact and homogenized fruits were from two consecutive years, the higher SSC can be explained by the effect of seasonal variation according to our previous studies (59, 60). The effect of higher temperature on lycopene content is larger and opposite to that of SSC, which may have been caused by extreme high-temperature events in 2020 (59, 61).

Calibration and Validation

Both instruments were used to perform reflectance and absorbance measurements on homogenized samples, but the intact fruits could only be measured with the ASD instrument as they do not fit in the Perten sample tray due to their size.

Soluble Solid Content Prediction

The results of SSC showed reliable correlation coefficient of cross-validation (R^2 = 0.68) originated from raw reflectance of intact fruits and absorbance preprocessed by MSC (R^2 = 0.72) with RMSECV 0.58 and 0.59, respectively in Vis-NIR spectra. In this spectral range, the absorbance data gave the smallest error (RMSECV 0.43) for the homogenized samples, which could not be improved by SNV (R^2 = 0.58; RMSECV 0.43). The predictive capability of the SWIR spectrum for SSC gave the best results when using the first derivative of reflectance spectra, better than in the Vis-NIR (R^2 = 0.61), and with lower error (RMSECV = 0.41).

Figure 6 shows the scatter plot of measured and predicted SSC using Vis-NIR PLSR models in the calibration and validation set of intact fruits by ASD. The best correlation was performed using the absorbance data + MSC spectral transformation of the samples for the determination of SSC agreed with others (32). The statistical parameters of prediction were R^2 = 0.7216 and RMSECV = 0.59°Brix.

The correlation coefficient (R^2 = 0.6821) and error (RMSECV = 0.58) of the first derivative of absorbance in homogenized fruit samples are only slightly different from the reflectance results (**Figure 7**), as has been described by others (51). Based on the graphical representations of the SSC calibrations and validations, the reflectance-based SWIR spectrum seems to be a better prediction method for homogenized samples.

Although DM is of major importance in tomato physiological research (62), the DM content of the fruit is closely related to its SSC (63). Therefore, SSC has been widely used in practice for the grading of raw tomatoes as it requires simpler sample preparation, cheaper devices, and less labor (4).

There are several difficulties to estimate SSC of tomato fruits non-destructively by spectral characteristic. Tomato fruits have a low SSC, which makes it more difficult to make a reliable prediction compared to fruits with a higher DM content (64). Especially, NIR-SSC predictions were heavily influenced by the correlation of inner and outer mesocarp SSC, which varied during fruit development (35).

Usually, the range of SSC in tomato fruit from the same cultivar and the same growing condition were relatively limited, so fruits using from different cultivars and production sites to achieve a wide range of fruit SSC are recommended for model development (65, 66). Therefore, we aimed to include more varieties and growing sites in the experiment to analyze a more representative sample population.

The scientific evidence generally agrees that Vis-NIR spectroscopy can be used to assess SSC in intact, thin-skinned fruit and is already being used in commercial practice. It is also expected that models based on transmittance will be more reliable in predicting SSC than models based on reflectance or absorbance as these methods are sensitive to surface reflectance variation (35, 67).

Lycopene Content Prediction

According to PLSR, based on Vis-NIR absorbance spectra, the best lycopene prediction of fruits resulted with MSC. The correlation coefficient of cross-validation was R^2 = 0.46 and RMSECV = 39.6 mg kg^{-1} (**Table 1**). The best calibration of homogenate absorbance spectra by ASD had a more reliable predictive capability,

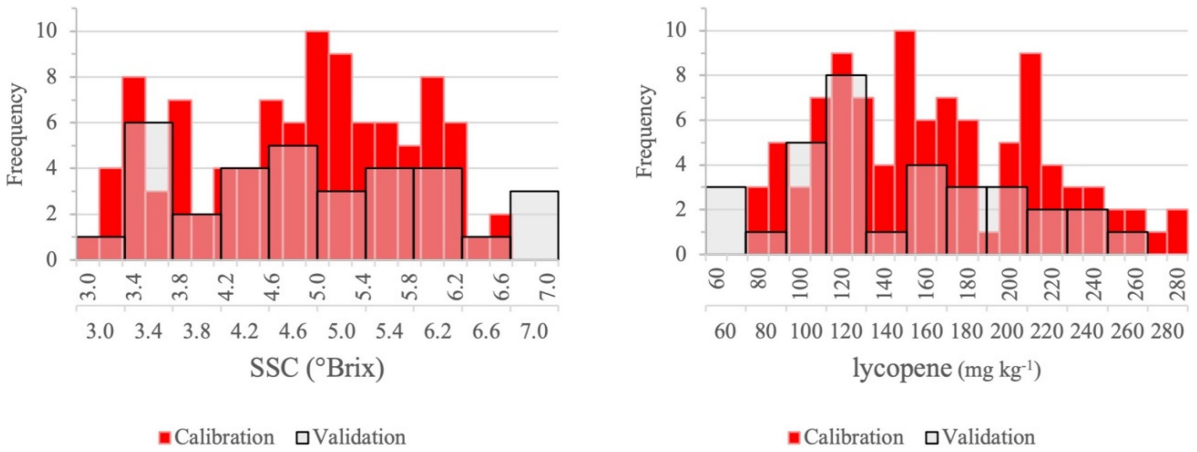


FIGURE 4 | Distribution of SSC and lycopene content of intact tomato fruit for the calibration (n = 99) and validation (n = 33) sets.

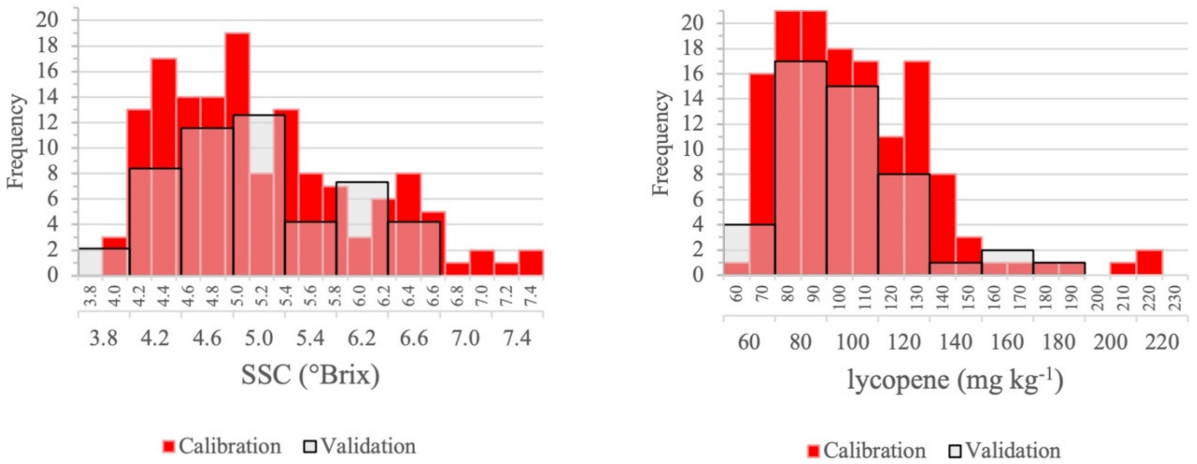


FIGURE 5 | Distribution of SSC and lycopene content of homogenized samples for the calibration (n = 144) and validation (n = 48) sets.

TABLE 2 | SSC and lycopene content of intact tomato fruits and homogenized samples in the calibration and validation sets.

	Total (n = 132)			Calibration (n = 99)			Validation (n = 33)		
Fruit	**Range**	**Mean**	***SD***	**Range**	**Mean**	***SD***	**Range**	**Mean**	***SD***
SSC (°Brix)	3.07–6.70	4.80	0.96	3.07–6.60	4.80	0.18	3.20–6.70	4.81	0.35
Lycopene (mg kg^{-1})	79.4–287.5	167.9	51.2	81.6–287.5	168.5	9.97	79.4–282.2	166.2	18.2
	Total (n = 192)			**Calibration (n = 144)**			**Validation (n = 48)**		
Homogenate	**Range**	**Mean**	***SD***	**Range**	**Mean**	***SD***	**Range**	**Mean**	***SD***
SSC (°Brix)	3.85–7.41	5.17	0.77	3.96–7.41	5.20	0.13	3.85–6.49	5.09	0.11
Lycopene (mg kg^{-1})	63–223	109.4	28.5	63.0–223.0	110.5	4.85	73.0–181.0	106.0	4.01

RMSECV = 15.07 mg kg^{-1}, where the correlation coefficient was R^2 = 0.68 (**Figure 8**).

The predictive ability in the SWIR range (R^2 = 0.47; RMSECV = 17.95 mg kg^{-1}) was lower for lycopene than in the Vis-NIR range, as represented in **Figure 9**.

The concentration of lycopene is not homogeneous in the fruit of tomatoes, being highest under the skin and much lower in the rest of the fruit (68), and recently bred tomato varieties with high lycopene content require more accurate methods to quantify lycopene content (69).

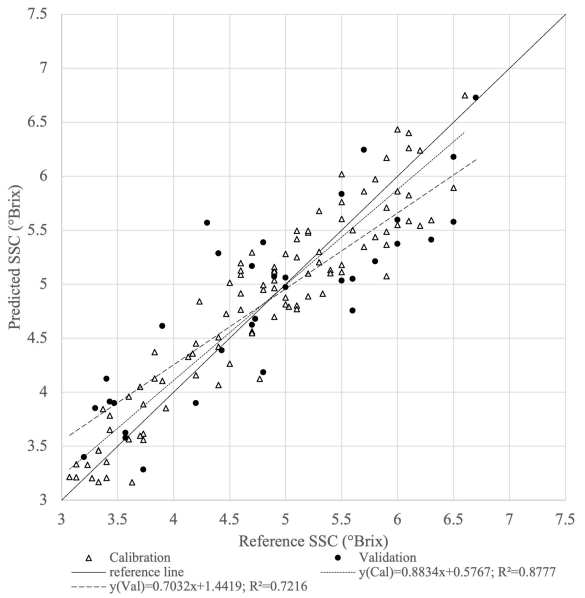


FIGURE 6 | Calibration (Cal) and validation (Val) set of reference (n = 99) vs. predicted (n = 33) SSC of intact tomato fruits derived from the best PLSR model from absorbance of Vis-NIR spectra with MSC preprocessing.

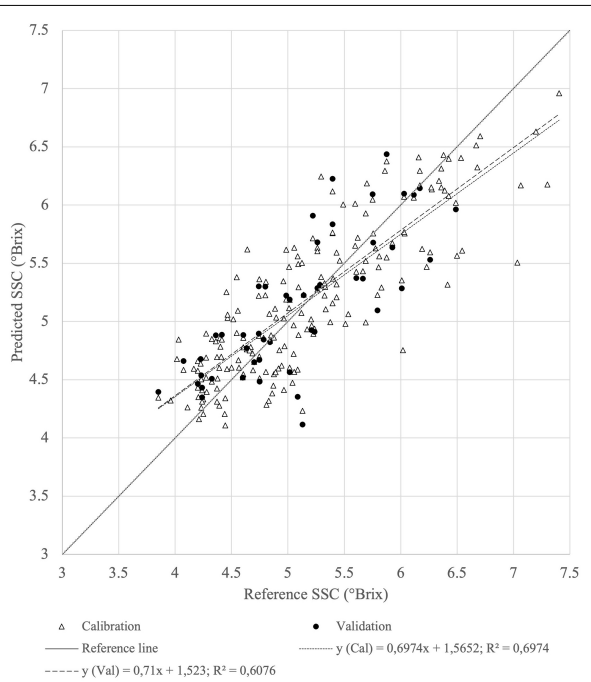


FIGURE 7 | Calibration (Cal) and validation (Val) set of reference (n = 99) vs. predicted (n = 33) SSC of tomato homogenates derived from the best PLSR model from reflectance of SWIR spectra with first derivative preprocessing.

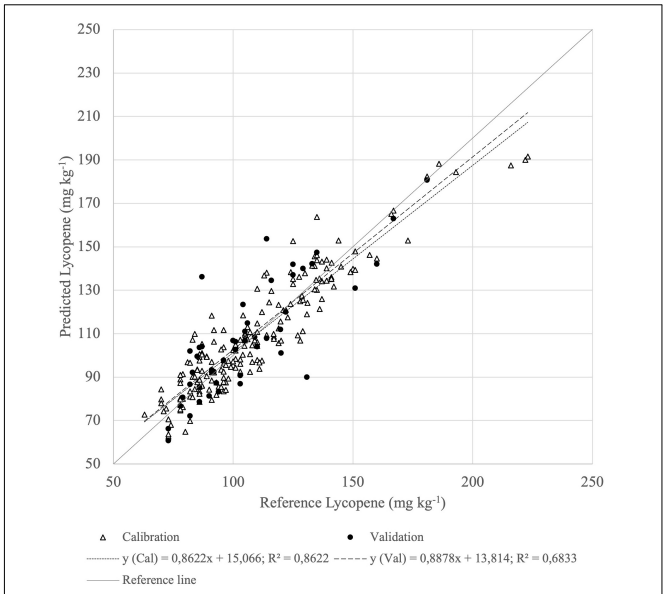


FIGURE 8 | Calibration (Cal) and validation (Val) set of reference (n = 144) vs. predicted (n = 48) lycopene content of homogenized tomato fruit samples derived from the best PLSR model from absorbance of Vis-NIR spectra.

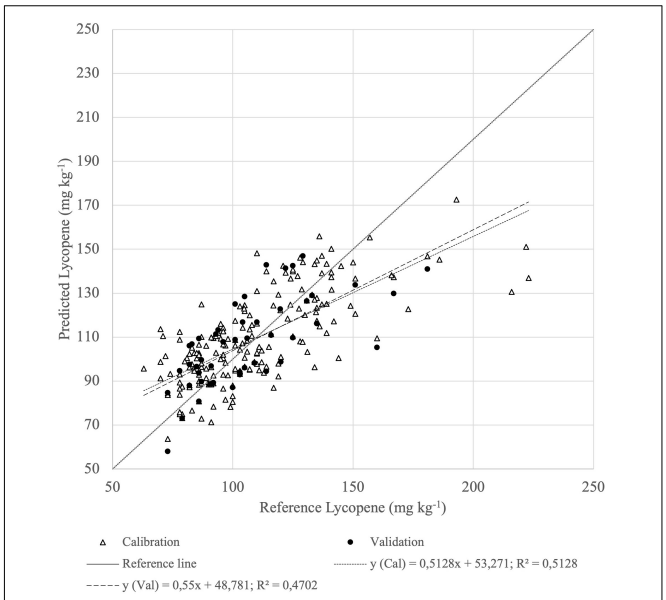


FIGURE 9 | Calibration (Cal) and validation (Val) set of reference (n = 144) vs. predicted (n = 48) lycopene content of homogenized tomato fruit samples derived from the best PLSR model from absorbance of SWIR spectra with first derivative preprocessing.

Lycopene content is well defined by non-destructively measured color values of tomato fruits, which in turn is highly dependent on variety and ripeness (56, 70, 71). VIS reflectance spectra can therefore be readily used to assess the main carotenoid in intact tomato fruit (72). Further investigation of NIR spectra on additional varieties to evaluate carotenoids in intact tomato fruit may help to develop more robust models (72, 73).

CONCLUSION

Vis-NIR spectroscopy is a rapid tool to assist the industry or the laboratory for the estimation of the quality of raw or homogenized tomato fruits.

The use of multiplicative scattering correction and the first derivative were efficient preprocessing techniques for the validation and resulted in the most accurate estimation models of ingredients in tomato. Calibration models of raw absorbance from Vis-NIR spectra resulted in reliable prediction of intact fruits' SSC (R^2_{VAL} = 0.72), but SWIR spectral instrument produced lower RMSECV (0.41°Brix). Raw absorbance by Vis-NIR spectral range resulted slightly lower RMSECV of homogenate lycopene content (15.07 mg kg^{-1}) comparing model of absorbance with first derivative in SWIR range (17.95 mg kg^{-1}).

Combination of the two techniques (spectral range) could result in a more accurate calibration model for intact berries, which could be used to select raw material before the processing and for monitoring its homogenate in analytical laboratory or during the quality check of incoming raw material at the receiving area of processing plant. More homogeneous samples would result in a more accurate calibration, but this would not be conducive to the wide applicability of these models in practice. Vis-NIR spectroscopy appears to be a rapid and cost-effective technique compared to laboratory analytics, but using raw spectra requires a high level of skill because preprocessing is necessary. Traditional chemometric methods are time-consuming with higher cost and environmental impact, especially for lycopene analytics. The accuracy of the prediction obtained in this study indicates that Vis-NIR spectroscopy offers a useful method for quick and convenient evaluation of quality traits of tomato fruit. Further studies may give perspective to obtain better calibration models involving middle infrared range with more diverse sample population in accurate predictive capability.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

ZP and MÉ: conceptualization and writing—original draft preparation. ST, HD, PS, and GaP: methodology and investigation. MÉ: software, data curation, and visualization. MÉ, ZP, and ST: validation. LH: resources and funding acquisition. ZP and GáP: writing—review and editing. GáP and LH: supervision. All authors have read and agreed to the published version of the manuscript.

FUNDING

This research was supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project. This study was supported by the ÚNKP-21-4 New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund.

REFERENCES

1. World Processing Tomato Council [WPTC]. *World Production Estimate of Tomatoes for Processing.* (2021). Available online at: https://www.wptc.to/pdf/releases/WPTC%20World%20Production%20estimate%20as%20of%2004-04-2022.pdf (accessed June 17, 2022).
2. Rocco CD, Morabito R. Robust optimisation approach applied to the analysis of production / logistics and crop planning in the tomato processing industry. *Int J Prod Res.* (2016) 54:5842–61. doi: 10.1080/00207543.2016.1181284
3. Martínez-Hernández GB, Boluda-Aguilar M, Taboada-Rodríguez A, Soto-Jover S, Marín-Iniesta F, López-Gómez A. Processing, packaging, and storage of tomato products: influence on the lycopene content. *Food Eng Rev.* (2016) 8:52–75. doi: 10.1007/s12393-015-9113-3
4. Rocco CD, Morabito R. Production and logistics planning in the tomato processing industry: a conceptual scheme and mathematical model. *Comput Electron Agric.* (2016) 127:763–74. doi: 10.1016/j.compag.2016.08.002
5. Branthôme F-X. *Hungary: Univer Doubles its Processing Capacity.* (2017). Available online at: http://www.tomatonews.com/en/hungary-univer-doubles-itsprocessing-capacity_2_128.html (accessed June 17, 2022).
6. Opara UL, Al-Ani MR, Al-Rahbi NM. Effect of fruit ripening stage on physico-chemical properties, nutritional composition and antioxidant components of tomato (*Lycopersicum esculentum*) cultivars. *Food Bioprocess Technol.* (2012) 5:3236–43. doi: 10.1007/s11947-011-0693-5
7. Raffo A, Leonardi C, Fogliano V, Ambrosino P, Salucci M, Gennaro L, et al. Nutritional value of cherry tomatoes (*Lycopersicon esculentum* cv. Naomi F1) harvested at different ripening stages. *J Agric Food Chem.* (2002) 50:6550–6. doi: 10.1021/jf020315t
8. Nour V, Ionica ME, Trandafir I. Bioactive compounds, antioxidant activity and color of hydroponic tomato fruits at different stages of ripening. *Not Bot Horti Agrobot Cluj Napoca.* (2015) 43:404–12. doi: 10.15835/nbha43210081
9. Grandillo S, Zamir D, Tanksley SD. Genetic improvement of processing tomatoes: a 20 years perspective. *Euphytica.* (1999) 110:85–97. doi: 10.1023/A:1003760015485
10. Baltazar A, Aranda JI, González-Aguilar G. Bayesian classification of ripening stages of tomato fruit using acoustic impact and colorimeter sensor data. *Comput Electron Agric.* (2008) 60:113–21. doi: 10.1016/j.compag.2007.07.005
11. Wati RK, Pahlawan MFR, Masithoh RE. Development of calibration model for pH content of intact tomatoes using a low-cost Vis/NIR spectroscopy. *IOP Conf Ser Earth Environ Sci.* (2021) 686:012049. doi: 10.1088/1755-1315/686/1/012049
12. Saad AG, Jaiswal P, Jha SN. Non-destructive quality evaluation of intact tomato using VIS-NIR spectroscopy. *Int J Adv Res.* (2014) 2:632–9.
13. Rahman A, Kandpal LM, Lohumi S, Kim MS, Lee H, Mo C, et al. Nondestructive estimation of moisture content, pH and soluble solid contents in intact tomatoes using hyperspectral imaging. *Appl Sci.* (2017) 7:109. doi: 10.3390/app7010109
14. Borba KR, Aykas DP, Milani MI, Colnago LA, Ferreira MD, Rodriguez-Saona LE. Portable near infrared spectroscopy as a tool for fresh tomato quality control analysis in the field. *Appl Sci.* (2021) 11:3209. doi: 10.3390/app11073209
15. Bureau S, Arbex de Castro Vilas Boas A, Giovinazzo R, Jaillais B, Page D. Toward the implementation of mid-infrared spectroscopy along the processing chain to improve quality of the tomato based products. *LWT Food Sci Technol.* (2020) 130:109518. doi: 10.1016/j.lwt.2020.109518

16. Kubo MTK, Rojas MI, Miano AC, Augusto PED. Rheological properties of tomato products. In: Porretta S editor. *Tomato Chemistry, Industrial Processing and Product Development*. London: The Royal Society of Chemistry (2019). p. 3–25. doi: 10.1039/9781788016247-00001
17. Rao VA, Young GL, Rao LG. *Lycopene and Tomatoes in Human Nutrition and Health*. Boca Raton, FL: CRC Press (2018). p. 204.
18. Jürkenbeck K, Spiller A, Meyerding SGH. Tomato attributes and consumer preferences – a consumer segmentation approach. *Br Food J.* (2020) 122:328–44. doi: 10.1108/BFJ-09-2018-0628
19. Kim DS, Lee DU, Lim JH, Kim S, Choi JH. Agreement between visual and model-based classification of tomato fruit ripening. *Trans ASABE.* (2020) 63:667–74. doi: 10.13031/TRANS.13812
20. Petropoulos SA, Fernandes Â, Xyrafis E, Polyzos N, Antoniadis V, Barros L, et al. The optimization of nitrogen fertilization regulates crop performance and quality of processing tomato (*Solanum lycopersicum* l. cv. heinz 3402). *Agronomy.* (2020) 10:10050715. doi: 10.3390/agronomy10050715
21. Goisser S, Wittmann S, Fernandes M, Mempel H, Ulrichs C. Comparison of colorimeter and different portable food-scanners for non-destructive prediction of lycopene content in tomato fruit. *Postharvest Biol Technol.* (2020) 167:111232. doi: 10.1016/j.postharvbio.2020.111232
22. Deák KJ, Szigedi T, Pék Z, Baranowski P, Helyes L. Carotenoid determination in tomato juice using near infrared spectroscopy. *Int Agrophys.* (2015) 29:275–82. doi: 10.1515/intag-2015-0032
23. Szuvandzsiev P, Helyes L, Lugasi A, Szántó C, Baranowski P, Pék Z. Estimation of antioxidant components of tomato using VIS-NIR reflectance data by handheld portable spectrometer. *Int Agrophys.* (2014) 28:521–7.
24. Goisser S, Krause J, Fernandes M, Mempel H. Determination of tomato quality attributes using portable NIR-sensors. In: Beyerer J, Puente León F, Längle T editors. *Optical Characterization of Materials: Conference Proceedings*. Karlsruhe: KIT Scientific Publishing (2019). p. 1–12.
25. Choudhary R, Bowser TJ, Weckler P, Maness NO, McGlynn W. Rapid estimation of lycopene concentration in watermelon and tomato puree by fiber optic visible reflectance spectroscopy. *Postharvest Biol Technol.* (2009) 52:103–9. doi: 10.1016/j.postharvbio.2008.10.002
26. Ciaccheri L, Tuccio L, Mencaglia AA, Mignani AG, Hallmann E, Sikorska-Zimny K, et al. Directional versus total reflectance spectroscopy for the in situ determination of lycopene in tomato fruits. *J Food Compos Anal.* (2018) 71:65–71. doi: 10.1016/j.jfca.2018.01.023
27. Deák KJ, Szigedi T, Palotás G, Daood HG, Helyes L. Determination of °Brix, lycopene, β-carotene and total carotenoid content of processing tomatoes using near infrared spectroscopy. *Acta Hortic.* (2015) 1081:253–8.
28. Ibrahim A, Daood HG, Bori Z, Helyes L. Using infrared spectroscopy for tracking and estimating antioxidant in tomato fruit fractions. *Eur J Eng Res Sci.* (2018) 3:21–30. doi: 10.24018/ejers.2018.3.5.736
29. Szuvandzsiev P, Daood HG, Posta K, Helyes L, Pék Z. Application of VIS-NIR reflectance spectra for estimating soluble solid and lycopene content of open-field processing tomato fruit juice from irrigation and mycorrhiza treatments. *Acta Hortic.* (2017) 1159:73–7. doi: 10.17660/ActaHortic.2017.1159.11
30. Eldin AB. Near infrared spectroscopy. In: Akyar I editor. *Wide Spectra of Quality Control*. Rijeka: InTech Europe (2011). p. 237–48.
31. Osborne BG. Near-infrared spectroscopy in food analysis. In: Meyers RA editor. *Encyclopedia of Analytical Chemistry*. Hoboken, NJ: John Wiley & Sons Ltd (2006). p. 1–14. doi: 10.1002/9780470027318.a1018
32. De Oliveira GA, Bureau S, Renard CMGC, Pereira-Netto AB, De Castilhos F. Comparison of NIRS approach for prediction of internal quality traits in three fruit species. *Food Chem.* (2014) 143:223–30. doi: 10.1016/j.foodchem.2013.07.122
33. Anderson NT, Walsh KB, Flynn JR, Walsh JP. Achieving robustness across season, location and cultivar for a NIRS model for intact mango fruit dry matter content. II. Local PLS and nonlinear models. *Postharvest Biol Technol.* (2021) 171:111358. doi: 10.1016/j.postharvbio.2020.111358
34. Pissard A, Marques EJN, Dardenne P, Lateur M, Pasquini C, Pimentel MF, et al. Evaluation of a handheld ultra-compact NIR spectrometer for rapid and non-destructive determination of apple fruit quality. *Postharvest Biol Technol.* (2021) 172:1111375. doi: 10.1016/j.postharvbio.2020.111375
35. Walsh KB, Blasco J, Zude-Sasse M, Sun X. Visible-NIR 'point' spectroscopy in postharvest fruit and vegetable assessment: the science behind three decades of commercial use. *Postharvest Biol Technol.* (2020) 168:111246. doi: 10.1016/j.postharvbio.2020.111246
36. Mishra P, Roger JM, Rutledge DN, Woltering E. SPORT pre-processing can improve near-infrared quality prediction models for fresh fruits and agro-materials. *Postharvest Biol Technol.* (2020) 168:111271. doi: 10.1016/j.postharvbio.2020.111271
37. Torres I, Pérez-Marín D, De la Haba MJ, Sánchez MT. Fast and accurate quality assessment of Raf tomatoes using NIRS technology. *Postharvest Biol Technol.* (2015) 107:9–15. doi: 10.1016/j.postharvbio.2015.04.004
38. Ibrahim A, Daood H, Friedrich L, Hitka G, Helyes L. Monitoring, by high-performance liquid chromatography, near-infrared spectroscopy, and color measurement, of phytonutrients in tomato juice subjected to thermal processing and high hydrostatic pressure. *J Food Process Preserv.* (2021) 45:e15370. doi: 10.1111/jfpp.15370
39. Golic M, Walsh K, Lawson P. Short-wavelength near-infrared spectra of sucrose, glucose, and fructose with respect to sugar concentration and temperature. *Appl Spectrosc.* (2003) 57:139–45. doi: 10.1366/000370203321535033
40. Vitális F, Zaukuu JLZ, Bodor Z, Aouadi B, Hitka G, Kaszab T, et al. Detection and quantification of tomato paste adulteration using conventional and rapid analytical methods. *Sensors.* (2020) 20:6059. doi: 10.3390/s20216059
41. Zhang L, Schultz MA, Cash R, Barrett DM, McCarthy MJ. Determination of quality parameters of tomato paste using guided microwave spectroscopy. *Food Control.* (2014) 40:214–23. doi: 10.1016/j.foodcont.2013.12.008
42. Horváth KZ, Andryei B, Helyes L, Pék Z, Neményi A, Nemeskéri E. Effect of mycorrhizal inoculations on physiological traits and bioactive compounds of tomato under water scarcity in field conditions. *Not Bot Horti Agrobot Cluj Napoca.* (2020) 48:1233–47. doi: 10.15835/nbha48311963
43. Nemeskéri E, Horváth K, Pék Z, Helyes L. Effect of mycorrhizal and bacterial products on the traits related to photosynthesis and fruit quality of tomato under water deficiency conditions. *Acta Hortic.* (2019) 1233:61–5. doi: 10.17660/ActaHortic.2019.1233.10
44. Nemeskéri E, Neményi A, Bőcs A, Pék Z, Helyes L. Physiological factors and their relationship with the productivity of processing tomato under different water supplies. *Water.* (2019) 11:586. doi: 10.3390/w11030586
45. Andryei B, Horváth KZ, Duah SA, Takács S, Égei M, Szuvandzsiev P, et al. Use of plant growth promoting rhizobacteria (PGPRs) in the mitigation of water deficiency of tomato plants (*Solanum lycopersicum* L.) Növekedést serkentõ rhizobaktériumok használata paradicsom növények vízhiányának mérséklésére. *J Cent Eur Agric.* (2021) 22:167–77.
46. Ayusto-Yuste MC, González-Cebrino F, Lozano-ruiz M, Fernández-León AM, Bernalte-García MJ. Influence of ripening stage on quality parameters of five traditional tomato varieties grown under organic conditions. *Horticulturae.* (2022) 8:313. doi: 10.3390/horticulturae8040313
47. Daood HG, Bencze G, Palotás G, Pék Z, Sidikov A, Helyes L. HPLC analysis of carotenoids from tomatoes using cross-linked C18 column and MS detection. *J Chromatogr Sci.* (2014) 52:985–91. doi: 10.1093/chromsci/bmt139
48. Daood HG, Ráth S, Palotás G, Halász G, Hamow K, Helyes L. Efficient HPLC separation on a Core-C30 column with MS2 characterization of isomers, derivatives and unusual carotenoids from tomato products. *J Chromatogr Sci.* (2021) 60:336–47. doi: 10.1093/chromsci/bmab085
49. Yao H, Lewis D. Spectral preprocessing and calibration techniques. In: Sun DW editor. *Hyperspectral Imaging for Food Quality Analysis and Control*. Cambridge, MA: Academic Press (2010). p. 45–78. doi: 10.1016/B978-0-12-374753-2.10002-4
50. Pedro AMK, Ferreira MMC. Nondestructive determination of solids and carotenoids in tomato products by near-infrared spectroscopy and multivariate calibration. *Anal Chem.* (2005) 77:2505–11. doi: 10.1021/ac048651r
51. Ścibisz I, Reich M, Bureau S, Gouble B, Causse M, Bertrand D, et al. Mid-infrared spectroscopy as a tool for rapid determination of internal quality parameters in tomato. *Food Chem.* (2011) 125:1390–7. doi: 10.1016/j.foodchem.2010.10.012
52. Clément A, Bacon R, Sirois S, Dorais M. Mature-ripe tomato spectral classification according to lycopene content and fruit type by visible, NIR reflectance and intrinsic fluorescence. *Qual Assur Saf Crop Foods.* (2015) 7:747–56. doi: 10.3920/QAS2014.0521

53. Ciaccheri L, Tuccio L, Mencaglia AA, Sikorska-Zimny K, Hallmann E, Kowalski A, et al. Prediction models for assessing lycopene in open-field cultivated tomatoes by means of a portable reflectance sensor: cultivar and growing-season effects. *J Agric Food Chem.* (2018) 66:4748–57. doi: 10.1021/acs.jafc.8b01570
54. Panjai L, Röhlen-Schmittgen S, Ellenberger J, Noga G, Hunsche M, Fiebig A. Effect of postharvest irradiation with red light on epidermal color and carotenoid concentration in different parts of tomatoes. *J Food Meas Charact.* (2021) 15:1737–46. doi: 10.1007/s11694-020-00770-0
55. Najjar K, Abu-Khalaf N. Non-destructive quality measurement for three varieties of tomato using VIS/NIR spectroscopy. *Sustainability.* (2021) 13:10747. doi: 10.3390/su131910747
56. Stinco CM, Rodríguez-Pulido FJ, Luisa E-GM, Gordillo B, Vicario IM, Meléndez-Martínez AJ. Lycopene isomers in fresh and processed tomato products: correlations with instrumental color measurements by digital image analysis and spectroradiometry. *Food Res Int.* (2013) 50:111–20.
57. Huang Y, Lu R, Chen K. Assessment of tomato soluble solids content and pH by spatially-resolved and conventional Vis/NIR spectroscopy. *J Food Eng.* (2018) 236:19–28. doi: 10.1016/j.jfoodeng.2018.05.008
58. Flores K, Sánchez MT, Pérez-Marín D, Guerrero JE, Garrido-Varo A. Feasibility in NIRS instruments for predicting internal quality in intact tomato. *J Food Eng.* (2009) 91:311–8. doi: 10.1016/j.jfoodeng.2008.09.013
59. Takács S, Pék Z, Csányi D, Daood HG, Szuvandzsiev P, Palotás G, et al. Influence of water stress levels on the yield and lycopene content of tomato. *Water.* (2020) 12:2165. doi: 10.3390/W12082165
60. Pék Z, Szuvandzsiev P, Neményi A, Tuan LA, Bakr J, Nemeskéri E, et al. Comparison of a water supply model with six seasons of cherry type processing tomato. *Acta Hortic.* (2019) 1233:41–6. doi: 10.17660/ActaHortic.2019.1233.7
61. Helyes L, Lugasi A, Pék Z. Effect of natural light on surface temperature and lycopene content of vine ripened tomato fruit. *Can J Plant Sci.* (2007) 87:927–9.
62. Heuvelink E, Okello RCO, Giovannoni JJ, Dorais M. Tomato. In: Wien HC, Stützel H editors. *The Physiology of Vegetable Crops*. Wallingford: CAB International (2020). p. 138–78.
63. Khuriyati N, Matsuoka T. Monitoring NIR internal spectroscopy properties soilless of on-plant culture tomato fruits in using for control of nutrient solution among the quality parameters considered for tomato fruits, soluble solids content (SSC) is the most important component. *Environ Control Biol.* (2005) 43:39–46.
64. Sohaib ASS, Zeb A, Qureshi WS, Arslan M, Ullah MA, Alasmary W, et al. Towards fruit maturity estimation using NIR spectroscopy. *Infrared Phys Technol.* (2020) 111:103479. doi: 10.1016/j.infrared.2020.103479
65. Acharya UK, Subedi PP, Walsh KB. Robustness of tomato quality evaluation using a portable Vis-SWNIRS for dry matter and colour. *Int J Anal Chem.* (2017) 2017:2863454. doi: 10.1155/2017/2863454
66. Brito AA, Campos F, Nascimento A dos R, Corrêa G, de C, Silva FA, et al. Determination of soluble solid content in market tomatoes using near-infrared spectroscopy. *Food Control.* (2021) 126:108068. doi: 10.1016/j.foodcont.2021.108068
67. Wang H, Zhang R, Peng Z, Jiang Y, Ma B. Measurement of SSC in processing tomatoes (*Lycopersicon esculentum* Mill.) by applying Vis-NIR hyperspectral transmittance imaging and multi-parameter compensation models. *J Food Process Eng.* (2019) 42:e13100. doi: 10.1111/jfpe.13100
68. Toor RK, Savage GP. Antioxidant activity in different fractions of tomatoes. *Food Res Int.* (2005) 38:487–94.
69. Ilahy R, Siddiqui MW, Piro G, Lenucci MS, Hdider C, Helyes L. A focus on high-lycopene tomato cultivars: horticultural performance and functional quality. *Acta Hortic.* (2017) 1159:57–64. doi: 10.17660/ActaHortic.2017.1159.9
70. Brandt S, Pék Z, Barna É, Lugasi A, Helyes L. Lycopene content and colour of ripening tomatoes as affected by environmental conditions. *J Sci Food Agric.* (2006) 86:568–72. doi: 10.1002/jsfa.2390
71. Ilahy R, Tlili I, Siddiqui MW, Hdider C, Lenucci MS. Inside and beyond color: comparative overview of functional quality of tomato and watermelon fruits. *Front Plant Sci.* (2019) 10:769. doi: 10.3389/fpls.2019.00769
72. Tilahun S, Park DS, Seo MH, Hwang IG, Kim SH, Choi HR, et al. Prediction of lycopene and β-carotene in tomatoes by portable chroma-meter and VIS/NIR spectra. *Postharvest Biol Technol.* (2018) 136:50–6. doi: 10.1016/j.postharvbio.2017.10.007
73. Yang HQ. Nondestructive prediction of optimal harvest time of cherry tomatoes using VIS-NIR spectroscopy and PLSR calibration. *Adv Eng Forum.* (2011) 1:92–6. doi: 10.4028/www.scientific.net/aef.1.92

Conflict of Interest: GáP, GaP, and PS were employed by Univer Product PLC.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Égei, Takács, Palotás, Palotás, Szuvandzsiev, Daood, Helyes and Pék. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 14 July 2022
doi: 10.3389/fnut.2022.916642

Flavor and Other Quality Traits of Tomato Cultivars Bred for Diverse Production Systems as Revealed in Organic Low-Input Management

Cut Erika[1†], Detlef Ulrich[2], Marcel Naumann[1], Inga Smit[1], Bernd Horneburg[3†] and Elke Pawelzik[1]*

[1] Division Quality of Plant Products, Department of Crop Sciences, University of Göttingen, Göttingen, Germany, [2] Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Julius Kühn-Institute (JKI), Federal Research Centre for Cultivated Plants, Quedlinburg, Germany, [3] Section of Genetic Resources and Organic Plant Breeding, Department of Crop Sciences, University of Göttingen, Göttingen, Germany

This study was conducted to determine the volatile organic compounds (VOCs) associated with fruit flavor in diverse tomato cultivars (salad and cocktail cultivars) under organic low-input production. For this objective, 60 cultivars deriving from very diverse breeding programs 1880–2015 were evaluated in 2015, and a subset of 20 cultivars was selected for further evaluation in 2016. The diversity of instrumentally determined traits, especially for VOCs concentration and sensory properties (fruit firmness, juiciness, skin firmness, sweetness, sourness, aroma, and acceptability), was investigated at two harvest dates. The evaluation of the cultivars exhibited a wide range of variation for all studied traits, with the exception of a few VOCs. Cultivar had the most important effect on all instrumentally determined traits, while the influence of cultivar × harvest date × year interaction was significant for 17 VOCs, but not for total soluble solid (TSS) and titratable acidity (TA). The VOCs with the highest proportion (>8%) were hexanal, 6-methyl-5-heptene-2-one, 2-isobutylthiazole, and (*E*)-2-hexenal, which were identified in all cultivars. Twelve VOCs significantly correlated with one or more sensory attributes and these VOCs also allowed differentiation of the fruit type. Among these VOCs, phenylethyl alcohol and benzyl alcohol positively correlated with acceptability in the cocktail cultivars, whereas 2-isobuthylthiazole and 6-methyl-5-hepten-2-ol negatively correlated with acceptability in the salad cultivars. As a result of this study, organic breeders are recommended to use cultivars from a wide range of breeding programs to improve important quality and agronomic traits. As examples, salad tomatoes "Campari F_1", "Green Zebra", and "Auriga", as well as cocktail tomatoes "Supersweet 100 F_1", "Sakura F_1", and "Black Cherry" showed higher scores for the sensory attributes aroma and acceptability under organic low-input growing conditions. It remains a challenge for breeders and growers to reduce the trade-off of yield and quality.

Keywords: tomato cultivars, VOC, organic low-input production, flavor, breeders' sensory test, phenylethyl alcohol, 2-isobutylthiazole

OPEN ACCESS

Edited by:
Spyridon Alexandros Petropoulos, University of Thessaly, Greece

Reviewed by:
Ivana Tomaz, University of Zagreb, Croatia
Subramanian Babu, VIT University, India

***Correspondence:**
Elke Pawelzik
epawelz@gwdg.de

†Present addresses:
Cut Erika, Department of Agricultural Product Technology, Universitas Syiah Kuala, Banda Aceh, Indonesia

Bernd Horneburg, Section of Organic Plant Breeding and Agrobiodiversity, University of Kassel, Witzenhausen, Germany

Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *09 April 2022*
Accepted: *07 June 2022*
Published: *14 July 2022*

Citation:
Erika C, Ulrich D, Naumann M, Smit I, Horneburg B and Pawelzik E (2022) Flavor and Other Quality Traits of Tomato Cultivars Bred for Diverse Production Systems as Revealed in Organic Low-Input Management. Front. Nutr. 9:916642. doi: 10.3389/fnut.2022.916642

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is currently one of the most important vegetable crops in the world (1), with consumption of fresh tomatoes and products at 20.2 kg capita^{-1} year^{-1} in 2019 (2). The tomato fruit constitutes an essential component of the human diet as it is a source of minerals, vitamins, and phytochemicals (3). For decades, domestication and the considerable attempts in tomato breeding have mainly focused on improving agronomic traits, such as fruit yield and weight, and, to a lesser extent, color and shape, firmness, disease resistance, and adaptation to different growing environments (4–6). While these approaches have significantly increased productivity, they have also shown negative impacts on the sensory and nutritional quality of commercial modern cultivars, which are often perceived by consumers as having less flavor (1, 7, 8). In recent years, the consumer demand for tomatoes with better flavor has increased, which is accompanied by ongoing breeding attempts toward the production of genotypes with improved sensory qualities and with multidisciplinary approaches to sensory assessment of the fruits (9, 10).

The production system can have a greater influence on fruit quality, including the concentration of bioactive compounds, such as phenols and lycopene, than on fruit yield, and the contents of these compounds are often higher in fruits from organic and low-input systems than in fruits from conventional production (11). Field-grown tomatoes have been reported to have higher levels of VOCs than greenhouse-grown fruits (12), but some cultivars e.g., Campari F_1, have been developed for superior flavor in protected cropping. Flavor properties are important breeding targets to meet consumer demands for tomatoes (13, 14). In addition, the increasing interest in environmentally friendly produced food (15) may encourage breeders to develop cultivars with excellent flavor characteristics for organic low-input cultivation as well. Flavor of a fresh fruit is the sum of an interaction between taste and olfaction that results from a complex interaction of sugars (glucose, fructose), organic acids (citrate, malate, ascorbate, glutamate), and volatile compounds (VOCs) (16, 17). Sugars and acids activate taste receptors, while various VOCs stimulate olfactory receptors (18, 19). Aroma is a very complex trait (20), and its perception is not the result of the action of a single VOC but the result of interactions between different VOCs (16). Texture is also connected to the formation of VOCs, as it is also related to the degradation of cell walls and membranes. At the basic level, cell wall disruption stimulates contact between enzymes and substrates involved in the release of VOCs (21). In addition to many primary and secondary metabolites, such as sugars, amino acids, fatty acids, and carotenoids that can directly influence the sensory properties of the fruit, they are also precursors of some important VOCs of tomato (22). A combination of taste, aroma, and mouth-feel characteristics had major contribution to its flavor. The mechanisms behind flavor characteristics and sensory variations in tomato fruit have been studied to a limited extent (23). Daoud et al. (24) found a good correlation of the sensory attribute aroma with the general taste perception and with texture and, also, with the instrumentally determined sum parameter VOCs. Texture perception is the sensation perceived when eating, and the greatest contributor to texture of tomato products is insoluble solids, which account for approximately 10–20% of the total solids in the cell wall of the fruit (25, 26).

The characteristic sweet–sour flavor of tomato fruit is not solely the result of the interactions of the non-volatile compounds (i.e., sugars, acids, and amino acids) in the fruit (22), but it is determined by a complex combination of volatile and nonvolatile metabolites, which is not yet well understood (1). In strawberry, flavor has apparently been declared an important breeding target after yield and shelf life, to which special attention is paid (27), while tomato has been described as an excellent and important model organism for studies on fleshy fruit to investigate flavor at the molecular level (7, 28). Previous sensory studies on tomato have shown that flavor is the most important characteristic to improve the sensory quality of fruits and is necessary to meet consumers' expectations (19, 25, 29).

While studies on the chemistry and variability of quality parameters of tomatoes, especially their flavor, compounds are widely available (30, 31); knowledge about chemical properties and VOC accumulation in tomatoes grown in organic low-input conditions and their correlation with general and specific sensory attributes is scarce. In this respect, the characterization of flavor-associated traits relevant to sensory properties is of interest, as they are increasingly valued by consumers.

This study is based on field trials under organic low-input conditions, in which 60 salad and cocktail tomato cultivars were cultivated in 2015, and a subset of 20 cultivars was grown in 2016. First results have been recently published by Chea et al. (32), focusing on identifying cultivars with better growth, yield, and selected fruit quality traits. The aim of the present study was to characterize the cultivars with respect to the variation of their sensory quality under low-input conditions, with the focus on the instrumentally determined traits and on the sensory quality, as perceived by human senses and the breeding background. It is expected that the results of the present study will provide information on the cultivars that have better flavor based on volatile and non-volatile organic components and are most suitable for organic low-input production and as parents in organic breeding.

MATERIALS AND METHODS

Plant Materials, Experimental Layout, and Crop Cultivation

The experiments were carried out with indeterminate cultivars corresponding to the group of salad (average fruit weight, 103 g) and cocktail (average fruit weight, 26 g) cultivars. Morphological, leaf nutrient, fruit yield, and selected fruit quality traits of the tested cultivars have been previously studied (32).

Sixty cultivars (33 salad and 27 cocktail cultivars) were selected, with information from extension services, research stations, breeders, and seed companies in Germany, Austria and Switzerland, and the IPK Genebank 2015 (**Supplementary Table 1**), a variety reduction selection process was carried out in which 60 cultivars were reduced to a subgroup of 20

cultivars (8 salad and 12 cocktail cultivars). These 20 selected cultivars were the best cultivars in terms of four traits: yield, total soluble solids (TSS), titratable acidity (TA), and the sensory attribute aroma. Reducing the number of cultivars to a few could increase the effectiveness of breeders in further selection of certain desirable traits among genotypes in breeding programs. The 60 cultivars and a subset of 20 cultivars were, respectively, grown in the field under low-input conditions at Reinshof Experimental Station, University of Göettingen, Germany, according to standard organic horticultural practices. In this study, the term "low-input" includes the avoidance of off-farm inputs during cultivation, especially fertilizer application and the use of moderate irrigation. The field had been used for organic farming since 2003, and faba beans and winter wheat were grown as preceding crops in 2015 and 2016, respectively.

The field trial was arranged in a randomized complete block design with eight biological replications (one and two plants per plot in 2015 and 2016, respectively). Further details of cultivation conditions and agronomic treatments are given in Chea et al. (32) and Erika et al. (33).

Preparation of Samples

Fruits were harvested fully mature between early August and October at two harvest dates [2015: 13 weeks after planting (WAP) and 18 WAP; 2016: 14 WAP and 19 WAP]. The healthy fruit samples were brought to the laboratory and immediately measured for fruit color. A tomato sample consisting of three to ten fruits per biological replication was used for each of the analyses. After washing, it was divided for five subsamples. One subsample was used for the immediate extraction of VOCs. Another subsample was used for sensory evaluation the following day, while the other subsamples were sliced and stored at −20°C before being analyzed for TSS and TA, and further traits.

Instrumental Analysis

The measurements of fruit color, TSS, and TA were performed according to Kanski et al. (34), whereas VOCs analysis was conducted by headspace solid phase micro-extraction, and subsequent gas chromatography equipped with a flame ionization detector for detection (HS-SPME-GC-FID), following the method of Ulrich and Olbricht (35). Fruits were rinsed with the ionized water, cut into wedges and homogenized in two parts of volume of 20% NaCl solution (w/v), with a hand mixer (Braun, Germany) at medium speed for 2 min. The homogenate was filled in 50-ml centrifuge tubes and centrifuged at 4°C and 3,000 rpm for 30 min (Centrifuge 5804 R, Eppendorf, Hamburg, Germany) to separate clear supernatant. Ten milliliters of the supernatant were mixed carefully with 20-μL internal standard (5 μL 1-octanol dissolved in 10-ml ethanol). Subsequently, an 8-ml aliquot was transferred into a 20-ml headspace vial (Gerstel GmbH, Germany) already containing 4-g NaCl and sealed with a screw cap septum. The VOC extract samples were vortexed for 10 s and stored at −80°C until analysis.

Prior HS-SPME analysis, the frozen VOC samples were incubated for 15 min at 35°C with a shaking operation mode of 300 rpm to allow equilibration of volatiles in the headspace, and then they were exposed to the vial headspace for another 15 min at 35°C under the same continuous shaking after an SPME fiber (100-μm poly-dimethylsiloxane/PDMS; Supelco, Bellefonte, PA) was inserted into the vial. Desorption was performed within 2 min in the splitless mode and 3 min with split at 250°C. An Agilent Technologies 6890 GC equipped with an HP-Wax column (0.25-mm i.d., 30-m length, and 0.5-μm film thickness) and FID were used for separation and detection. Carrier gas was hydrogen using a flow rate of 1.1 ml min^{-1}. The temperature program was the following: 45°C (5 min) from 45 to 210°C at 3°C min^{-1} and held 25.5 min at 210°C. All samples were run with two technical replications.

The commercial software ChromStat2.6 (Analyt, Műllheim, Germany) was used for raw data processing. Data inputs for ChromStat 2.6 were raw data from the percentage reports (retention time/peak area data pairs) performed with the software package ChemStation [version Rev.B.02.01.-SR1 (260)] by Agilent. Using ChromStat2.6, the chromatograms were divided in up to 200 time intervals, each of which represented a peak (substance) occurring in at least one chromatogram of the analysis set. The peak detection threshold was set on the 10-fold value of noise. The values are given as raw data (the peak area in counts), which also can be described as relative concentration because of the normalized sample preparation. Afterward, the relative concentrations of VOC values were normalized to the mean total abundance of identified compounds and expressed in norm %, calculated according to the following formula:

$$norm\% = \frac{A^i}{\sum_1^n A^i}$$

where I = substance i; A^i = relative concentration of substance i (dimensionless); and n = number of observations (identified VOCs). All the analyses were performed using the Statistica 13.3 package (TIBCO Software Inc., Chicago, United States).

The volatiles were identified by parallel runs of selected samples on an identically equipped GC-MS. The VOCs were identified by comparison of their mass spectra with library entries (NIST 14; the National Institute of Standards and Technology, Wiley, Nbs75k, United States), as well as by comparing RI with authentic references. CAS-numbers of the VOCs (**Supplementary Table 2**) were retrieved from the web-based chemical search engine[1] and from the online edition of the "specifications for flavorings" database[2].

Sensory Evaluation

Due to the high number of cultivars in 2015, a sensory evaluation, using the breeders' sensory test (36) was carried out. Three panelists (male, 25–50 years of age) with experience in sensory evaluation of tomatoes, knowledge of the cultivars to be evaluated, and the terminology to be used were trained in preparation for the sensory evaluation. Sensory attributes that contribute to the fruit flavor (fruit firmness, juiciness, skin firmness, sweetness, sourness, aroma), and the acceptability were assessed. The scoring was based on a 9-point scale

[1]https://commonchemistry.cas.org/

[2]http://www.fao.org/ag/agn/jecfa-flav/

(0 = minimum intensity and 9 = maximum intensity), with the detailed description of the assessed attributes, following Hagenguth et al. (36) for most of the attributes. Fruit firmness referred to resistance of the pericarp during first chewing, and skin firmness referred to the degree to which the epidermis remained intact during chewing. Juiciness was described as amount of liquid that escapes during initial chewing. The aroma was associated with the retronasal impression of the fruit slices. The samples were labeled with a three-digit code and served to the panelists on transparent plastic trays. To avoid fruit type bias, the different fruit types were evaluated in separate groups.

In 2016, quantitative descriptive analysis (QDA) was used to evaluate the sensory attributes of 20 cultivars. Eleven panelists aged 25–55 years (45% male and 65% female) were trained weekly for 22 months in descriptive evaluation of tomatoes, which involved eight training sessions according to the ISO (International Organization for Standardization) standards 8586 (37). The technical procedure for panel training and sensory evaluation sessions was the same as the protocol of sensory methods described in Daoud et al. (24) with modifications. The sensory attributes used in this panel test were similar to those used in the breeders' sensory test (36). The panelists were trained in eight sessions during 2-week training prior to the evaluation. The first three sessions introduced general sensory techniques and established the descriptive terms used to characterize flavor (fruit firmness, juiciness, skin firmness, sweetness, sourness, and aroma) and the overall acceptability of tomatoes. In the following sessions, each of the attributes was defined, and different references to represent each attribute were presented to the panelist. During the training session, the panel leader mediated group discussions to reach a consensus. The panelists were asked to rate samples in terms of the intensity of the individual flavor attributes (fruit firmness, juiciness, skin firmness, sweetness, sourness, aroma) and the overall acceptance of each sample on a score sheet with an unstructured line scale (0 = minimum intensity to 100 = maximum intensity). Each analysis was carried out in individual booths in the sensory laboratory at the University of Göettingen under daylight-equivalent lighting conditions that met the specifications of ISO 8589 (38). About 30 min before the evaluation, the samples were removed from storage at 7°C to adjust to room temperature and cut into equal sample sizes or to 1/8 wedges, depending on fruit size. The samples were presented to the panelists in small bowls. Water and plain crackers were provided to cleanse the palates.

Statistical Analysis

Overall mean abundance of instrumental and sensory data was subjected to one-way ANOVA and Tukey's test for honestly significant difference (Tukey's HSD) to determine possible differences in means at $p < 0.05$, $p < 0.01$, and $p < 0.001$. Pearson correlation analysis was performed between and within VOCs significantly associated ($p < 0.05$ and $p < 0.01$) with instrumental and sensory parameters. Principal component analysis (PCA) was conducted to identify the principal components responsible for the most of the variations within the dataset. PCA was performed on the instrumentally determined data and the mean sensory ratings (based on the correlation matrix) over both crop years. All the analyses were performed using the Statistica 13.3 package (TIBCO Software Inc., Chicago, United States).

RESULTS

With the aim of identifying cultivars best suited for organic low-input production and as parents for organic breeding, the present study assessed tomato fruit flavor quality and its relationship with sensory traits, breeding background and agronomic characteristics, instrumental data, and sensory properties of 60 tomato cultivars. To achieve the objective, 33 salad and 27 cocktail cultivars grown in 2015, and a subset of eight salad and 12 cocktail cultivars grown in 2016 were determined, with a focus on flavor-associated VOCs. The PCA, performed with the mean values of the concentration of 32 identified VOCs (**Supplementary Table 2**), color parameters, TA, TSS, and sensory data from every year in 2015 and 2016, showed a large diversity (**Figure 1**). In 2015, all tested samples are distributed across the parameter space, with a more and less distinct clustering, distinguishing mainly cocktail and salad cultivars, with the exception of the salad cultivar "Green Zebra" dislocated in the left quadrant and the cocktail cultivar "König Humbert" in an opposite quadrant. In addition, a separated group in the upper left quadrant of the plot, which included most of the cultivars with yellow and orange fruits as well as with red fruits, could be clearly distinguished (**Figure 1**). No clear clustering occurs for other subgroups, but a number of cultivars with superior aroma-like "Green Zebra" and "Black Cherry" plotted to the left of the main cluster. The corresponding loading plot (**Figure 2**) illustrates that fruit firmness, yield (FY), average fruit weight (AFW), intensity index (IDX), and VOCs as geranial and octanal are associated in the cultivars studied. A higher IDX indicates a higher input in the production systems suitable for a cultivar (**Supplementary Table 1**). Sensory traits regarded as positive (sweetness, sourness, aroma, firmness) clustered together with the related analytical traits TA and TSS, and skin firmness.

Volatile Organic Compounds of 60 Cultivars

The concentration of VOCs was analyzed in 60 cultivars grown in 2015. Twenty four VOCs were identified in both the salad and cocktail cultivars (**Supplementary Tables 3**, **4**) belonging to five substance classes, such as aldehyde (ALD), ketone (KET), alcohol (ALC), sulfur-derived compound (SDC), and aliphatic acid (ALA). Significant differences were found between cultivars for most VOCs.

The group of ALD accounted for the largest proportion of the total VOCs in 60 cultivars, followed by KET, ALC, SDC, and ALA. The levels of these chemical groups varied from cultivar to cultivar, with the highest proportions of 72.5, 32.3, 25.8, 25.5, and 5.4% found in "Philovita F1", "Green Zebra", "Golden Currant", "Clou", and "Goldene Königin", respectively (**Figure 3**). Hexanal (ALD) had the highest share of the total measured VOCs in salad and cocktail cultivars, with 27 and 30%, respectively. Methylheptadione (KET) and decadienal (ALD) could only be detected in some cultivars of both groups, while benzyl alcohol

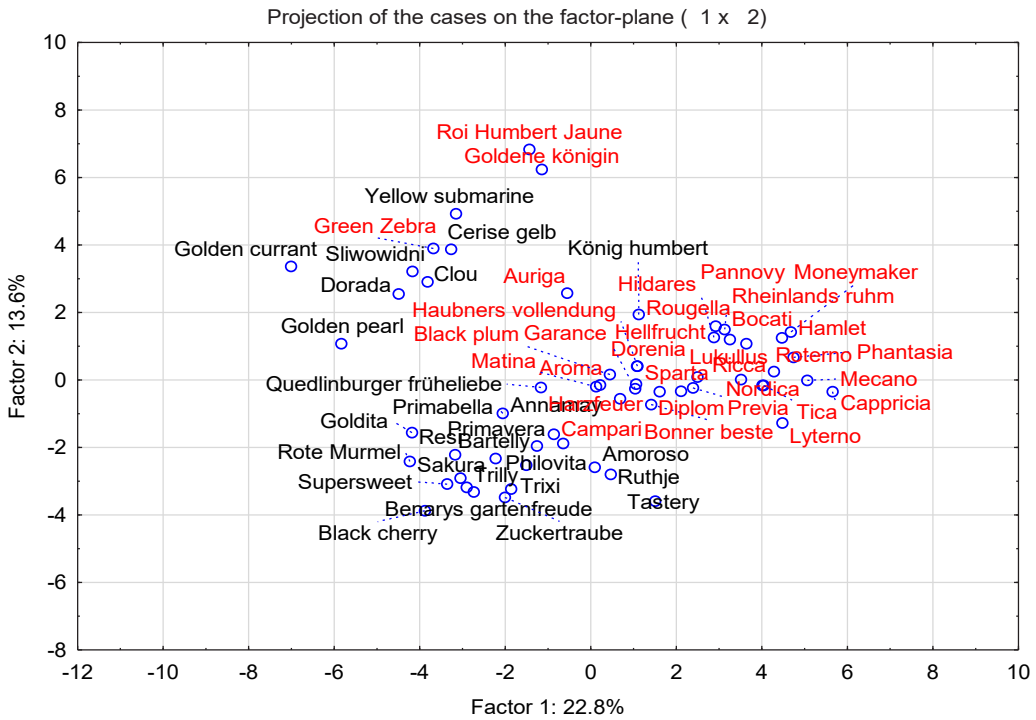


FIGURE 1 | A PCA score plot of the instrumental and sensory attributes of 60 cultivars (33 salad and 27 cocktail cultivars) grown in 2015. Salad cultivars are indicated in red; and cocktail cultivars are shown in black. The full names of cultivar are listed in **Supplementary Table 1**.

(ALC) was present in all salad cultivars and in most cocktail cultivars (**Supplementary Tables 3**, **4**).

Influence of the Year and Harvest Date on Volatile Organic Compounds

The main effects on the concentration of VOCs in the 20 cultivars grown in 2015 and 2016 are shown in **Supplementary Table 5**. The effect of cultivar on all identified VOCs, except of α-terpineol, was always significant at different confidence levels. Of the total 31 VOCs identified, the interaction of cultivar (C) × harvest date (H) × year (Y) showed significant effects on 17 VOCs. The VOCs were composed of hexanal (35.2%), 6-methyl-5-heptene-2-one (17.4%), 2-isobutylthiazole (11.1%), (E)-2-hexenal (8.8%), geranylacetone (5.3%), octanal (3.7%), and β-damascenone (3.3%). Other VOCs were detected in relative concentrations of less than 3% of the total quantified VOCs.

The Student's T-test showed that the relative concentrations of 2-isobutylthiazole, linalool, phenylethyl alcohol, and nonanoic acid were significantly different ($p < 0.001$) between cocktail and salad cultivars, highlighting the differences in tomato aroma between the groups of fruit type. **Tables 1**, **2** show the variation of concentration for each of the 31 VOCs within the individual salad and cocktail cultivars. There were 15 VOCs in the salad cultivars and 23 VOCs in the cocktail cultivars, each with significant differences at different confident levels. However, these differences were not consistent within the fruit type. For example, hexanal, octanal, and 1-hexanol differed significantly between the cocktail but not between the salad cultivars. Hexanal was predominant in both salad and cocktail cultivars, followed by (*E*)-2-hexenal and octanal. The concentration of hexanal was not significantly different among salad cultivars but varied within the cocktail cultivars, ranging from 25.1% ("Supersweet 100 F_1") to 48.7% ("Black Cherry") of total VOCs. The range of (*E*)-2-hexenal from 7.08 to 13.64% was also high but not statistically different in cocktail cultivars, while significant variation of (*E*)-2-hexenal was found in salad cultivars (3.37–9.94%). Between the fruit type, most of salad cultivars were characterized with higher concentrations of 2-isobutylthiazole, 6-methyl-5-hepten-2-ol, linalool, and 2-isobutylthiazole, while benzyl alcohol and phenylethyl alcohol were found in a considerable level in most of cocktail cultivars (**Tables 1**, **2**).

Sensory Evaluation

The analysis of sensory characteristics of the tested cultivars focused on the following attributes: texture (fruit firmness, juiciness, and skin firmness), taste (sweetness, sourness), aroma, and overall acceptability. Two types of sensory tests were carried out in order to characterize the fruit quality of the cultivars. In 2015, the breeders' sensory test was performed on 60 cultivars, whereas, in 2016, a QDA test with a trained panel was used to access the 20 cultivars.

Breeders' Sensory Test of 60 Cultivars

Sensory parameters analyzed on fruits of salad and cocktail cultivars in 2015 varied considerably, with the coefficients of variation (CV) ranging from 15.5 to 26.4 and from 9.6 to 14.8%,

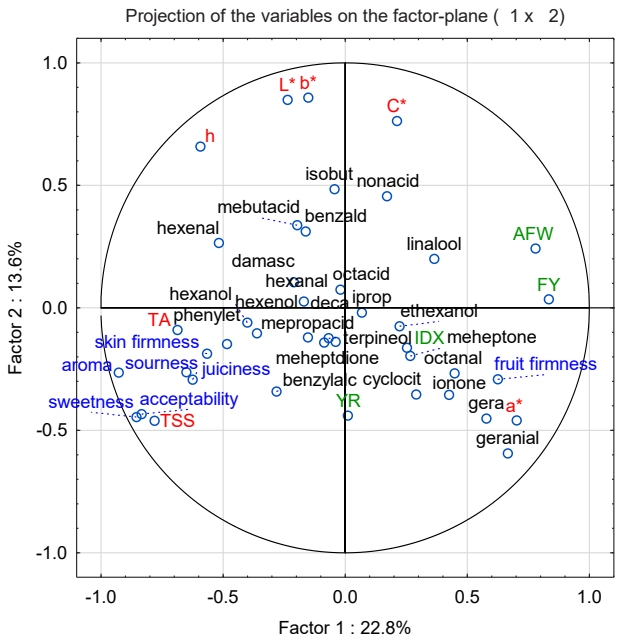


FIGURE 2 | A PCA loading plot of the instrumental parameters (in red and black) and sensory attributes (in blue) as well as other cultivar-related parameters (in green) of 60 cultivars (33 salad and 27 cocktail cultivars) grown in 2015. Instrumental parameters: TSS - total soluble solids; TA - titratable acid; L* – lightness; a* – red (+)/green (–); b*: yellow (+)/blue (–); C* – chroma; h – hue. Sensory attributes: fruit firmness, juiciness, skin firmness, sweetness, sourness, aroma, and acceptability. Other parameters: AFW – average fruit weight, FY – fruit yield, IDX – intensity index, YR – year of release. VOCs are named according to **Supplementary Table 2**.

respectively (**Table 3**). Among the salad cultivars, fruit firmness was rated highest for the cultivars "Lyterno F_1", "Cappricia F_1", "Roterno F_1", and "Bocati F_1". The highest rating for juiciness was obtained for "Green Zebra", which were not significantly different from "Harzfeuer F_1" and "Auriga", while skin firmness was rated at highest for "Harzfeuer F_1", "Auriga", and "Campari F_1". In terms of sweetness, "Campari F_1", "Harzfeuer F_1", "Green Zebra", and "Auriga" were evaluated as the sweetest among the salad cultivars, whereas "Green Zebra", "Auriga", and "Campari F_1" had the highest score of sourness. Regarding aroma, "Green Zebra", "Campari F_1", "Auriga", and "Harzfeuer F_1" were characterized as the cultivars with the most intensive aroma. In term of overall acceptability among salad cultivars, "Campari F_1" seems to be the most accepted cultivar by the breeders' panelists, with the highest value from 5.3 to 6.2 (scale, 0: minimum intensity, - 9: maximum intensity), but these cultivars did not significantly differ from "Green Zebra" and "Auriga".

Among the cocktail cultivars, almost all of the selected cultivars used for further evaluation in 2016 were the cultivars that performed highest in at least one of the tested sensory attributes. Exemplary, "Sakura F_1", "Supersweet 100 F_1", "Benarys Gartenfreude", and "Goldita" were scored at highest for sweetness and acceptability in the breeders' sensory test in 2015. In spite of these, some cultivars that were not selected for investigation in 2016 also scored high on certain sensory attributes, such as "Trilly F_1" for juiciness, sweetness, aroma, and overall acceptability. The so-called wild cultivars "Rote Murmel" and "Golden Currant" were highly appreciated in terms of juiciness, taste, and aroma, but not for firmness. "Trilly F_1" and "Golden Pearl F_1" were rated as the most acceptable cultivars in 2015 (**Table 3**).

Panel Sensory Test of Selected 20 Cultivars

The CV of sensory attributes analyzed in the fruit of salad and cocktail cultivars in 2016 ranged from 5.7 to 17.3 and 5.4 to 12.0%, respectively (**Table 4**). In terms of the highest rated cultivars, the results of the sensory panel in 2016 were relatively similar to those of the breeders' sensory test in 2015, e.g., "Supersweet 100 F1", "Benarys Gartenfreude", "Sakura F_1", and "Bartelly F_1" were ranked as those with the highest acceptability by both sensory panels. In addition, "Supersweet 100 F_1", "Sakura F_1", "Bartelly F_1", "Goldita", and "Black Cherry", which received the highest score of aroma in the sensory panel test, were also rated as the most aromatic cultivars by the breeders' sensory team. In contrast, "Primavera" had the lowest scores for aroma and acceptability, indicating the least appreciated cultivar among the studied cocktail cultivars.

Correlations Between Traits

The correlations between all traits based on 60 cultivars are given in **Supplementary Table 6**. The year of release did not have significant influence on quality traits with few exceptions: the content of nonanoic acid decreased; TSS, fruit firmness, and acceptability slightly increased. A significant negative correlation of quality traits with yield was observed. The compounds octanal, linalool, geranial, β-ionone showed positive correlation with yield; whereas (*E*)-2-hexenal, 1-hexanol, (*Z*)-3-hexen-1-ol, and phenylethyl alcohol were negatively correlated with yield formation. Yield and intensity index correlated positively with fruit firmness and negatively correlated with skin firmness.

For the 20 cultivars, significant correlations between the VOCs and instrumental and sensory traits are shown in **Table 5**. Of the 31 VOCs, 12 compounds were correlated with at least one sensory attribute. Phenylethyl alcohol and (*E*)-2-hexenal, both strongly correlated with TSS, were also positively correlated with sweetness, aroma, and acceptability ($r > 0.55$), whereas 2-isobutylthiazole and 6-methyl-5-hepten-2-ol were negatively correlated with these sensory traits. Both geranial and 6-methyl-5-hepten-2-ol showed a negative correlation with TA and sourness. Geranial was positively correlated ($r > 0.60$) with fruit firmness and negatively with juiciness. The strongest positive correlation was determined between (*Z*)-3-hexen-1-ol and skin firmness ($r = 0.80$). Among instrumental traits, the strongest negative correlation was found between 2-isobutylthiazole and TSS content ($r = -0.68$). A few correlations were significant between VOCs and color components. For example, citral correlated negatively with fruit color Parameters L* and b*, and geranial correlated positively with h value. In addition, **Supplementary Table 7** shows that some correlations between

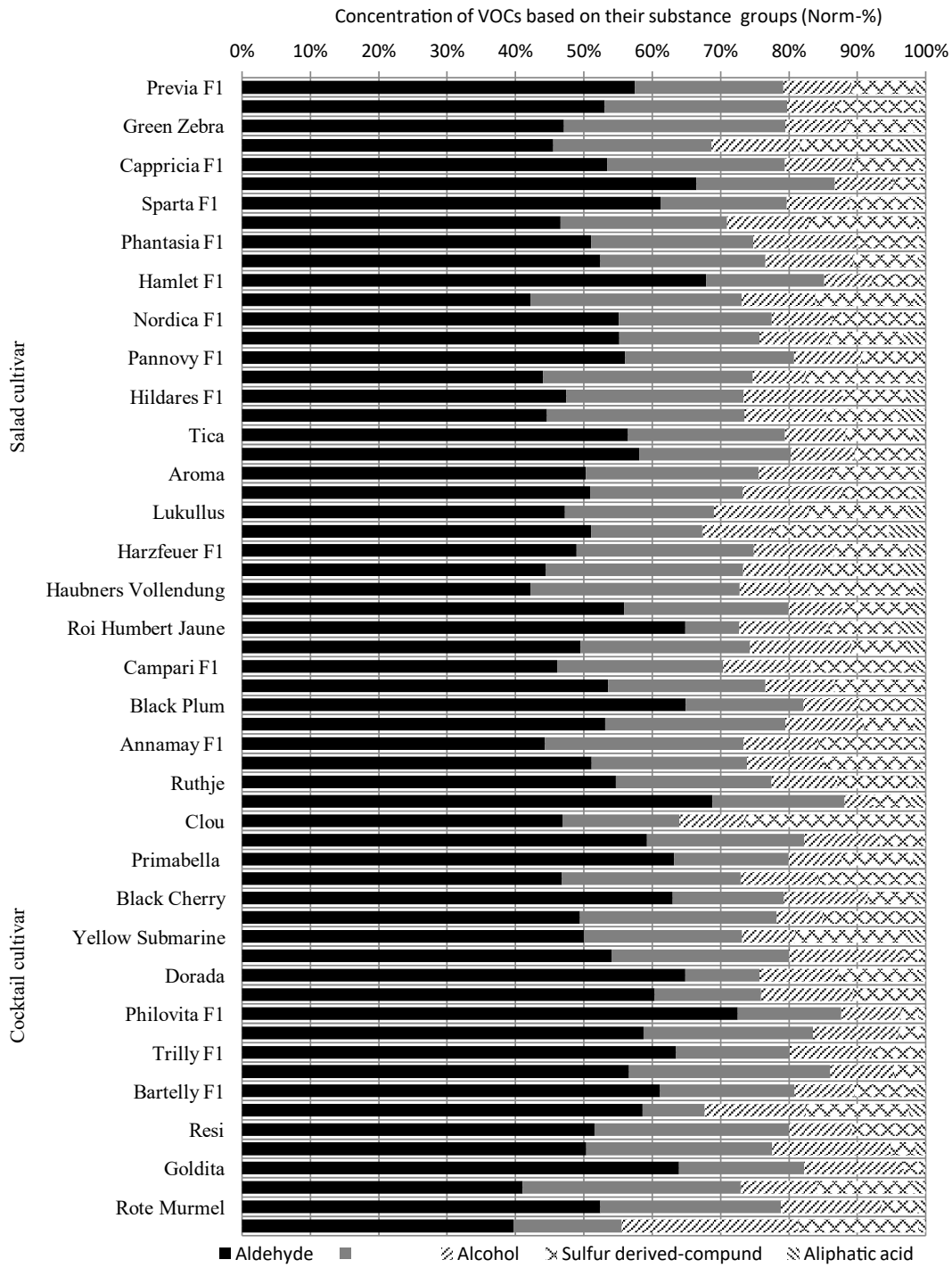


FIGURE 3 | Concentration of VOCs accumulated in the 60 cultivars grown in 2015 compiled to their substance groups. The cultivars are ordered based on average fruit weight from high to low. Cultivar names "Goldene Koenigin", "Quedlinburger Fruehe Liebe", and "Koenig Humbert" were written as "Goldene Königin", "Quedlinburger Frühe Liebe", and "König Humbert", respectively. The substance groups of the VOCs: Aldehydes: β-cyclocitral, benzaldehyde, citral, decadienal, (*E*)-2-hexenal, geranial, hexanal and octanal; ketones: 6-methyl-5-hepten-2-one, β-damascenone, β-ionone, (*E*)-geranylacetone, farnesylacetone, and methylheptadione); alcohols: 1-hexanol, 2-ethyl-1-hexanol, α-terpineol, benzyl alcohol, eugenol, linalool, phenylethyl alcohol, and (*Z*)-3-hexen-1-ol; sulfur-derived compound: 2-isobutylthiazole; aliphatic acids: 2-methylpropanoic acid, 3-methylbutanoic acid, octanoic acid, and nonanoic acid; and another substance groups, ester (2-methylbutylacetate, isopropylmyristate, and methylsalicylate) are not included in the figure due to their minor abundance in the tomato fruit samples.

TABLE 1 | Concentration of volatile organic compounds (VOCs) (norm-%) of eight salad tomato cultivars grown in 2015 and 2016.

VOCs	Cultivar								Significance	Tukey's HSD	Concentration (mean ± SD)	
	Campari F_1	Auriga	Harzfeuer F_1	Roterno F_1	Lyterno F_1	Bocati F_1	Cappricia F_1	Green Zebra			2015	2016
Hexanal	29.60	29.28	31.52	37.43	28.24	36.61	37.01	36.13	ns	20.74	22.4 ± 11.1	44.1 ± 8.60
2-methylbutylacetate	Nd	0.12	Nd	Nd	0.09	Nd	Nd	0.06	***	0.21	0.00 ± 0.00	0.28 ± 1.65
(*E*)-2-hexenal	9.94	9.74	9.03	3.37	6.20	5.79	7.72	9.78	*	5.58	8.86 ± 4.82	6.62 ± 3.16
octanal	4.77	2.93	3.97	3.48	4.86	2.95	4.23	2.30	ns	3.02	5.47 ± 1.75	2.11 ± 0.97
6-methyl-5-heptene-2-one	16.08	13.24	18.75	20.80	17.86	15.90	15.80	24.67	*	9.75	20.7 ± 7.66	15.7 ± 6.13
1-hexanol	1.59	1.65	2.23	1.14	0.82	1.62	0.72	1.31	ns	1.63	1.91 ± 1.38	1.06 ± 0.71
(*Z*)-3-hexen-1-ol	2.85	3.48	3.27	1.49	2.00	1.96	1.65	1.47	ns	2.51	3.59 ± 1.58	1.19 ± 0.95
2-isobutylthiazole	17.32	13.25	11.45	17.88	18.27	17.19	10.82	12.02	***	5.09	13.1 ± 4.04	13.2 ± 5.49
6-methyl-5-hepten-2-ol	0.35	Nd	0.38	0.37	0.55	0.42	0.50	Nd	ns	0.55	0.00 ± 0.00	0.56 ± 0.39
2-ethyl-1-hexanol	0.85	0.72	0.73	0.69	1.05	0.66	0.95	0.68	ns	0.70	0.95 ± 0.58	0.67 ± 0.28
benzaldehyde	0.16	0.28	0.20	0.14	0.10	0.10	0.00	0.14	ns	0.39	0.28 ± 0.33	0.00 ± 0.00
linalool	1.93	2.83	2.48	1.67	3.23	2.98	3.47	2.46	**	2.08	3.51 ± 1.54	1.60 ± 1.04
methylheptadione	0.08	Nd	0.05	0.05	Nd	Nd	Nd	0.02	ns	0.16	0.05 ± 0.15	0.00 ± 0.00
β-cyclocitral	1.03	5.57	1.16	0.97	1.63	0.97	1.47	0.22	***	1.19	2.08 ± 2.08	1.18 ± 0.93
3-mebutanoic acid	0.21	0.48	0.34	0.33	0.19	0.27	0.40	0.55	ns	0.68	0.52 ± 0.55	0.13 ± 0.25
α-terpineol	0.73	0.19	0.30	0.04	0.09	0.11	0.48	0.26	ns	0.99	0.28 ± 0.91	0.27 ± 0.28
geranial	0.94	0.09	1.14	0.98	1.62	0.71	1.17	Nd	*	1.59	1.66 ± 1.21	0.00 ± 0.00
citral	0.75	0.09	0.79	0.72	0.89	0.56	0.37	Nd	***	1.11	0.00 ± 0.00	1.52 ± 1.05
decadienal	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	–	–	0.00 ± 0.00	0.00 ± 0.00
methylsalicylate	0.39	0.51	2.00	0.31	0.07	1.43	0.22	0.66	ns	1.95	0.00 ± 0.00	1.28 ± 1.75
β-damascenone	3.21	5.97	2.35	1.62	3.26	2.25	4.97	5.00	**	3.99	5.34 ± 3.20	1.48 ± 1.48
(*E*)-geranylacetone	4.44	2.77	5.28	4.92	6.07	5.67	5.88	1.11	***	2.75	5.79 ± 2.53	4.49 ± 5.29
2-mepropanoic acid	0.27	0.54	0.24	0.21	0.40	0.15	0.13	0.18	ns	0.56	0.20 ± 0.45	0.27 ± 0.31
benzyl alcohol	Nd	0.09	0.04	Nd	Nd	Nd	Nd	0.05	***	0.17	0.00 ± 0.00	0.16 ± 0.32
phenylethyl alcohol	0.81	0.38	0.20	0.21	0.34	0.19	0.25	0.26	***	0.50	0.38 ± 0.46	0.55 ± 0.60
β-ionone	0.98	4.15	0.95	0.92	1.51	0.82	1.29	Nd	***	1.05	1.80 ± 1.66	0.97 ± 0.74
eugenol	0.19	0.08	0.13	0.05	0.15	0.18	0.03	0.03	***	0.26	0.00 ± 0.00	0.24 ± 0.24
farnesylacetone	Nd	0.64	0.17	0.04	0.08	0.17	0.24	Nd	***	0.37	0.00 ± 0.00	0.34 ± 0.39
isopropylmyristate	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	–	–	Nd	Nd
octanoic acid	0.33	0.50	0.43	0.17	0.42	0.02	0.20	0.14	ns	0.61	0.55 ± 0.49	Nd
nonanoic acid	0.20	0.42	0.41	Nd	Nd	0.35	Nd	0.48	ns	0.67	0.46 ± 0.60	Nd

*Each mean represents six biological replicates (over 2 years) and three biological replicates (within 1 year); SD: standard deviation; Nd: not detectable or present at low levels; ns indicates a nonsignificant difference; *, ** and *** indicate significance differences of each factor and interaction at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; HSD (0.05) = critical value for comparisons by Tukey's honestly significant difference (HSD) tests at $p < 0.05$.*

VOCs were also observed, such as β-ionone, which had the strongest positive correlation with β-cyclocitral (r = 0.98), followed by phenylethyl alcohol, which correlated positively with benzyl alcohol (r = 0.83).

Principal Component Analysis of the Instrumental and Sensory Parameters in 20 Cultivars

Classification between fruit types, the relative importance of each variable (instrumental and sensory traits), and the relationships between these variables and fruit type are shown in **Figure 4**. The cultivars were classified into four PCs with respect to their instrumental and sensory characteristics: (i) PC1 distinguished the characteristics of cocktail cultivars (i.e., "Goldita" and "Supersweet 100 F_1") from salad cultivars (i.e., "Bocati F_1", "Cappricia F_1", "Lyterno F_1", "Roterno F_1"), (ii) PC2-contained data of "Auriga" and "Green Zebra", and separated these salad cultivars from others in the same PC, (iii) in PC3, "Resi" was distinguished from other cultivars, and PC4 contained only "Tastery F_1" differed from the other CVs (**Supplementary Table 8**). PC1 was assigned a higher score for TSS, sweetness, aroma, and acceptability, as well for variation in 2-isobuthylthiazole, 6-methyl-5-hepten-2-ol, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, phenylethyl alcohol, and benzyl alcohol. High loading of TA; color parameters (L*, b*, C*, and h); sensory attributes (sourness and juiciness); and a number of VOCs, including nonanoic acid, 3-mebutanoic acid, β-damascenone, farnesylacetone, and benzaldehyde, contributed to the separation of PC2. While PC1 and PC2 were generally associated with higher scores of sensory attributes, PC3 and PC4 were distinguished from the other PCs mainly by higher loading values of VOCs (**Supplementary Table 9**). Overall, the classification of the two fruit types was mainly influenced by the highest loading score of

TABLE 2 | Concentrations of volatile organic compounds (VOCs) (norm-%) of 12 cocktail tomato cultivars grown in 2015 and 2016.

VOCs	Cultivars												Significance	Tukey's HSD	Concentration (mean ± SD)	
	Goldita	Super-sweet 100 F_1	Resi	Bartelly F_1	Benarys Garten-freude	Prima-vera	Black Cherry	Sakura	Prima-bella	Tastery F_1	Annamay F_1	Amoroso F_1			2015	2016
Hexanal	30.06	25.11	34.64	40.44	37.16	40.10	48.67	26.26	47.50	42.17	30.31	37.71	***	16.94	29.1 ± 11.2	43.9 ± 9.90
2-methylbutylacetate	Nd	Nd	5.54	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	***	2.65	0.00 ± 0.00	0.80 ± 3.03
(*E*)-2-hexenal	10.07	12.62	7.25	11.50	7.08	9.23	9.00	13.64	9.59	9.01	7.24	7.92	ns	8.94	12.8 ± 7.07	6.19 ± 2.80
octanal	3.94	4.32	3.26	3.13	3.68	4.19	3.63	3.46	1.90	4.73	3.74	4.53	***	2.34	4.83 ± 1.49	2.44 ± 1.16
6-methyl-5-heptene-2-one	14.61	22.43	22.51	14.26	28.97	13.41	11.48	16.99	10.57	10.80	20.28	17.67	***	6.87	16.7 ± 6.68	17.0 ± 7.52
1-hexanol	2.13	1.35	2.37	1.12	2.25	2.87	2.01	1.38	1.43	1.54	1.71	1.65	***	1.34	2.27 ± 1.08	1.23 ± 0.61
(*Z*)-3-hexen-1-ol	3.53	3.98	2.83	2.04	3.70	4.10	2.45	2.61	2.34	3.16	2.60	2.31	ns	2.94	4.30 ± 2.00	1.48 ± 0.91
2-isobutylthiazole	2.99	3.74	8.74	8.89	3.89	10.52	7.18	14.09	11.41	7.33	16.34	8.06	***	3.39	8.38 ± 4.44	11.1 ± 6.49
6-methyl-5-hepten-2-ol	0.18	0.07	0.12	0.07	Nd	Nd	0.28	0.21	0.29	0.05	0.17	0.09	***	0.27	0.00 ± 0.00	0.30 ± 0.34
2-ethyl-1-hexanol	1.10	0.84	0.54	0.80	0.60	1.22	0.78	0.92	0.55	1.28	0.67	0.96	*	0.69	0.97 ± 0.63	0.72 ± 0.30
benzaldehyde	Nd	0.11	0.07	0.16	0.04	0.04	0.07	0.13	0.02	Nd	0.05	0.21	ns	0.26	0.15 ± 0.24	0.00 ± 0.00
linalool	1.08	4.54	0.65	0.97	0.51	0.25	3.63	2.22	0.53	1.06	1.44	2.30	***	1.41	2.11 ± 1.79	1.17 ± 1.08
methylheptadione	Nd	Nd	Nd	0.10	Nd	Nd	Nd	Nd	Nd	Nd	0.04	Nd	ns	0.12	0.02 ± 0.11	0.00 ± 0.00
β-cyclocitral	0.73	1.84	0.97	1.55	1.17	2.53	0.88	1.56	1.16	2.24	1.19	1.37	***	0.73	1.47 ± 0.76	1.39 ± 0.88
3-mebutanoic acid	Nd	0.14	0.55	0.24	0.16	0.07	0.24	0.08	0.37	Nd	0.12	0.21	**	0.46	0.31 ± 0.42	0.08 ± 0.19
α-terpineol	Nd	0.37	0.14	0.06	0.32	0.84	0.63	0.20	0.06	Nd	0.10	0.32	ns	1.33	0.37 ± 1.23	0.14 ± 0.26
Geranial	0.32	0.81	0.87	0.66	1.03	0.46	0.77	0.80	0.61	1.20	0.57	0.82	ns	1.29	1.48 ± 0.73	0.00 ± 0.00
Citral	0.72	1.94	1.16	1.32	1.51	1.41	0.76	0.81	0.87	0.66	1.64	0.74	ns	1.85	0.00 ± 0.00	1.95 ± 1.17
Decadienal	Nd	Nd	Nd	0.04	0.24	Nd	Nd	Nd	Nd	Nd	Nd	0.05	*	0.21	0.05 ± 0.21	0.00 ± 0.00
Methylsalicylate	0.16	0.08	0.08	0.20	0.54	0.26	0.34	0.14	1.05	2.97	0.13	0.16	***	1.51	0.00 ± 0.00	1.06 ± 1.60
β-damascenone	2.61	4.44	2.80	2.94	0.72	1.50	2.77	6.32	2.32	1.90	4.62	3.83	***	3.70	5.01 ± 2.41	1.37 ± 1.91
(*E*)-geranylacetone	20.17	5.39	3.48	4.43	4.43	4.23	2.63	4.28	4.42	6.42	4.73	5.69	***	3.40	6.19 ± 4.92	4.73 ± 3.57
2-mepropanoic acid	0.22	0.26	Nd	0.36	0.06	0.31	0.40	0.28	0.09	0.47	0.20	0.32	ns	0.63	0.30 ± 0.54	0.23 ± 0.30
Benzyl alcohol	1.25	0.59	0.08	0.47	Nd	Nd	0.24	0.48	Nd	Nd	Nd	Nd	***	0.40	0.16 ± 0.37	0.28 ± 0.50
Phenylethyl alcohol	2.96	3.10	0.26	2.39	0.30	Nd	0.18	1.60	1.14	0.43	0.74	1.29	***	1.00	1.29 ± 1.38	0.96 ± 1.14
β-ionone	0.88	1.35	0.82	1.35	1.12	2.19	0.67	1.23	0.91	2.24	1.08	1.23	***	0.64	1.25 ± 0.72	1.18 ± 0.59
Eugenol	Nd	Nd	0.04	0.11	Nd	0.06	0.06	Nd	0.21	0.30	0.10	0.08	**	0.21	0.00 ± 0.00	0.14 ± 0.20
Farnesylacetone	0.23	0.20	0.19	0.12	0.27	0.07	Nd	0.12	0.14	Nd	0.05	0.12	**	0.34	0.00 ± 0.00	0.25 ± 0.40
Isopropylmyristate	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	–	–	Nd	Nd
Octanoic acid	0.03	0.36	0.05	0.30	0.26	0.13	0.24	0.20	0.04	0.05	0.16	0.36	*	0.40	0.36 ± 0.33	0.00 ± 0.00
Nonanoic acid	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	0.49	Nd	Nd	Nd	***	0.30	0.08 ± 0.35	0.00 ± 0.00

*Each mean represents six biological replicates (over 2 years) and three biological replicates (within 1 year); SD: standard deviation; Nd: not detectable or present at low levels; ns indicates a nonsignificant difference; *, ** and *** indicate significance differences of each factor and interaction at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; HSD (0.05) = critical value for comparisons by Tukey's honestly significant difference (HSD) tests at $p < 0.05$.*

TABLE 3 | Breeders' sensory scores of 33 salad and 27 cocktail tomato cultivars grown in 2015.

Cultivar	Fruit firmness	Juiciness	Skin firmness	Sweetness	Sourness	Aroma	Acceptability
Salad cultivar							
Previa F_1	4.25	5.67	4.54	4.21	5.33	5.02	4.46
Garance F_1	4.86	4.48	5.79	4.38	5.75	4.96	4.04
Green Zebra	2.86	6.58	4.90	4.94	7.31	6.69	6.09
Diplom F_1	4.34	6.31	5.46	4.40	4.83	4.29	3.75
Cappricia F_1	6.85	4.98	3.88	2.71	3.79	2.29	2.00
Rougella F_1	5.98	3.88	5.29	3.31	3.71	3.27	2.33
Sparta F_1	5.42	6.06	4.96	4.38	5.56	5.12	4.96
Bocati F_1	5.86	5.29	3.56	2.77	4.60	3.10	2.67
Phantasia F_1	7.35	3.77	4.38	2.60	4.56	2.52	2.00
Mecano F_1	6.96	4.44	3.79	2.44	3.83	2.17	1.87
Hamlet F_1	6.65	3.39	4.65	2.33	3.34	2.42	1.92
Lyterno F_1	7.04	5.06	4.63	3.29	5.04	3.48	3.17
Nordica F_1	6.85	5.08	3.75	3.29	4.17	3.08	2.48
Moneymaker	6.17	4.67	4.94	2.13	3.52	2.02	1.50
Pannovy F_1	5.06	4.25	4.54	2.50	4.96	2.75	2.46
Roterno F_1	6.46	5.33	3.63	3.52	3.52	3.15	2.73
Hildares F_1	4.56	5.08	4.96	3.56	4.12	3.54	2.56
Bonner Beste	4.66	6.17	6.45	4.17	5.95	4.83	4.57
Tica	7.02	4.58	4.09	2.79	4.50	2.81	2.54
Ricca	7.23	4.61	4.00	2.60	4.13	2.62	2.23
Aroma	4.92	5.86	5.71	4.54	4.94	4.96	3.94
Rheinlands Ruhm	5.67	5.54	4.79	2.65	3.67	2.56	1.92
Lukullus	5.21	5.19	4.73	3.31	4.42	3.56	2.44
Goldene Königin	3.60	5.08	5.38	3.92	4.54	4.67	3.19
Harzfeuer F_1	4.63	5.69	6.11	5.15	5.10	5.04	4.34
Auriga	3.58	5.65	5.67	4.73	6.02	5.94	5.33
Haubners Vollendung	3.37	5.42	5.58	4.29	5.17	4.98	3.50
Dorenia	5.09	5.19	4.90	4.17	5.00	4.54	4.00
Roi Humbert Jaune	3.83	4.56	5.83	3.42	4.46	4.33	3.02
Hellfrucht	4.92	5.85	5.40	3.77	4.56	3.67	3.14
Campari F_1	4.77	5.27	5.56	6.23	5.33	6.25	6.21
Matina	4.83	5.96	6.29	4.91	4.57	4.95	4.43
Black Plum	3.08	3.12	4.83	4.71	3.88	5.21	2.67
Mean	5.3	5.1	4.9	3.7	4.7	4.0	3.3
SD	1.5	1.2	1.1	1.2	1.3	1.5	1.5
CV (%)	15.52	15.87	18.43	20.82	18.8	23.15	26.36
HSD (0.05)	1.68	1.66	1.87	1.58	1.81	1.89	1.77
Significance							
Cultivar (C)	***	***	***	***	***	***	***
Harvest (H)	**	***	ns	*	***	*	***
Interaction (C × H)	***	ns	ns	ns	ns	ns	ns
Cocktail Cultivar							
Amoroso F_1	6.46	5.69	4.08	5.71	5.46	5.38	5.27
Annamay F_1	6.00	5.50	4.81	6.17	5.38	5.93	6.24
Quedlinburger Frühe Liebe	3.56	6.65	5.94	5.19	5.92	5.00	4.00
Ruthje	5.79	6.02	5.44	5.98	5.08	5.40	5.06
König Humbert	3.50	2.58	4.46	4.15	3.42	4.29	1.75
Clou	4.38	6.75	7.04	5.42	5.84	5.88	4.88
Tastery F_1	8.10	5.75	5.13	6.44	4.38	4.38	4.96
Primabella	6.27	5.52	5.61	5.73	6.33	5.98	5.42
Sakura F_1	5.88	6.63	5.92	7.48	5.98	7.15	7.44
Black Cherry	3.27	6.46	6.92	6.63	6.61	7.73	7.33

(Continued)

TABLE 3 | (Continued)

Cultivar	Fruit firmness	Juiciness	Skin firmness	Sweetness	Sourness	Aroma	Acceptability
Cerise gelb	5.21	4.75	5.52	5.67	5.08	6.06	4.77
Yellow Submarine	3.79	4.48	5.38	5.29	5.04	5.77	3.96
Zuckertraube	4.02	6.75	5.69	6.63	5.73	6.36	6.15
Dorada	4.21	6.44	5.25	6.23	5.54	6.31	6.11
Primavera	4.00	6.46	5.63	5.50	5.27	5.21	4.79
Philovita F_1	6.79	5.54	5.15	6.13	5.10	5.94	6.10
Trixi	5.75	6.04	6.73	6.90	4.73	6.17	5.77
Trilly F_1	6.65	5.38	5.50	7.58	5.31	6.69	7.17
Benarys Gartenfreude	4.63	5.19	7.50	7.13	5.56	6.56	5.44
Bartelly F_1	3.79	5.60	4.90	6.65	5.19	6.65	6.29
Golden Pearl F_1	4.54	6.63	6.63	7.46	5.12	7.06	7.00
Resi	2.69	6.94	6.63	6.36	6.11	7.52	6.15
Supersweet 100 F_1	3.79	6.79	6.06	7.35	6.02	7.19	7.13
Goldita	3.88	6.33	6.52	7.10	6.12	7.29	6.96
Sliwowidnij	2.98	6.77	5.96	5.73	5.85	6.10	5.42
Rote Murmel	2.58	7.38	5.02	6.67	5.88	7.04	6.65
Golden Currant	3.33	7.15	4.23	7.06	4.79	6.83	5.46
Mean	4.7	6.0	5.7	6.3	5.4	6.2	5.7
SD	1.6	1.2	1.1	1.0	1.0	1.1	1.4
CV (%)	14.8	10.2	12.4	9.6	11.7	11.0	14.1
HSD (0.05)	1.39	1.23	1.41	1.21	1.28	1.37	1.61
Significance							
Cultivar (C)	***	***	***	***	***	***	***
Harvest (H)	ns	***	**	***	***	**	**
Interaction (C × H)	*	***	ns	**	ns	**	ns

*The breeders' sensory scoring was based on a 9-point scale where 0 = not detectable and 9 = maximum intensity; mean values are given for each of the 33 salad cultivars and the 27 cocktail cultivars as mean from samples grown in 2015; SD = standard deviation; CV = coefficient of variation; ns indicates a nonsignificant difference; *, ** and *** indicate significance differences of each factor and interaction at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; HSD (0.05) = critical value for comparisons by Tukey's honestly significant difference (HSD) test at $p < 0.05$. The cultivars are arranged in descending order according to their average single fruit weight. The cultivars shown in bold were selected in 2015 for further evaluation in 2016.*

"sweet-taste factor" (i.e., TSS/sweetness), the intensity of aroma, and the flavor-related VOCs mentioned above.

DISCUSSION

Fruit flavor is a complex trait influenced by interaction of both fruit biochemical characteristic and consumer sensory perception (35, 39). In terms of fruit quality, high variability was observed between tomato cultivars, with salad cultivars having 10–70% lower values for minerals, dry matter, TSS, and total phenolics than cocktail cultivars (31).

Volatile Organic Compounds Profile of the Tomato Cultivars

A total of 31 VOCs, with aldehydes accounting the largest proportion of the total VOCs concentration, were detected in the samples. This result is in agreement with Wang et al. (40), who reported that ALD accounted for the highest proportion of the total VOCs concentration in tomato fruits, followed by ALC and KET, with all three compound classes compromising more than 95% of total VOCs concentration. Selli et al. (41) reported that 77.2% of the total VOCs in tomatoes were ALD. Of more than 400 VOCs identified in tomato fruits (42), most studies agreed that about 16 VOCs were likely to contribute significantly to tomato aroma (14, 18, 43). Of these, nine VOCs were identified in this study, which are believed to contribute to tomato aroma, such as ALD (hexanal and trans-2-hexenal), ALC (cis-3-hexenol and 2-phenylethyl alcohol), KET (6-methyl-5-hepten-2-one, β-damascenone, and β ionone), SDC (2-isobutylthiazole), and EST (methylsalicylate).

In both years, hexanal derived from C6 fatty acid was the most abundant VOC found, with a mean value of 35.05%. Hexanal is often associated with the perception of "green" and "grassy" in tomato fruits (41), as well as with the evaluation of sweetness of ripe tomatoes (44). The open chain apocarotenoid cleavage product, 6-methyl-5-heptene-2-one, derived from lycopene (14), was the second most abundant VOC (17.77%), followed by the branched chain amino acid-derived VOC 2-isobutylthiazole, with a relative concentration of 11.37%. The latter is one of the most important and interesting aroma components of tomato, as its concentration remains stable during fruit ripening (20) and is not affected by crushing and exposure to oxygen (45). Two esters, namely, farnecylacetone and isopropilmyristate have been identified in the VOCs of the tomato fruits. The latter was detected in trace amounts, and its concentration was not

TABLE 4 | Panel sensory scores of eight salad and 12 cocktail tomato cultivars grown in 2016.

Cultivar	Fruit firmness	Juiciness	Skin firmness	Sweetness	Sourness	Aroma	Acceptability
Salad cultivar							
Green Zebra	25.01	72.02	43.02	30.80	61.60	53.24	46.01
Cappricia F_1	62.61	57.09	36.10	17.76	32.63	26.08	22.09
Bocati F_1	55.46	60.71	38.70	20.41	31.01	28.41	22.38
Lyterno F_1	63.91	58.93	40.33	20.68	32.95	32.00	26.95
Roterno F_1	57.70	60.14	35.51	21.20	26.54	28.43	25.40
Harzfeuer F_1	33.58	57.96	49.10	32.30	37.29	39.99	27.75
Auriga	29.93	63.02	56.33	32.42	56.64	45.46	33.31
Campari F_1	43.86	59.83	45.18	36.09	44.42	46.17	43.32
Mean	46.5	61.2	43.0	26.5	40.4	37.5	30.9
SD	15.3	5.7	8.1	7.9	12.6	10.3	10.1
CV (%)	9.4	5.75	11.55	16.91	7.17	10.1	17.31
HSD (0.05)	7.89	6.35	8.97	8.08	5.23	6.83	9.66
Significance							
Cultivar (C)	***	***	***	***	***	***	***
Harvest (H)	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Interaction (C × H)	*ns*	*ns*	*ns*	*ns*	*	*	*ns*
Cocktail cultivar							
Amoroso F_1	54.39	60.08	39.97	45.59	41.16	50.73	51.98
Annamay F_1	55.68	59.78	45.23	40.75	46.35	51.50	49.24
Tastery F_1	78.02	59.98	45.12	51.08	26.92	40.49	45.08
Primabella	49.28	62.35	42.86	38.54	49.63	51.29	45.69
SakuraF_1	43.90	61.39	49.15	48.09	47.96	57.08	56.56
Black Cherry	33.09	63.61	53.03	42.00	55.06	54.79	51.10
Primavera	26.22	67.67	50.35	41.81	37.36	43.87	35.36
Benarys Gartenfreude	43.21	57.81	56.94	46.13	46.20	51.82	42.00
Bartelly F_1	32.30	57.15	45.70	48.85	41.21	56.85	52.88
Resi	29.80	66.22	53.53	42.87	49.62	54.32	45.59
Supersweet 100 F_1	29.93	58.89	54.03	53.94	46.18	59.11	57.47
Goldita	28.41	59.96	56.88	47.53	48.54	55.58	49.15
Mean	42.0	61.2	49.4	45.6	44.7	52.3	48.5
SD	15.5	4.7	6.5	7.2	8.4	6.3	8.0
CV (%)	10.68	5.42	7.7	12.05	10.46	6.11	9.96
HSD (0.05)	8.47	6.27	7.18	10.38	8.83	6.04	9.13
Significance							
Cultivar (C)	***	***	***	***	***	***	***
Harvest (H)	***	**	*	***	***	**	*ns*
Interaction (C × H)	*ns*	**	*ns*	*ns*	*ns*	**	***

*The panel sensory was rated based on a 0 to 100 sensory perceptible scale (0 = minimum intensity and 100 = maximum intensity); mean values are given for each of the eight salad and the 12 cocktail cultivars as mean from samples grown in 2016; SD = standard deviation; CV = coefficient of variation; ns indicates a nonsignificant difference; *, ** and *** indicate significance differences of each factor and interaction at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; HSD (0.05) = critical value for comparisons by Tukey's honestly significant difference (HSD) test at $p < 0.05$. The cultivars are arranged in descending order according to their average single fruit weight.*

influenced by the interaction of cultivar and environmental factors (**Supplementary Table 5**). This is in agreement with Goulet (46), who reported that red-fruited tomato cultivars have relatively low levels of acetate esters compared with green-fruited cultivars. In the present study, farnecylacetone was shown to correlate positively with color (L*, b*, and C* values), but not with sensory perceptions (**Table 5**). However, their overall impact on sensory properties is negligible, and they are not relevant to tomato aroma (16). The fruity esters that are important for flavor in most fruits, e.g., in strawberry (47) do not seem to have the same role in tomato fruits, which may explain the lack of contribution and relevance of esters for sensory evaluation of flavor (16, 46).

Cultivar and Harvest Season Effects on Instrumental Traits

Breeders have made considerable efforts to improve the quality characteristics of tomato. The suitability of cultivars for a particular location may not be similar to another location (48). Usually, inconsistent quality performance in different growing

TABLE 5 | Correlation coefficients between volatile organic compounds (VOCs) and instrumental parameters and between VOCs and sensory attributes in the 20 cultivars grown in 2015 and 2016.

VOCs	Instrumental parameters[#]							Sensory attributes						
	TSS	TA	L*	a*	b*	C*	h	Fruit firmness	Juiciness	Skin firmness	Sweetness	Sourness	Aroma	Acceptability
(*E*)-2-hexenal	0.59**	0.54*	−0.10	−0.33	−0.03	−0.18	0.23	−0.49*	0.12	0.48*	0.64**	0.50*	0.67**	0.69**
octanal	0.00	−0.45*	−0.35	0.23	−0.32	−0.33	−0.30	0.34	−0.50*	−0.04	0.11	−0.51*	−0.22	−0.07
1-hexanol	0.31	0.22	−0.19	−0.12	−0.20	−0.29	0.02	−0.49*	0.38	0.65**	0.39	0.26	0.33	0.12
(*Z*)-3-hexen1-ol	0.54*	0.27	−0.15	0.04	−0.08	−0.17	−0.09	−0.39	0.00	0.80**	0.58**	0.20	0.37	0.26
2-isobutylthiazole	−0.68**	−0.41	0.16	0.31	0.22	0.37	−0.17	0.34	−0.03	−0.61**	−0.69**	−0.31	−0.55*	−0.49*
6-methyl-5-hepten-2-ol	−0.52*	−0.56*	−0.11	0.51*	−0.12	0.07	−0.42	0.44	−0.45*	0.52*	−0.69**	−0.48*	−0.64**	−0.59**
3-methylbutanoic acid	−0.45*	0.13	0.44	−0.09	0.39	0.47*	0.21	−0.25	0.36	−0.18	−0.50*	0.28	−0.15	−0.29
geranial	−0.08	−0.60**	−0.53*	0.58**	0.51*	−0.34	−0.66**	0.63**	−0.67**	−0.30	−0.19	−0.68**	−0.47*	−0.35
citral	0.64**	0.08	−0.67**	0.34	−0.60**	−0.53*	−0.54*	−0.11	−0.25	0.35	0.55*	−0.07	0.37	0.35
benzyl alcohol	0.51*	0.40	0.10	−0.24	0.08	−0.05	0.24	−0.43	−0.06	0.49*	0.49*	0.29	0.52*	0.49*
phenylethyl alcohol	0.63**	0.41	−0.10	−0.04	−0.03	−0.09	0.00	−0.27	−0.25	0.31	0.6**	0.20	0.56**	0.63**
eugenol	−0.24	−0.41	−0.10	0.28	−0.07	0.01	−0.24	0.55*	−0.21	−0.39	−0.15	−0.40	−0.36	−0.22
farnesylacetone	0.01	0.21	0.51*	0.18	0.55*	0.53*	0.02	−0.27	−0.13	0.42	−0.07	0.25	−0.01	−0.16
nonanoic acid	−0.35	0.25	0.5*	−0.16	0.54*	0.57**	0.33	−0.22	0.33	−0.06	−0.37	0.33	−0.11	−0.24

*Data used for the Pearson correlation are derived from each of the 20 cultivars as mean from both years. [#]The instrumental parameters used for Pearson correlation analysis were taken from Chea et al. (33). Significant correlation is indicated by asterisks: *$p < 0.05$ and **$p < 0.01$. VOCs – volatile organic compounds; TSS – total soluble solid; TA – titratable acids; color parameters: L* – lightness; a* – red (+)/green (–); b* – yellow (+)/blue (–); C* – chroma; h – hue. Sensory attributes: fruit firmness, juiciness, skin firmness, sweetness, sourness, aroma and acceptability. Only the VOCs, which have significant correlations with at least one of the other parameters, are shown.*

environments is the result of genotype by environment (G × E) interaction (GEI) (49). Cultivar-by-harvest and year (C × H × Y) interactions were significant for 17 VOCs and not significant for 14 VOCs (**Supplementary Table 5**), illustrating the stability performance of the cultivars at different harvest dates, years, and their interactions. The information would facilitate the selection of stable/unstable genotypes that perform well in different environments (39). Generally, breeders use GEI to select genotypes with high and stable performance in different environments, and the genotypes whose GEI is insignificant are considered stable (50).

The wide cultivar variation in fruit VOCs concentration in salad and cocktail cultivars (**Tables 1**, **2**) indicates that some cultivars could be used as parents to develop well-adapted cultivars with improved flavor in different environments, such as harvest date (H) and year (Y) in organic low-input management. The effect of cultivar alone was much more important, and it was significant for almost all the compounds, excluding α-terpineol. Paolo et al. (8) and Rambla et al. (16) reported that, although oxygenated terpenoides are among the most abundant volatiles in vegetative tissues of tomato plants and, particularly, in trichomes, only a few of them, including α-terpineol, are present in the ripe fruit, and their influence on fruit flavor is negligible.

The significant C × H × Y interaction effects on VOCs underline the need in breeding for these traits, as plant breeders have to develop cultivars that perform consistently well under different environmental conditions and seasons. Bauchet and Causse (51) reported that VOCs exhibiting a variable pattern of heritability indicate a high sensitivity of these compounds to environmental conditions. As an example, even when fruits of the same genotypes are grown with identical field management, consumers usually complain that off-season tomato fruits are not as good as in-seasonal ones in terms of overall flavor and eating quality (22). This can be a problem for plant breeders as it is too labor-intensive to develop cultivars for each specific site (48) or each specific growing season.

In our previous study, no C × Y interaction was found for TSS and TA within both salad and cocktail cultivar groups (32). This result is consistent with other studies (10, 52) and thus indicates a high stability of these taste-related traits to environmental variation. This is also in line with Gautier et al. (53), who found that the influence of temperature and irradiance on the level of secondary metabolites was more pronounced than on the primary metabolites (e.g., TSS). Based on heritability and G × E interaction, TA was the least environmentally sensitive trait and was much more likely to be retained when the cultivar was grown in a different location (50). Basically, the extent of a G × E interaction is influenced by the genetic structure of the genotype (54).

The fruit color analyzed instrumentally was less variable as the fruits of most cultivars were red ($a^* > 10$), with exception of the salad cultivar "Green Zebra" (green – yellow) and the cocktail cultivar "Black Cherry", which was characterized by a red – brown fruit skin color (32). The ALD geranial correlated

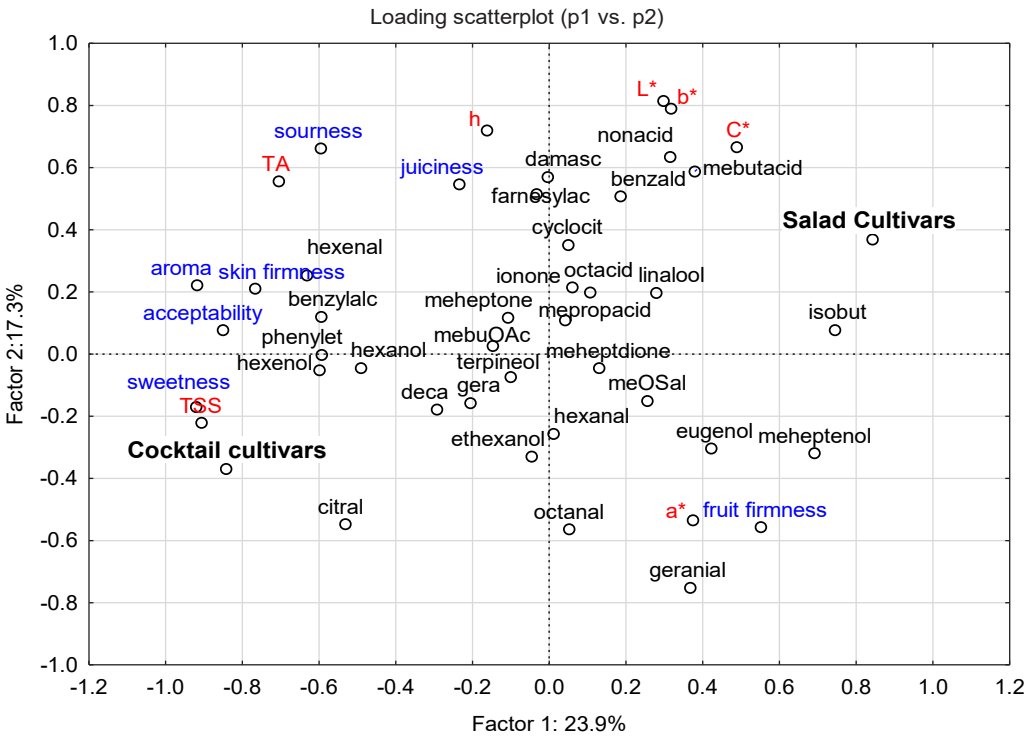


FIGURE 4 | PCA bi-plot of the instrumental parameters (in red and black) and sensory attributes (in blue) of the 20 cultivars (eight salad and 12 cocktail cultivars) grown in 2015 and 2016. Instrumental parameters: TSS – total soluble solids; TA – titratable acid; L* – lightness; a* – red (+)/green (–); b* – yellow (+)/blue (–); C* – chroma; h – hue. Sensory attributes: fruit firmness, juiciness, skin firmness, sweetness, sourness, aroma, and acceptability. VOCs are named according to **Supplementary Table 2**.

positively with a* and b* levels, while the ALD citral and the EST farnecyl acetone showed a positive relationship with b* (**Table 5**), so there seems to be a relationship between these VOCs and carotenoids. Genotype is an important determinant of the extent of variability in carotenoid content of ripe tomato fruits (12). The red color value (a*) of our cultivars was lower than that of the cocktail cultivars studied in Sonntag et al. (55), while the color values of the red-fruited salad cultivars ("Bocati F_1", "Cappricia F_1", "Roterno F_1") were comparable to the results of Sonntag et al. (55). The red color of the tomato fruits is due to the synthesis of lycopene and degradation of chlorophyll (56), while an orange genotype accumulates high levels of β-carotene in addition to a low-lycopene content (57). Increasing a* value, lycopene and ß-carotene were observed during ripening of red-fruited cultivars (55). Based on heritability and genotype by environment interaction, lycopene, which accounts for more than 85% of total carotenoids in many red-fruited cultivars (58), was the most environmentally sensitive trait (50). The difference in the levels of a* and b* in the cultivar (32) could be due to the accumulation of derivatives of carotenoid metabolism, such as C8-ketone 6-methyl-5-hepten-2-one, C10-aldehyde geranial, β-ionone, and β-damascenone, which were present in higher concentration in the studied cultivars (**Tables 1**, **2**). With the exception of geranial, these carotenoid derivatives did not significantly influence the flavor perception of the fruit, despite their high content. The higher amount of 6-methyl-5-hepten-2-one might be mainly due to a higher lycopene content in the pericarp (40); however, there was no significant correlation between the amount of the carotenoid derivate and the sensory attributes in our study.

Among the salad cultivars, "Campari F_1" had the highest TSS, and "Green Zebra" yielded the highest TA. In the cocktail cultivars, "Benarys Gartenfreude" gave the highest value of taste components, followed by "Supersweet 100 F_1" [see data in Chea et al. (32)]. The TSS, commonly used as an indirect measure of sugar content and sweetness, is considered to be the trait with the highest influence on cherry tomato purchase preference (30). The fact that fruit size and yield per plant are lower in cocktail cultivars than in salad cultivars (32) is, probably, a possible explanation for the higher concentration of TSS observed in this fruit type group. In contrast, TA levels do not seem to be related to fruit type. From a commercial perspective, the content of organic acids in fruits is one of the most important characteristics influencing the sensorial qualities of the product (31).

Sensory Characteristics of the Cultivars

Improving the sensory quality of fresh market tomato is of great interest to breeders, but it is a very complex goal (6). From the breeder's perspective, analysis of aroma compounds in fruit crops demands expensive equipment and training (i.e. sensory analysis). Although breeders can use sensory analysis, it is often difficult to perform and requires access to a panel

and considerable expertise (18). In the present study, breeders' sensory test was used as a tool to evaluate organoleptic quality in 2015, as a breeders' sensory panel is better suited to evaluate a large number of small samples with a small team. Despite their obvious advantages regarding number of cultivars and, more importantly, the time required for analysis, field evaluations are generally subjective and prone to error because they are usually based on the sensory preferences of one or few individuals (39). However, Hagenguth et al. (36) demonstrated a highly significant correlation of sweetness and TSS and sourness and TA, indicating the efficiency of the breeders' sensory test. A trained panel may not able to assess a large number of samples in a short time, which is typical for early breeding generations, for example, that also require replications (36).

Sensory quality of tomato fruit thus includes taste, aroma, and texture (referred to as flavor), as well as color (24). It seems that a high rating of sensory aroma and measured a* value were important factors that enhanced the acceptability of "Supersweet 100 F_1" and "Sakura F_1", regardless of their superiority in sweetness as the most important criterion. Red-colored tomatoes are more familiar and attractive to consumers than other colored fruits (59, 60). Regarding the aroma, both sensory tests confirmed that "Supersweet 100 F_1", "Black Cherry", "Sakura F_1", and "Goldita" had the most intensive aroma. Zörb et al. (58) found that, besides aroma, other sensory attributes, such as sweetness, juiciness, and a fruit-like appearance, are more important for cocktail tomatoes, which are usually eaten raw. The highest scores for aroma and juiciness were obtained for "Black Cherry", which performed best in the sensory tests in 2015 and 2016 (**Tables 3**, **4**). "Black Cherry" is known as a traditional or "heirloom" type and is usually appreciated for its distinct aroma, especially compared to modern cultivars (21, 61). The high consumer acceptance of "heirloom" tomatoes is due to their excellent fruit quality in terms of TSS, TA, TSS/TA ratio, and sensory properties in terms of sweetness, sourness, and tomato-like taste (62). On the other hand, the increasing demand for "heirloom" cultivars is often associated with a better flavor, offering a "taste of the past" that modern cultivars lack (63). "Black Cherry" not only performed best in terms of aroma but also featured a unique red-deep brown fruit color. Barry and Pandey (64) suggested that the red-brown color of a tomato fruit may be the result of the retention of chlorophyll and the simultaneous accumulation of lycopene during fruit ripening. It was also interesting that the two sensory tests showed a consistent result for all attributes studied. Across all tested cultivars, "Supersweet 100 F_1", "Sakura F_1", and "Black Cherry" were characterized by very satisfactory overall performance and were superior to other cultivars in terms of sensory quality.

Correlation Between Traits

Correlations between all traits were calculated to identify relationships between these parameters (**Figure 2** and **Table 5**). No general trend that indicated a superiority of older cultivars was observed, although senior consumers often believe that tomatoes "tasted better in the past." The cultivars used in this study derived from largely diverse backgrounds, including breeding for intensive indoor production to low-input organic management. Particularly, growers using low-input systems frequently concentrate on high organoleptic quality. This may be the reason why important sensory and other traits do not depend on the age of a cultivar.

Although hexanal had the highest concentration in the cultivars, it did not show a significant correlation with other traits, confirming that the compounds with high abundance do not always necessarily characterize the fruit aroma (65). However, Cebolla-Cornejo et al. (52) pointed out that hexanal is one of most important VOC contributing to tomato aroma. The Pearson correlation coefficient was used to infer which VOCs contribute to each sensory attribute (**Table 5**). Although several compounds have been shown to contribute to other instrumental parameters and sensory traits, our results highlight that few VOCs, including (*E*)-2-hexenal, phenylethyl alcohol, and benzyl alcohol, are important flavor components. Wang and Seymour (21) reported a lower concentration of (*E*)-2-hexenal in tomato, which they consider as a reason of poor aroma in modern tomato cultivars and which is confirmed by the present study. Colantonio et al. (39) found that fatty-acid-derived VOCs [e.g., (*E*)-2-hexenal and (*Z*)-3-hexen-1-ol] and 2-phenylethanol had positive contribution to sweetness perception of the tomato, which was also observed in our study. Also, an apparent negative correlation was found between geranial and TA, and geranial and sourness, but not with overall acceptability. Geranial was reported as one of the volatile compounds that contribute to perceived sweetness independent of sugar concentration and, therefore, strongly associated with tomato flavor intensity (19). This suggests that consumer acceptance of tomatoes could be improved by selecting for optimal concentrations of these VOCs in the fruit. Despite their high relevance as a flavor compound, acids were reported to be less important than sugars in improving overall liking in tomato (39) and strawberry (47).

The compound 2-isobutylthiazole correlated negatively with sweetness (**Table 5**), which was also shown by Vogel et al. (17), who found that increasing levels of 2-isobutylthiazole correlated with TSS content and lower perception of sweetness. The concentration of 2-isobutylthiazole was lower in the cocktail cultivars than in the salad cultivars. Our result is in line with that of Ursem et al. (66), who found a lower relative content of 2-isobutylthiazole in cherry tomatoes compared to cultivars with higher fruit weight (i.e., beef and round tomatoes). In tomato homogenate, 2-isobutylthiazole was reported as bitter/pungent (67, 68). Although 6-methyl-5-hepten-2-one had the highest concentration after hexanal (**Tables 1**, **2**), a Pearson correlation analysis showed no statistical correlation with sensory attributes. The lack of this correlation in the present study is interesting, as this VOC has typically been associated with fruit-like flavor and overall acceptability (18, 69). Also, negative correlations were found between 6-me-5-hepten-2-ol and all sensory attributes, except fruit firmness. This result was in contradiction with the results from another study (70) that identified 6-me-5-hepten-2-ol as corresponding aromatic alcohol of 6-me-5-hepten-2-one, which was detected only in overripe tomatoes. It was suggested that these two compounds were

carotenoid-related VOCs, whose accumulation in the tomato fruit was indirectly affected by ethylene (71). Although 6-methyl-5-hepten-2-one had the highest concentration after hexanal (**Tables 1** and **2**), a Pearson correlation analysis showed no statistical correlation with sensory attributes. The lack of this correlation in the present study is interesting, as this VOC has typically been associated with fruit-like flavor and overall acceptability (18, 69). Also, negative correlations were found between 6-me-5-hepten-2-ol and all sensory attributes, except fruit firmness. This result was in contradiction with the results from another study (70) that identified 6-me-5-hepten-2-ol as corresponding aromatic alcohol of 6-me-5-hepten-2-one, which was detected only in overripe tomatoes. It was suggested that these two compounds were carotenoid-related VOCs, whose accumulation in the tomato fruit was indirectly affected by ethylene (71). Thus, lower levels of 2-isobutylthiazole and 6-me-5-hepten-2-ol could lead to higher sensory scores, especially for sweetness, sourness, and the aroma, as well as to higher acceptance of the cultivars as found in both sensory tests. Phenylethyl alcohol, reported in a previous study as the most important metabolite to distinguish between cherry and non-cherry cultivars (66), was positively correlated with TSS content, sweetness, aroma, and overall acceptance. Benzyl alcohol was described as an important flavor component that has been shown to contribute to overall liking and flavor intensity of the tomato (39). The lack of high correlation coefficients between VOCs and sensory attributes can be partly due to complex synergistic and antagonistic interaction dynamics of chemical constituents with human receptors, which can alter the level to which individual chemicals are detectable during sensory assessment, especially in complex food crops, such as tomatoes (72).

Pearson correlation analysis of the VOC concentrations across all cultivars revealed that some correlations between metabolites were supported by the biochemical pathway from which they originated. For example, there were strong associations between the apocarotenoid-derived (β-cyclocitral and β-ionone) and the phenylalanine-derived VOCs (phenylethyl alcohol and benzyl alcohol). Apocarotenoid-derived VOCs are synthesized by the oxidative cleavage of carbon–carbon double bonds in carotenoids (73), and their contents increase substantially during the conversion of chloroplasts into chromoplasts (74). They were, in addition, described as fruity and/or floral and are commonly not abundant in tomato fruits but have very low-odor thresholds that allow humans to perceive their odor characteristics (75). Positive correlation between phenylethyl alcohol and benzyl alcohol, which were assigned to phenylpropanoids metabolic pathways originated from phenylalanine (76), was confirmed in our study. Phenylalanine-derived VOCs were expected to contribute to the increase of the perception of sweet taste and overall liking (65). Colantonio (39) highlighted the important role of VOCs as part of the fruit flavor profile in the perception of sweetness, and, therefore, not only sugar acid parameters but also selected VOCs should be included in breeding programs.

Although Vogel et al. (17) (showed a positive relationship between β-cyclocitral and β-ionone and taste-related traits and acceptability, no correlation was found between the accumulation of these VOCs and cultivar acceptability, neither in the breeder's nor in the sensory panel tests. These contradictory results may be a result of the particular sets of tomato genotypes that were analyzed.

The apparent positive correlation of yield and intensity index with fruit firmness (r = 0.53 and r = 0.49) and negative correlation with skin firmness may be biased by the assessment. This implied that fruits of a high yielding-cultivar were firmer than those of lower-yielding cultivars, but not for the skin. The results could be biased by the assessment, as a similar correlation trend was not confirmed for the skin firmness. While the firmness of the fruit (i.e., the pericarp) can be well distinguished between the tester's teeth in the sensory assessment, the skin (i.e., the epidermis) breaks more easily when supported by a firm pericarp and may, therefore, appear to be less firm.

Principal Component Analysis Between Instrumental and Sensory Data

The location of the sensory attributes in the loadings plot (**Figure 2**) shows very close correlations between the attributes sweetness, aroma, and acceptability, while the attribute fruit firmness was located in the opposite quadrant of the plot. The results of PCA are consistent with the Pearson correlation analysis (**Supplementary Table 6**). The acceptability of the cultivars is positively correlated with the sensory attributes sweetness (r = 0.94) and aroma (r = 0.93). Fruit firmness is negatively correlated (r = 0.34), while the other sensory attributes, such as sensory sourness, juiciness, and skin firmness, have a lower influence on the formation of the acceptability (r values of 0.76, 0.72, and 0.53, respectively).

Among the identified VOCs, 2-isobutylthiazole, 6-methyl-5-hepten-2-ol, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, 2-phenylethyl alcohol, and benzyl alcohol can be considered as the most authentic volatile marker compounds for the cultivars, as they have high loading scores in the PC1 (**Supplementary Table 9**). A high loading score of 2-phenylethyl alcohol, which was measured in 6- to 13-fold higher concentrations in small-fruited tomato cultivars than in large-fruited cultivars (66), might indicate the contribution of this VOC to tomato aroma (16, 20). "Auriga" and "Green Zebra", which differed from other cultivars by a higher loading score for sourness and color components (i.e., L*, b*, and h), were described by Chea et al. (32) as cultivars with high Mg concentrations in both leaves and fruits, which, in turn, correlated positively with TA content in fruits. Among the important VOCs in our study, three compounds correlated positively [(*E*)-2-hexenal, phenylethyl alcohol, and benzyl alcohol)] and two negatively (2-isobutylthiazole and 6-me-5-hepten-2-ol), with the acceptability of the cultivars (**Table 5**). These VOCs distinguish the cocktail cultivars ("Supersweet 100 F_1" and "Goldita") from the salad cultivars ("Roterno F_1", "Lyterno F_1", "Bocati F_1", and "Cappricia F_1"). Overall, the results of the PCA suggest that the magnitude of the effect of each individual VOC is much smaller than the individual effect of other instrumental traits (especially TSS and

TA) on the sample separation, highlighting the complexity of breeding for tomato flavor.

CONCLUSION

Cultivar was the most important factor influencing the concentration of all measured instrumental and sensory traits. Results from the present work indicate that the main cultivar effect on VOCs and other traits was generally stronger than the cultivar and environmental condition (C × H × Y interaction) effect. The C × H × Y interaction had a significant effect on most color components, but not on TSS and TA. We observed 31 significant correlations between individual VOC and sensory attributes in tomato cultivars. The VOCs (*E*)-2-hexenal and phenylethyl alcohol were positively associated with sensory scores of sweetness, aroma, and acceptability. On the other hand, 2-isobutylthiazole and 6-methyl-5-hepten-2-ol were negatively correlated with TSS and the sensory parameters sweetness, aroma, and acceptability. This information may allow determining aroma compound composition distinctive of both within and between the cultivar groups, the diversity of the VOCs, as well as to identify cultivars with enhanced levels of the targeted traits. As a result of this study, organic breeders should use cultivars from a wide range of breeding programs to improve important quality and agronomic traits. As examples, salad tomatoes "Campari F_1", "Green Zebra", and "Auriga" and cocktail tomatoes "Supersweet 100 F_1", "Sakura F_1", and "Black Cherry" showed important organoleptic attributes under organic low-input growing conditions. It remains a challenge for breeders and growers to reduce the trade-off of yield and quality.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

CE, BH, and EP planned and designed the experimental setup. CE performed the experiments and analyzed the data. DU supervised the VOCs measurements and data analysis. CE, DU, MN, IS, BH, and EP wrote the manuscript. All authors read and approved the manuscript.

FUNDING

CE received a scholarship from the Ministry of Research, Technology and Higher Education of the Republic of Indonesia (RISTEK-DIKTI).

ACKNOWLEDGMENTS

We thank Janna Henrike Groeneveld for her contribution on-farm and Bettina Egger, Gunda Jansen, and Evelyn Krüger from the Division Quality of Plant Products for technical assistance. We greatly appreciate the efforts of the technical staff as well in Julius Kühn-Institute for helping with the analyses of VOCs. We thank the Ministry of Research, Technology and Higher Education of the Republic of Indonesia (RISTEK-DIKTI) for the financial support of CE. We are grateful to Barbara Wedemeyer-Kremer for her technical assistance during the field experiment. The Software AG Foundation supported the Section of Genetic Resources and Organic Plant Breeding. We acknowledge support by the Open Access Publication Funds of the Göttingen University.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.916642/full#supplementary-material

REFERENCES

1. Cortina PR, Santiago AN, Sance MM, Peralta IE, Carrari F, Asis R. Neuronal network analyses reveal novel associations between volatile organic compounds and sensory properties of tomato fruits. *Metabolomics.* (2018) 14:57. doi: 10.1007/s11306-018-1355-7
2. Faostat. *FAOSTAT [Internet]*. (2022). Available online at: https://www.fao.org/faostat/en/#data/FBSH (accessed December 9, 2021)
3. Uluisik S, Chapman NH, Smith R, Poole M, Adams G, Gillis RB, et al. Genetic improvement of tomato by targeted control of fruit softening. *Nat Biotechnol.* (2016) 34:950–2. doi: 10.1038/nbt.3602
4. Blanca J, Montero-Pau J, Sauvage C, Bauchet G, Illa E, Díez MJ, et al. Genomic variation in tomato, from wild ancestors to contemporary breeding accessions. *BMC Genomics.* (2015) 16:257. doi: 10.1186/s12864-015-1444-1
5. Lin T, Zhu G, Zhang J, Xu X, Yu Q, Zheng Z, et al. Genomic analyses provide insights into the history of tomato breeding. *Nat Genet.* (2014) 46:1220–6. doi: 10.1038/ng.3117
6. Saliba-Colombani V, Causse M, Langlois D, Philouze J, Buret M. Genetic analysis of organoleptic quality in fresh market tomato. 1. Mapping QTLs for physical and chemical traits. *Theor Appl Genet.* (2001) 102:259–72. doi: 10.1007/s001220051643
7. Klee HJ, Tieman DM. Genetic challenges of flavor improvement in tomato. *Trends Genet.* (2013) 29:257–62. doi: 10.1016/j.tig.2012.12.003
8. Paolo D, Bianchi G, Scalzo RL, Morelli CF, Rabuffetti M, Speranza G. The chemistry behind tomato quality. *Nat Prod Commun.* (2018) 13:1225–32. doi: 10.1177/1934578X1801300927
9. Baldina S, Picarella ME, Troise AD, Pucci A, Ruggieri V, Ferracane R, et al. Metabolite profiling of Italian tomato landraces with different fruit types. *Front Plant Sci.* (2016) 7:664. doi: 10.3389/fpls.2016.00664
10. Causse M, Buret M, Robini K, Verschave P. Inheritance of nutritional and sensory quality traits in fresh market tomato and relation to consumer preferences. *J Food Sci.* (2003) 68:2342–50. doi: 10.1111/j.1365-2621.2003.tb05770.x
11. Ghorbani R, Poozesh V, Khorramdel S. Tomato production for human health, not only for food. In: Lichtfouse E editor. *Organic Fertilisation, Soil Quality and Human Health*. Dordrecht: Springer (2012). p. 187–225. doi: 10.1007/978-94-007-4113-3_8

12. Ilahy R, Tlili I, Siddiqui MW, Hdider C, Lenucci MS. Inside and beyond color: comparative overview of functional quality of tomato and watermelon fruits. *Front Plant Sci.* (2019) 10:769. doi: 10.3389/fpls.2019.00769
13. Kimbara J, Ohyama A, Chikano H, Ito H, Hosoi K, Negoro S, et al. QTL mapping of fruit nutritional and flavor components in tomato (*Solanum lycopersicum*) using genome-wide SSR markers and recombinant inbred lines (RILs) from an intra-specific cross. *Euphytica.* (2018) 214:2–12. doi: 10.1007/s10681-018-2295-z
14. Klee HJ. Improving the flavor of fresh fruits: genomics, biochemistry, and biotechnology: Tansley review. *New Phytol.* (2010) 187:44–56. doi: 10.1111/j.1469-8137.2010.03281.x
15. Araujo J, Telhado S. Organic food: a comparative study of the effect of tomato cultivars and cultivation conditions on the physico-chemical properties. *Foods.* (2015) 4:263–70. doi: 10.3390/foods4030263
16. Rambla JL, Tikunov YM, Monforte AJ, Bovy AG, Granell A. The expanded tomato fruit volatile landscape. *J Exp Bot.* (2013) 65:4613–23. doi: 10.1093/jxb/eru128
17. Vogel JT, Tieman DM, Sims CA, Odabasi AZ, Clark DG, Klee HJ. Carotenoid content impacts flavor acceptability in tomato (*Solanum lycopersicum*). *J Sci Food Agric.* (2010) 90:2233–40. doi: 10.1002/jsfa.4076
18. Baldwin EA, Scott JW, Shewmaker CK, Schuch W. Flavor trivia and tomato aroma: biochemistry and possible mechanisms for control of important aroma components. *HortScience.* (2000) 35:1013–22. doi: 10.21273/HORTSCI.35.6.1013
19. Tieman D, Bliss P, McIntyre LM, Blandon-Ubeda A, Bies D, Odabasi AZ, et al. The chemical interactions underlying tomato flavor preferences. *Curr Biol.* (2012) 22:1035–9. doi: 10.1016/j.cub.2012.04.016
20. Wang L, Baldwin EA, Bai J. Recent advance in aromatic volatile research in tomato fruit: the metabolisms and regulations. *Food Bioprocess Technol.* (2015) 9:203–16. doi: 10.1007/s11947-015-1638-1
21. Wang D, Seymour GB. Tomato flavor: lost and found? *Mol Plant.* (2017) 10:782–4. doi: 10.1016/j.molp.2017.04.010
22. Liu T, Zhu W, Huang J, Chen H, Nie R, Li CM. Comparison of the nutritional as well as the volatile composition of in-season and off-season Hezuo 903 tomato at red stage. *Eur Food Res Technol.* (2017) 243:203–14. doi: 10.1007/s00217-016-2736-7
23. Tikunov YM, Roohanitaziani R, Meijer-Dekens F, Molthoff J, Paulo J, Finkers R, et al. The genetic and functional analysis of flavor in commercial tomato: the FLORAL4 gene underlies a QTL for floral aroma volatiles in tomato fruit. *Plant J.* (2020) 103:1189–204. doi: 10.1111/tpj.14795
24. Daoud B, Naumann M, Ulrich D, Pawelzik E, Smit I. Assessment of sensory profile and instrumental analyzed attributes influenced by different potassium fertilization levels in three tomato cultivars. *J Appl Bot Food Qual.* (2021) 94:182–91.
25. Causse M, Friguet C, Coiret C, Lépicier M, Navez B, Lee M, et al. Consumer preferences for fresh tomato at the european scale: a common segmentation on taste and firmness. *J Food Sci.* (2010) 75:S531–41. doi: 10.1111/j.1750-3841.2010.01841.x
26. Waldron KW, Parker ML, Smith AC. Plant cell walls and food quality. *Comp Rev Food Sci Food Safety.* (2003) 2:128–46. doi: 10.1111/j.1541-4337.2003.tb00019.x
27. Olbricht K, Grafe C, Weiss K, Ulrich D. Inheritance of aroma compounds in a model population of *Fragaria* × *ananassa* duch. *Plant Breed.* (2007) 127:87–93. doi: 10.1111/j.1439-0523.2007.01422.x
28. Pesaresi P, Mizzotti C, Colombo M, Masiero S. Genetic regulation and structural changes during tomato fruit development and ripening. *Front Plant Sci.* (2014) 5:124. doi: 10.3389/fpls.2014.00124
29. El Hadi M, Zhang FJ, Wu FF, Zhou CH, Tao J. Advances in fruit aroma volatile research. *Molecules.* (2013) 18:8200–29. doi: 10.3390/molecules18078200
30. Casals J, Rivera A, Sabaté J, Romero del Castillo R, Simó J. Cherry and fresh market tomatoes: differences in chemical, morphological, and sensory traits and their implications for consumer acceptance. *J Agron.* (2018) 9:9. doi: 10.3390/agronomy9010009
31. Quinet M, Angosto T, Yuste-Lisbona FJ, Blanchard-Gros R, Bigot S, Martinez JP, et al. Tomato fruit development and metabolism. *Front Plant Sci.* (2019) 10:1554. doi: 10.3389/fpls.2019.01554
32. Chea L, Erika C, Naumann M, Smit I, Horneburg B, Pawelzik E. Morphological, leaf nutrient, and fruit quality characteristics of diverse tomato cultivars under organic low-input management. *Sustainability.* (2021) 13:12326. doi: 10.3390/su132112326
33. Erika C, Griebel S, Naumann M, Pawelzik E. Biodiversity in tomatoes: is it reflected in nutrient density and nutritional yields under organic outdoor production? *Front Plant Sci.* (2020) 11:589692. doi: 10.3389/fpls.2020.589692
34. Kanski L, Naumann M, Pawelzik E. Flavor-related quality attributes of ripe tomatoes are not significantly affected under two common household conditions. *Front Plant Sci.* (2020) 11:472. doi: 10.3389/fpls.2020.00472
35. Ulrich D, Olbricht K. Diversity of volatile patterns in sixteen *Fragaria vesca* L. accessions in comparison to cultivars of *Fragaria* × *ananassa*. *J Appl Bot Food Qual.* (2013) 86:295–302.
36. Hagenguth J, Kanski L, Kahle H, Naumann M, Pawelzik E, Becker HC, et al. Breeders' sensory test: a new tool for early selection in breeding for tomato (*Solanum lycopersicum*) flavour. *Plant Breed.* (2022) 141:96–107. doi: 10.1111/pbr.12994
37. ISO. *ISO 8586:2012: Sensory Analysis – General Guidelines for the Selection, Training and Monitoring of Selected Assessors and Expert Sensory Assessors; German Version EN ISO 8586, 2014*. Geneva: ISO (2012).
38. ISO. *ISO 8589:2007 + A1:2014: Sensory Analysis – General Guidance for the Design of Test Rooms; German Version EN ISO 8589:2010 + A1:2014*. Geneva: ISO (2007).
39. Colantonio V, Ferrão LFV, Tieman DM, Bliznyuk N, Sims C, Klee HJ, et al. Metabolomic selection for enhanced fruit flavor. *Proc Natl Acad Sci USA.* (2022) 119:e2115865119. doi: 10.1073/pnas.2115865119
40. Wang L, Bai J, Yu ZF. Difference in volatile profile between pericarp tissue and locular gel in tomato fruit. *J Integr Agric.* (2016) 15:2911–20. doi: 10.3390/molecules24142594
41. Selli S, Kelebek H, Ayseli MT, Tokbas H. Characterization of the most aroma-active compounds in cherry tomato by application of the aroma extract dilution analysis. *Food Chem.* (2014) 165:540–6. doi: 10.1016/j.foodchem.2014.05.147
42. Buttery RG, Teranishi R, Ling LC. Fresh tomato aroma volatiles: a quantitative study. *J Agric Food Chem.* (1987) 35:540–4. doi: 10.1021/jf00076a025
43. Buttery RG, Teranishi R, Flath RA, Ling LC. Fresh tomato volatiles: composition and sensory studies. In: Teranishi R, Buttery RG, Shahidi F editors. *Flavor Chemistry [Internet]*. Washington, DC: American Chemical Society (1989). p. 213–22. doi: 10.1021/bk-1989-0388.ch017
44. Maul F, Sargent SA, Sims CA, Baldwin EA, Balaban MO, Huber DJ. Tomato flavor and aroma quality as affected by storage temperature. *J Food Sci.* (2000) 65:1228–37. doi: 10.1111/j.1365-2621.2000.tb10270.x
45. Piombino P, Sinesio F, Moneta E, Cammareri M, Genovese A, Lisanti MT, et al. Investigating physicochemical, volatile and sensory parameters playing a positive or a negative role on tomato liking. *Food Res Int.* (2013) 50:409–19. doi: 10.1016/j.foodres.2012.10.033
46. Goulet C, Mageroy MH, Lam NB, Floystad A, Tieman DM, Klee HJ. Role of an esterase in flavor volatile variation within the tomato clade. *Proc Natl Acad Sci USA.* (2012) 109:19009–14. doi: 10.1073/pnas.1216515109
47. Fan Z, Hasing T, Johnson TS, Garner DM, Schwieterman ML, Barbey CR, et al. Strawberry sweetness and consumer preference are enhanced by specific volatile compounds. *Hortic Res.* (2021) 8:66. doi: 10.1038/s41438-021-00664-2
48. Panthee DR, Cao C, Debenport SJ, Rodríguez GR, Labate JA, Robertson LD, et al. Magnitude of genotype × environment interactions affecting tomato fruit quality. *HortScience.* (2012) 47:721–6. doi: 10.21273/HORTSCI.47.6.721
49. Kwabena Osei M, Annor B, Adjebeng- Danquah J, Danquah A, Danquah E, Blay E, et al. Genotype × environment interaction: a prerequisite for tomato variety development. In: Tatu Nyaku S, Danquah A editors. *Recent Advances in Tomato Breeding and Production [Internet]*. London: IntechOpen (2019). doi: 10.5772/intechopen.76011
50. Ssemakula G, Dixon A. Genotype X environment interaction, stability and agronomic performance of carotenoid-rich cassava clones. *Sci Res Essays.* (2007) 2:390–9.
51. Bauchet G, Causse M. Genetic diversity in tomato (*Solanum lycopersicum*) and its wild relatives. In: Caliskan M editor. *Genetic Diversity in Plants [Internet]*. London: InTech (2012). doi: 10.5772/33073
52. Cebolla-Cornejo J, Roselló S, Valcárcel M, Serrano E, Beltrán J, Nuez F. Evaluation of genotype and environment effects on taste and aroma flavor components of Spanish fresh tomato varieties. *J Agric Food Chem.* (2011) 59:2440–50. doi: 10.1021/jf1045427

53. Gautier H, Diakou-Verdin V, Bénard C, Reich M, Buret M, Bourgaud F, et al. How does tomato quality (sugar, acid, and nutritional quality) vary with ripening stage, temperature, and irradiance? *J Agric Food Chem.* (2008) 56:1241–50. doi: 10.1021/jf072196t
54. Baye TM, Abebe T, Wilke RA. Genotype–environment interactions and their translational implications. *Per Med.* (2011) 8:59–70. doi: 10.2217/pme.10.75
55. Sonntag F, Naumann M, Pawelzik E, Smit I. Improvement of cocktail tomato yield and consumer-oriented quality traits by potassium fertilization is driven by the cultivar. *J Sci Food Agric.* (2019) 99:3350–8. doi: 10.1002/jsfa.9552
56. Arias R, Lee TC, Logendra L, Janes H. Correlation of lycopene measured by HPLC with the L*, a*, b* color readings of a hydroponic tomato and the relationship of maturity with color and lycopene content. *J Agric Food Chem.* (2000) 48:1697–702. doi: 10.1021/jf990974e
57. Lewinsohn E, Sitrit Y, Bar E, Azulay Y, Meir A, Zamir D, et al. Carotenoid pigmentation affects the volatile composition of tomato and watermelon fruits, as revealed by comparative genetic analyses. *J Agric Food Chem.* (2005) 53:3142–8. doi: 10.1021/jf047927t
58. Zörb C, Piepho HP, Zikeli S, Horneburg B. Heritability and variability of quality parameters of tomatoes in outdoor production. *Research.* (2020) 2020:6707529. doi: 10.34133/2020/6707529
59. Oltman AE, Jervis SM, Drake MA. Consumer attitudes and preferences for fresh market tomatoes. *J Food Sci.* (2014) 79:S2091–7. doi: 10.1111/1750-3841.12638
60. Pagliarini E, Monteleone E, Ratti S. Sensory profile of eight tomato cultivars (lycopersicon esculentum) and its relationship to consumer preference. *Ital J Food Sci.* (2001) 13:285–96.
61. Bai Y, Lindhout P. Domestication and breeding of tomatoes: what have we gained and what can we gain in the future? *Ann Bot.* (2007) 100:1085–94. doi: 10.1093/aob/mcm150
62. Gioia FD, Serio F, Buttaro D, Ayala O, Santamaria P. Influence of rootstock on vegetative growth, fruit yield and quality in 'Cuore di Bue', an heirloom tomato. *J Hortic Sci Biotechnol.* (2010) 85:477–82. doi: 10.1080/14620316.2010.11512701
63. Brugarolas M, Martínez-Carrasco L, Martinez-Poveda A, Ruiz J. A competitive strategy for vegetable products: traditional varieties of tomato in the local market. *Span J Agric Res.* (2009) 7:294–304. doi: 10.5424/sjar/2009072-420
64. Barry CS, Pandey P. A survey of cultivated heirloom tomato varieties identifies four new mutant alleles at the green-flesh locus. *Mol Breed.* (2009) 24:269–76. doi: 10.1007/s11032-009-9289-4
65. Lubes G, Goodarzi M. Analysis of volatile compounds by advanced analytical techniques and multivariate chemometrics. *Chem Rev.* (2017) 117:6399–422. doi: 10.1021/acs.chemrev.6b00698
66. Ursem R, Tikunov Y, Bovy A, van Berloo R, van Eeuwijk F. A correlation network approach to metabolic data analysis for tomato fruits. *Euphytica.* (2008) 161:181. doi: 10.1007/s10681-008-9672-y
67. Tandon KS, Baldwin EA, Shewfelt RL. Aroma perception of individual volatile compounds in fresh tomatoes (Lycopersicon esculentum, Mill.) as affected by the medium of evaluation. *Postharvest Biol Technol.* (2000) 20:261–8. doi: 10.1016/S0925-5214(00)00143-5
68. Xu Y, Barringer S. Comparison of tomatillo and tomato volatile compounds in the headspace by selected ion flow tube mass spectrometry (SIFT-MS). *J Food Sci.* (2010) 75:C268–73. doi: 10.1111/j.1750-3841.2010.01537.x
69. Krumbein A, Peters P, Brückner B. Flavour compounds and a quantitative descriptive analysis of tomatoes (Lycopersicon esculentum Mill.) of different cultivars in short-term storage. *Postharvest Biol Technol.* (2004) 32:15–28. doi: 10.1016/j.postharvbio.2003.10.004
70. Güler Z, Emre Y. Distribution of volatile compounds in organic tomato (Lycopersicon esculentum) at different ripening stages. *Acad Food J.* (2013) 11:6–13. doi: 10.1021/jf3028528
71. Gao H, Zhu H, Shao Y, Chen A, Lu C, Zhu B, et al. Lycopene accumulation affects the biosynthesis of some carotenoid-related volatiles independent of ethylene in tomato. *J Integr Plant Biol.* (2008) 50:991–6. doi: 10.1111/j.1744-7909.2008.00685.x
72. Tieman D, Zhu G, Resende MFR, Lin T, Nguyen C, Bies D, et al. A chemical genetic roadmap to improved tomato flavor. *Science.* (2017) 355:391–4. doi: 10.1126/science.aal1556
73. Ahrazem O, Gómez-Gómez L, Rodrigo MJ, Avalos J, Limón MC. Carotenoid cleavage oxygenases from microbes and photosynthetic organisms: features and functions. *Int J Mol Sci.* (2016) 17:1–38. doi: 10.3390/ijms1711 1781
74. Tieman DM, Zeigler M, Schmelz EA, Taylor MG, Bliss P, Kirst M, et al. Identification of loci affecting flavour volatile emissions in tomato fruits. *J Exp Bot.* (2006) 57:887–96. doi: 10.1093/jxb/erj074
75. Kreissl J, Schieberle P. Characterization of aroma-active compounds in Italian tomatoes with emphasis on new odorants. *J Agric Food Chem.* (2017) 65:5198–208. doi: 10.1021/acs.jafc.7b01108
76. Dudareva N, Klempien A, Muhlemann JK, Kaplan I. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytol.* (2013) 198:16–32. doi: 10.1111/nph.12145

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Erika, Ulrich, Naumann, Smit, Horneburg and Pawelzik. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 25 July 2022
DOI 10.3389/fnut.2022.906227

Check for updates

OPEN ACCESS

EDITED BY
José Pinela,
Polytechnic Institute of Bragança (IPB), Portugal

REVIEWED BY
Yuquan Duan,
Chinese Academy of Agricultural Sciences, China
Han Xiao,
National Key Laboratory of Plant Molecular Genetics, Center for Excellence in Molecular Plant Sciences (CAS), China

*CORRESPONDENCE
Xiangbin Xu
xbxuibcas@126.com

SPECIALTY SECTION
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

RECEIVED 28 March 2022
ACCEPTED 20 June 2022
PUBLISHED 25 July 2022

CITATION
Zhao K, Chen R, Duan W, Meng L, Song H, Wang Q, Li J and Xu X (2022) Chilling injury of tomato fruit was alleviated under low-temperature storage by silencing Sly-miR171e with short tandem target mimic technology. *Front. Nutr.* 9:906227. doi: 10.3389/fnut.2022.906227

COPYRIGHT
© 2022 Zhao, Chen, Duan, Meng, Song, Wang, Li and Xu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Chilling injury of tomato fruit was alleviated under low-temperature storage by silencing Sly-miR171e with short tandem target mimic technology

Keyan Zhao[1], Rulong Chen[1], Wenhui Duan[1], Lanhuan Meng[1], Hongmiao Song[1], Qing Wang[2], Jiangkuo Li[3] and Xiangbin Xu[1,4]*

[1]School of Food Science and Engineering, Hainan University, Haikou, China, [2]Beijing Academy of Agriculture and Forestry Sciences, Beijing, China, [3]Tianjin Key Laboratory of Postharvest Physiology and Storage of Agricultural Products, National Engineering and Technology Research Center for Preservation of Agricultural Products, Tianjin, China, [4]Key Laboratory of Food Nutrition and Functional Food of Hainan Province, Haikou, China

In this study, the role of Sly-miR171e on post-harvest cold tolerance of tomato fruit was researched. The results showed that overexpression of Sly-miR171e (miR171e-OE) promoted postharvest chilling injury (CI) of tomato fruit at the mature red (MR) and mature green (MG) stage. Contrasted with the wild type (WT) and miR171e-OE fruit, the knockdown of Sly-miR171e (miR171e-STTM) showed a lower CI index, lower hydrogen peroxide (H_2O_2) content, and higher fruit firmness after harvest. In the fruit of miR171e-STTM, the expression level of *GRAS24*, *CBF1*, *GA2ox1*, and *COR*, and the GA3 content were ascended, while the expression levels of *GA20ox1* and *GA3ox1* were descended. The research demonstrated that CI in tomato fruit was alleviated at low temperature storage by silencing Sly-miR171e with short tandem target mimic (STTM) technology. Furthermore, it also provided helpful information for genetic modification of miR171e and control of CI in the postharvest fruit.

KEYWORDS

microRNA, tomato fruit, chilling injury, GRAS, GA

Introduction

Storage at low temperature is an effective method for controlling the quality of postharvest fruit, however, the chilling injury (CI) caused by low temperature often leads to a decline in fruit quality. The tomato fruit at the mature red (MR) and mature green (MG) stage is prone to CI when stored at temperature lower than 5 and 10°C, respectively. Tomato fruit stored at a low temperature usually shows symptoms like surface damage, rot, and tepbib1. In addition, low temperature affects the conformation and structure of the cell membranes of fruit, reduces consumer

acceptability, and finally leads to significant economic losses (2, 3). So, improving the cold tolerance of postharvest fruit is a research hotspot in genetics and breeding.

MicroRNA (miRNA), which comprises 18–25 nucleotides, is a kind of endogenous single-stranded RNA. It plays a significant role in plant growth and development, resistance to stress (low temperature, drought, and salt stress), and maintenance of genomic integrity (4–8). In *Arabidopsis thaliana*, northern blot analysis showed that the overexpression of miR397 affected the expression of the cold-regulated *CBF* and *COR* genes, enhanced the cold tolerance in plants, and indicated that miR397 was involved in cold resistance (4). The results of small RNA sequencing and expression analysis in cold-treated grapevine plants showed that miR395 was significantly up-regulated, indicating that miRNA might be involved in cold stress in plants (5). Under low-temperature storage, compared with WT fruit, silencing of miR164a reduced the CI index and H_2O_2 content of tomato and improved the cold tolerance of fruit, indicating that miRNA could respond to cold stress (6). The miR528 improved the cold resistance of the rice by curbing the expression level of *MYB30* and increasing ascorbate peroxidase (9). During low temperature storage, overexpression of miR319 in tomatoes reduced the electrolyte leakage and malondialdehyde (MDA) content and played a role in cold resistance (10). It was demonstrated that miR164 played a role in regulating NAC expression in strawberry fruit stored at low temperatures (7, 8).

miR171 is one of the conservative miRNA families in plants and participates in the development and stress responses by down-regulating the expression of GRAS family genes (9, 10). In *A. thaliana*, protein localization and yeast one-hybrid analysis showed that miR171c negatively regulated the gene family members of *GRAS*, *SCL6-II*, *SCL6-III*, and *SCL6-IV*, and caused the bud-reducing branching phenotype in mutant plants, indicated that miR171c plays a vital role in plant development (11). In the peach tree (*Prunus persica* L.), quantitative real-time PCR (qRT-PCR) results showed that miR171 was highly expressed under drought and salt stress, indicating that it played an essential role in resisting abiotic stress (12). High-throughput analysis results showed that miR171 was highly expressed in celery plants under drought, indicating that it was responsive to drought stress (13). GRAS is one of the transcriptional regulators induced in plants to adapt to cold stress. As an important family of plant-specific proteins, GRAS is usually present in the C-terminal part of the protein and plays important roles in plant development and abiotic stresses (14–16). In *A. thaliana*, the DELLA protein of the GRAS subfamily reduced hydrogen peroxide (H_2O_2) accumulation and enhanced stress resistance by increasing the expression of genes encoding active oxygen detoxification enzymes (ROS) (17). In tomato fruit, *SlGRAS4* showed a significant increase under low temperature stress, and the overexpression of *SlGRAS4* increased the cold tolerance (15, 18).

In tomatoes, *SlGRAS24* is the target gene of miR171 (15, 19, 20). So far, there was no report about the role of miR171e on the cold tolerance of fruit by negatively regulating the target gene of *GRAS*. In the present research, silence and overexpression of Sly-miR171e tomato fruit were gained, and the function of Sly-miR171e on low temperature stress in postharvest tomato fruit was explored. The results showed that silencing Sly-miR171e with a short tandem target mimic (STTM) in tomato fruit led to less CI at low temperature storage.

Materials and methods

The construction of plant vector

The STTM structure of miR171e was constructed according to the method of Yan et al. (21) (Supplementary Material 1). The miR171e overexpression vector was constructed by the method of Qin et al. (22) (Supplementary Material 2). The target fragment of miR171e was obtained from miRbase and transferred into pBWA (V) HS and pBI121 vectors containing CaMV35S promoter. The sequences of candidate vectors were identified and then the vectors were introduced into *Agrobacterium* GV3101. The primers that appeared in the experiment are listed in Supplementary Material 3.

Fruit and treatment

The tomato fruit (*Solanum lycopersicum cv.* Micro-Tom) at MR and MG stage without physical damage and infection was selected, put into the foam box, and then classified according to the size. The fruit at the MR stage was stored at 4°C, and the fruit at the MG stage was stored at 9°C. During the period of low temperature storage, three fruit were extracted from each group for 0, 5, 10, 15, 20, and 25 days, and the middle part of tomato fruit was selected for sampling.

H_2O_2 content and CI index

The CI index of tomato fruit was determined by the method of Zhao et al. (23), in which 0 = no pitting; 1 = the depression covers < 25% of the fruit surface; 2 = pitting coverage > 25% and < 50% of the surface; 3 = pitting coverage >50% and < 75% of the surface, 4 = pitting coverage > 75% Surface. Calculate using the following formula:

$$\text{CIindex} = \frac{\Sigma(\text{CI level}) \times (\text{number of fruit at the CI level})}{\text{total number of fruit}} \times 4.$$

The H_2O_2 content was measured by the analytical kit (Solarbio Inc Beijing, China), and the results of the test were expressed as μ mol kg^{-1} fresh weight (FW).

Electrolyte leakage and firmness of tomato fruit

The electrolyte leakage was measured according to Zhao et al. (24). The peel was separated using a 0.5 cm diameter separator, placed in the solution, and shaken on a shaker for 2 h, measuring the conductivity L1 using a conductivity meter (FE30), boiling for 10 min, cooling, and finally determining L2. Ion leakage was calculated as the ratio of L1 to L2.

The equatorial position in the fruit was measured three times with texture analysis (TA. XT Plus). The insertion speed was 50 mm s^{-1}, the insertion depth was 5 mm, and the results of the test were expressed as N.

The measure of GA3 content

The GA3 content was measured with the detection kit (Jiangsu Meibiao Biotechnology Co., Ltd.). The result was expressed as mg L^{-1}.

qRT-PCR analysis and RNA extraction

The total RNA of WT, miR171e-STTM, and miR171e-OE tomato fruit was extracted by using the plant RNA extraction kit (Tiangen Biotech Inc. Beijing, China), respectively. And then the concentration and quality of RNA were determined. The first-strand cDNA was synthesized using the FastKing RT kit (Tiangen Biotech Inc. Beijing, China), and the expression of Sly-miR171e, *GRAS24*, *GA3ox1*, *GA20ox1*, *GA2ox1*, *GAI*, *CBF1*, and *COR* was analyzed by the CFX Connect Real-Time System (Bio-Rad Laboratories, Inc. USA). The expression level was calculated using the formula: $2^{-\Delta\Delta Ct}$.

Statistical analysis

GraphPad Prism 9 (GraphPad, San Diego, CA, USA) and a two-way analysis of variance (ANOVA) were used for statistical analysis. $^{*}p < 0.05$ indicate significant differences in comparison with wild type (WT). All values were expressed as the average value ± standard deviation (SD).

Results

Characterization of MiR171e-STTM and MiR171e-OE fruit

The STTM and overexpression vectors were introduced into WT to obtain the miR171e-STTM and miR171e-OE lines through genetic transformation, which can express stably. In the first generation (T1), 23 STTM-171e lines and 17 miR171e-OE lines were tested (Supplementary Figures S1A,B). In the second generation (T2), 16 STTM-171e lines and 17 miR171e-OE lines were tested (Supplementary Figures S1C,D). The results of PCR amplification and agarose gel electrophoresis showed that both STTM and overexpression vectors had been inserted into the correct target sites (Supplementary Figure S1).

H_2O_2 content and CI index

Figures 1A,B showed that the fruit of miR171e-OE#7-1-3, miR171e-OE#8-3-2, and miR171e-OE#10-2-7 at the MR stage were shrunken at 15 d and showed obvious CI at 25 d. After 20 d at low temperature, CI appeared in WT fruit at the MR stage. However, the fruit of miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1 at the MR stage showed less cold damage after 25 d of storage. In addition, during low temperature storage of 4°C, the H_2O_2 content of the miR171e-STTM fruit was often less than that in WT (Figure 1C).

As shown in Figures 2A,B, after 20 days of storage, the tomato fruit of miR171e-OE#8-3-2 at the MG stage was shrunken and exhibited CI, and the miR171e-OE#10-2-7 line showed CI at 25 d. However, after being stored at 9°C for 30 d and the fruit was transferred to 25°C, the fruit of WT and miR171e-STTM at the MG stage showed no cold damage. The H_2O_2 content in miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1 fruit at MG stage was 0.82, 0.85 and 0.84 times less than that in WT at 25 days, respectively (Figure 2C).

Electrolyte leakage and firmness of tomato fruit

In contrast to WT fruit at the MR stage, the electrolyte leakage was reduced in the miR171e-STTM and was increased in miR171e-OE (Figure 3A). After 5 d of storage, the electrolyte leakage in fruit of miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, miR171e-STTM#8-2-1, miR171e-OE#7-1-3, miR171e-OE#8-3-2 and miR171e-OE#10-2-7 were 43, 42, 43, 56, 57, and 56%, respectively (Figure 3A). As shown in Figure 3A, after 25 days of storage, electrolyte leakage in WT fruit at the MR stage was 70%, which was 1.18, 1.2, and 1.18 times more than that in miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1, respectively. Contrary to the WT fruit at the MR stage, the firmness of miR171e-STTM fruit was increased, and the firmness of the miR171e-OE fruit was reduced. After storing for 25 days at low temperature, the firmness of WT fruit was 3.09 N, which was 1.15, 1.14, and 1.12 times more than that of miR171e-STTM#1-5-1,

10.3389/fnut.2022.906227

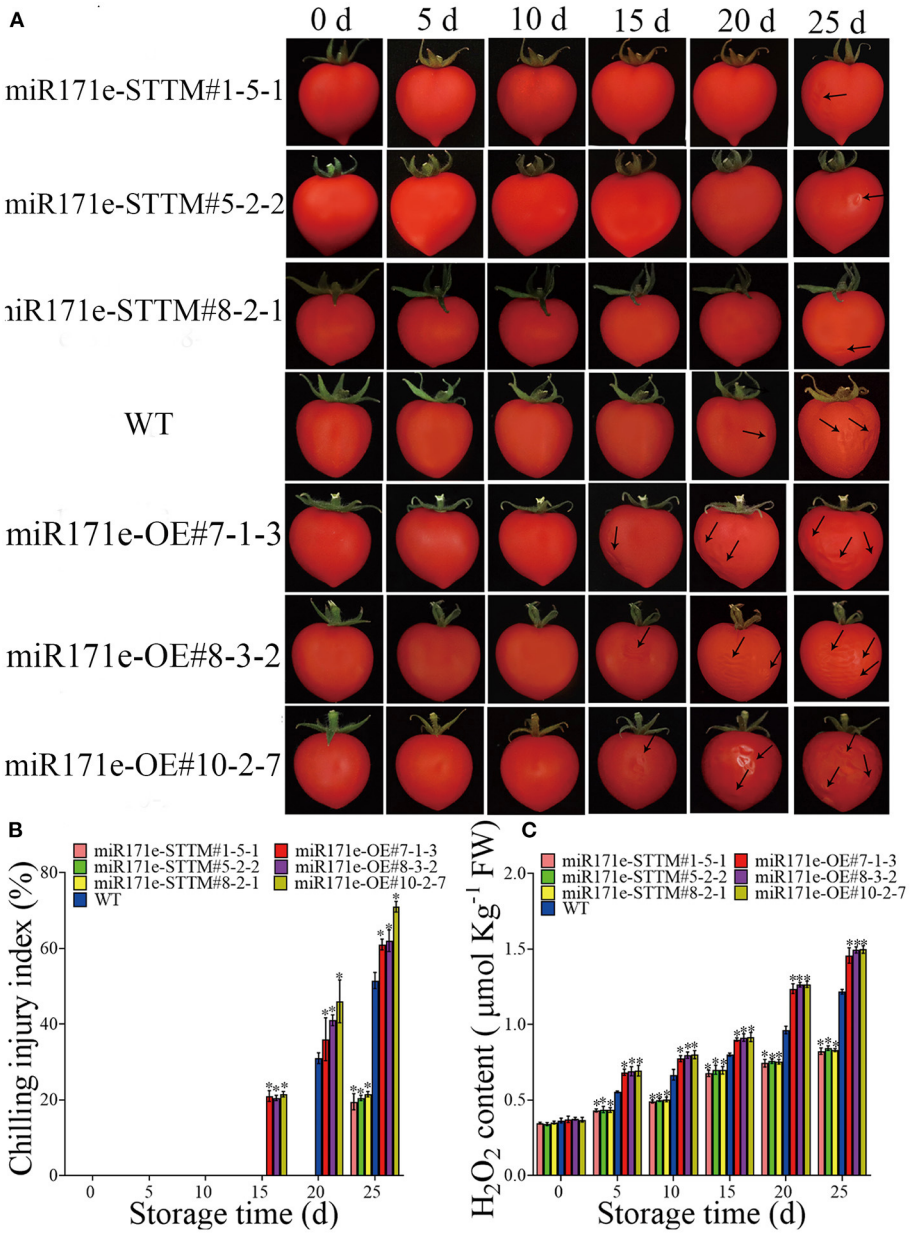


FIGURE 1
The CI and phenotypic characterization in tomato fruit at MR stage. **(A)** The H_2O_2 content of tomato fruit at MR stage. **(B)** The CI index of tomato fruit at MR stage. **(C)** Vertical bars represent standard deviations of the means, $n = 3$. Asterisks indicate the statistical difference in the significance at $^*p < 0.05$.

miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1, respectively (Figure 3A).

As shown in Figure 3B, in contrast with WT fruit, the electrolyte leakage in miR171e-STTM tomato fruit at the MG stage was reduced and increased in miR171e-OE fruit. After 5 days of storage, the electrolyte leakage of WT fruit was 41%, which was 1.17, 1.13, and 1.12 times more than that in miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1 of the tomato fruit at MG stage, respectively. The electrolyte leakage in WT fruit was 65%, which was 1.18, 1.16, and 1.16 times more than that in miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1 at 25 days, respectively. As shown in Figure 3B, after being stored for 0 to 25 days at low temperature, the firmness of miR171e-STTM fruit diminished from 10.69 to 6.14 N, which was more than that of WT fruit.

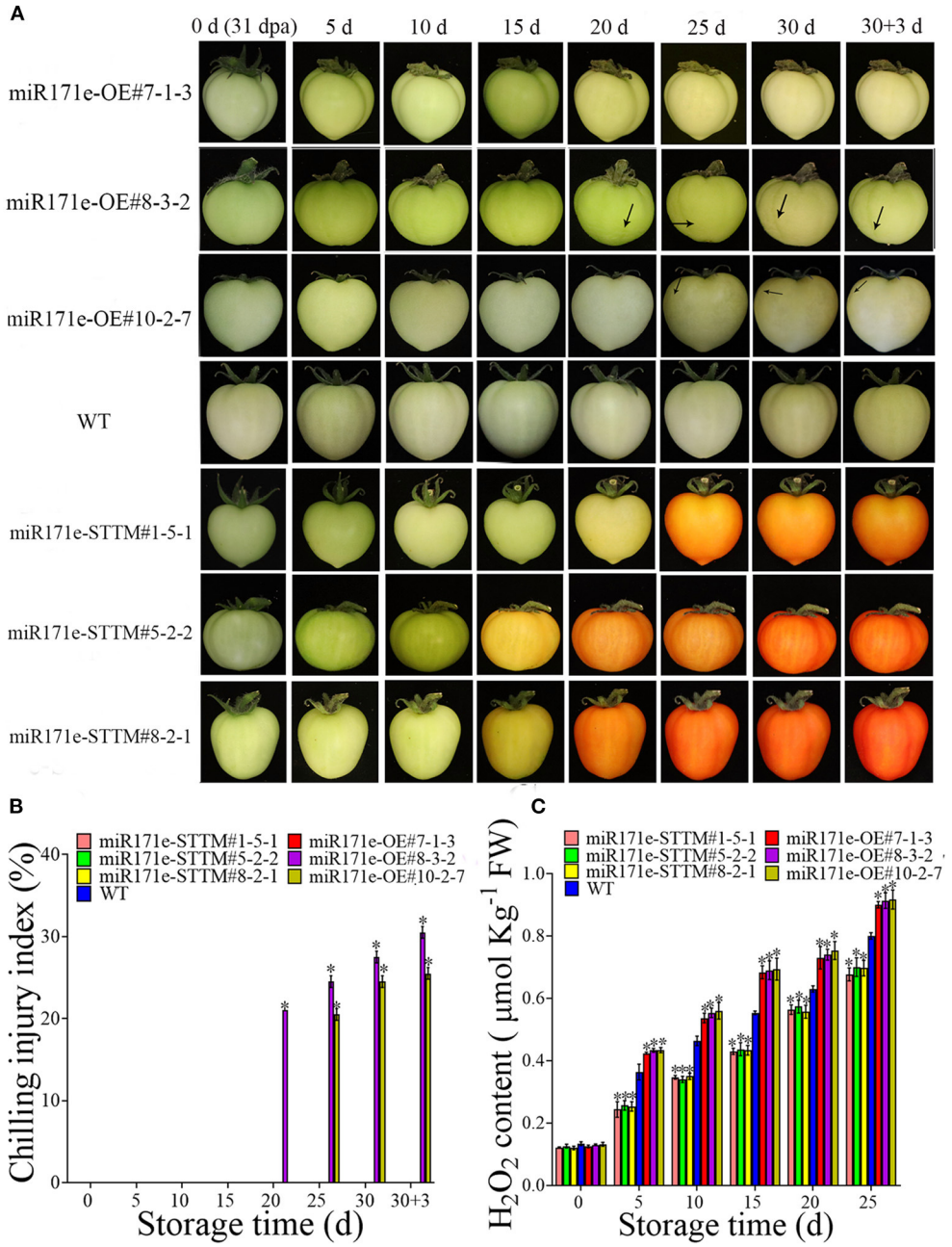


FIGURE 2
The CI and phenotypic characterization in tomato fruit at MG stage. **(A)** The H_2O_2 content of tomato fruit at MG stage. **(B)** The CI index of tomato fruit at MG stage. **(C)**. Vertical bars represent standard deviations of the means, $n = 3$. Asterisks indicate the statistical difference in the significance at $^*p < 0.05$.

The GA3 content and related genes expression

During storage from 0 to 25 days, contrary to the WT fruit at the MR stage, the GA3 content of the miR171e-STTM fruit was increase, and the GA3 content of the miR171e-OE fruit was reduced. The GA3 content was 0.0885 mg L^{-1} in WT fruit, which was 0.93, 0.9, and 0.93 times less than that in miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1 fruit at 25 days, respectively (Figure 4A). As shown in Figure 4A, the expression level of the DELLA protein gene (*GAI*) in miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and

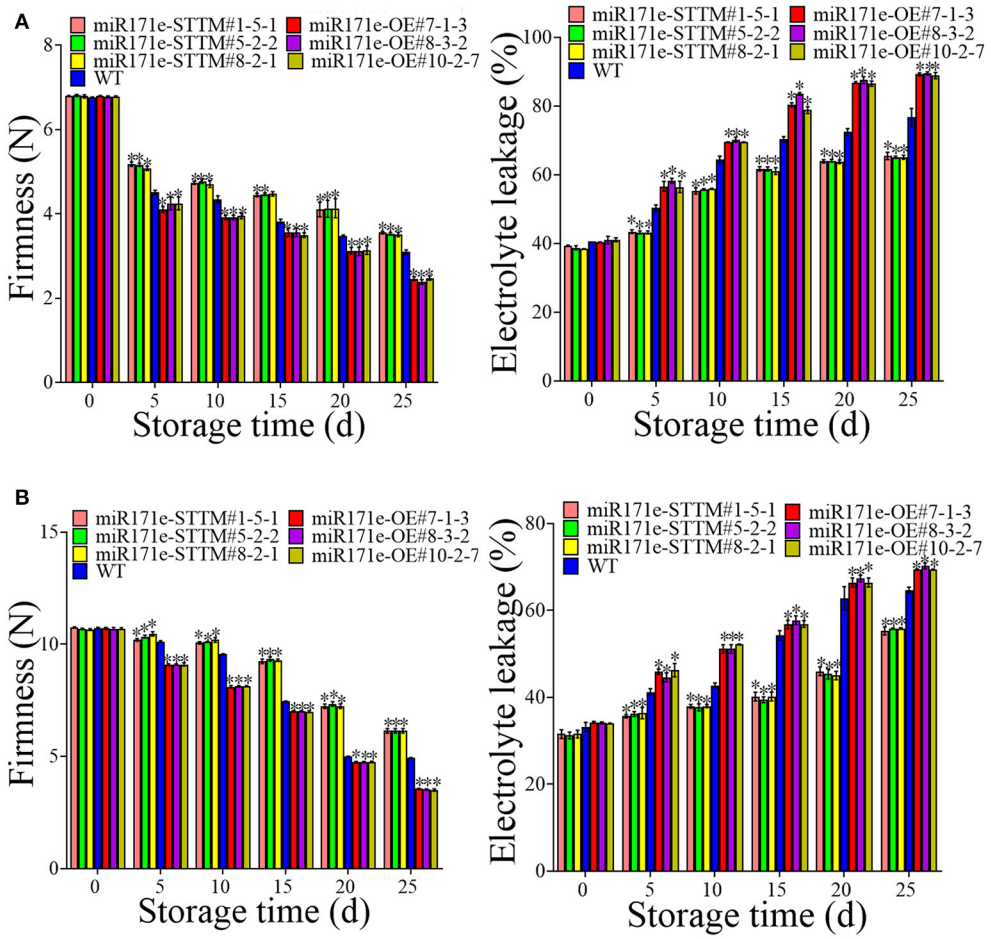


FIGURE 3
Electrolyte leakage and firmness in tomato fruit at MR **(A)** and MG stage **(B)**. Vertical bars represent standard deviations of the means, $n = 3$. Asterisks indicate the statistical difference in the significance at $^*p < 0.05$.

miR171e-STTM#8-2-1 fruit at MR stage was about 0.82, 0.87, and 0.83 times less than that in WT at 25 days. Moreover, the expression level of the critical GA synthesis genes *GA3ox1* and *GA20x1* in miR171e-STTM tomato fruit at the MR stage was about 0.78 and 0.61 times less than that in WT (Figure 4A). As shown in Figure 4A, at 25 days, the expression level of *GA2ox1*, which was the critical gene of GA metabolism of miR171e-STTM fruit at the MR stage, was about 2.06 times of WT fruit.

As shown in Figure 4B, contrary to the WT fruit at the MG stage, the GA3 content of the miR171e-STTM was increased, and the miR171e-OE was reduced. After 25 days of storage, the GA3 content in WT fruit was 0.077 mg L^{-1}, which was 0.76, 0.74, and 0.76 times less than that in miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1 fruit, respectively. The expression level of the *GAI* in miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1 tomato fruit at the MG stage was about 0.84, 0.85, and 0.85 times less than that in WT at 25 days. After 25 days at low temperature storage, the expression level of *GA3ox1* and *GA20x1* in the critical GA synthesis genes of miR171e-STTM fruit at the MG stage was about 0.70 and 0.56 times less than that in WT (Figure 4B), and the expression level of *GA2ox1* was about 1.97 times of WT (Figure 4B).

Expression level of MiR171e and *GRAS24*

As shown in Figure 5A, after 25 days of storage, the expression level of Sly-miR171e of miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1 fruit at MR stage was about 0.53, 0.27, and 0.14 times less than that in WT. Contrasted with WT fruit at the MR stage, the expression level of *GRAS24* in miR171e-STTM fruit was increased and decreased in the miR171e-OE fruit. After 25 days at low temperature, the expression of *GRAS24* in WT fruit was 1.94, which was 2.64, 2.7, and 2.27

10.3389/fnut.2022.906227

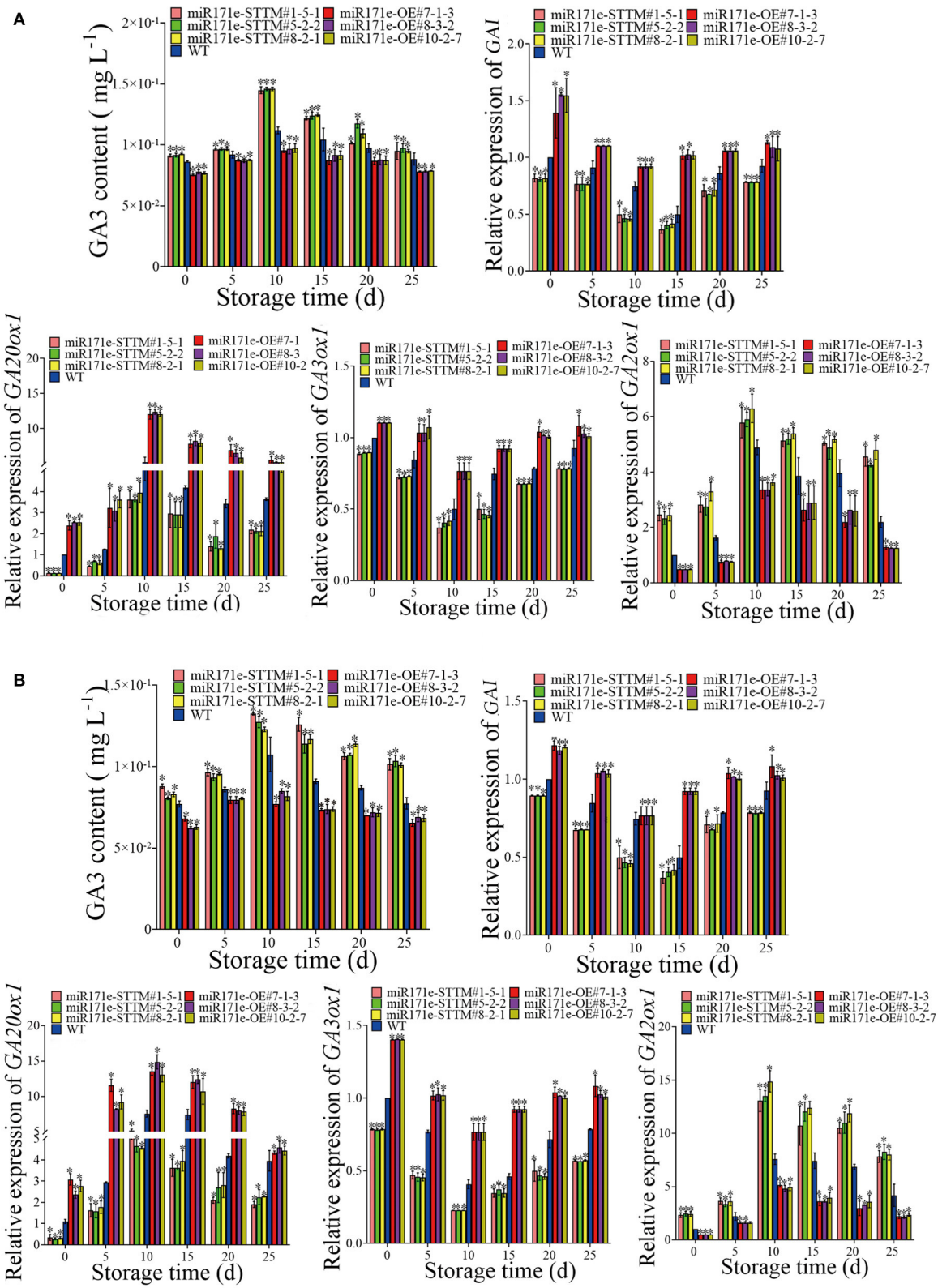


FIGURE 4
The GA3 content and the related gene expression in tomato fruit at MR **(A)** and MG stage **(B)**. Vertical bars represent standard deviations of the means, $n = 3$. Asterisks indicate the statistical difference in the significance at $^*p < 0.05$.

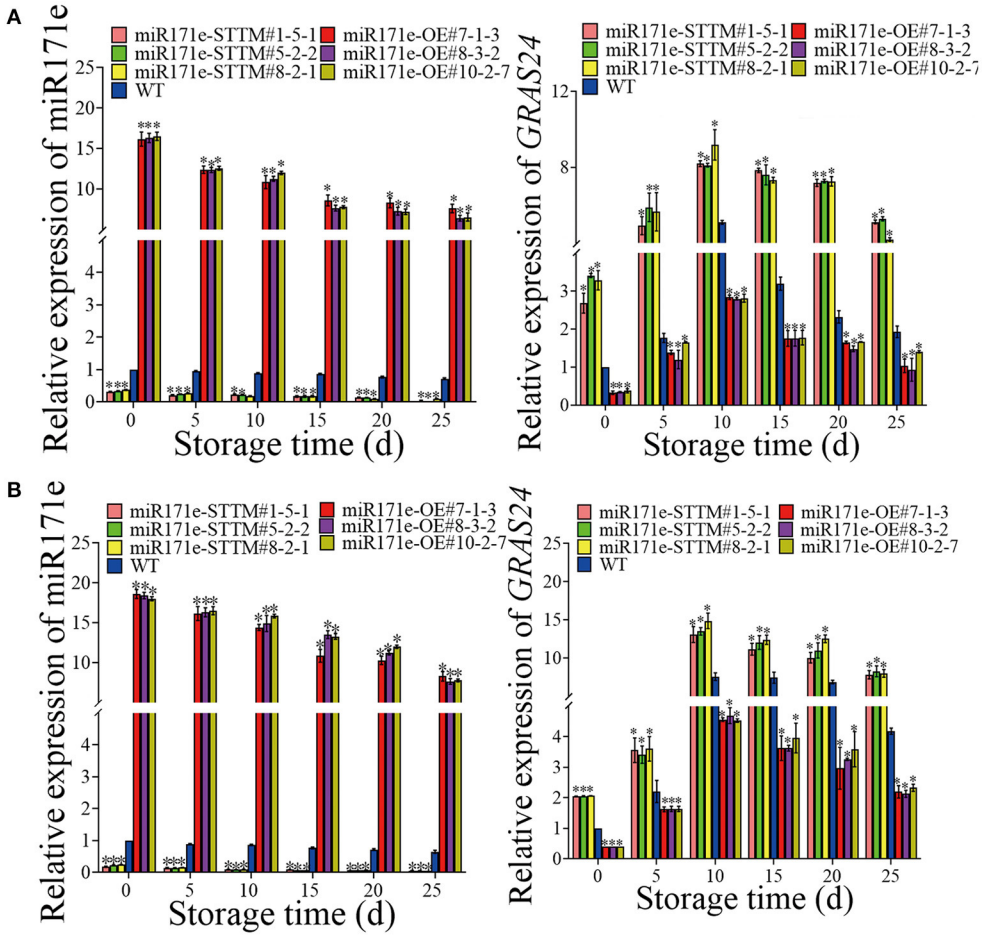


FIGURE 5
The relative expression of Sly-miR171e, *GRAS24* in tomato fruit at MR **(A)** and MG stage **(B)**. Vertical bars represent standard deviations of the means, $n = 3$. Asterisks indicate the statistical difference in the significance at $^*p < 0.05$.

times more than that in miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1 fruit, respectively (Figure 5A).

The expression level of Sly-miR171e in miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1 fruit at MG stage was about 0.15, 0.13 and 0.14 times less than that in WT at 25 d (Figure 5B). After 25 days of storage, the expression level of *GRAS24* in WT fruit was 4.18, which was 1.9, 2.0, and 2.0 times more than that in miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1 fruit, respectively (Figure 5B).

Expression level of *CBF1* and *COR*

As shown in Figure 6A, during storage from 0 to 25 d, contrasted with WT fruit at the MR stage, the expressions of *CBF1* and *COR* in the miR171e-STTM fruit were ascending and descending in the miR171e-OE fruit. After 25 d of storage, the expression level of *CBF1* of miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1 fruit was about 5.14, 4.85, and 4.85 times more than that in WT and the expression level of *COR* in miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1 fruit were about 1.46, 1.4, and 1.39 times more than that in WT (Figure 6A).

After 25 days of storage, the expression level of *CBF1* in miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1fruit at the MG stage was about 8.5, 8.5, and 8.6 times more than that in WT (Figure 6B), and the expression level of *COR* in miR171e-STTM#1-5-1, miR171e-STTM#5-2-2, and miR171e-STTM#8-2-1 fruit were about 2.29, 2.25, and 2.3 times more than that in WT (Figure 6B).

10.3389/fnut.2022.906227

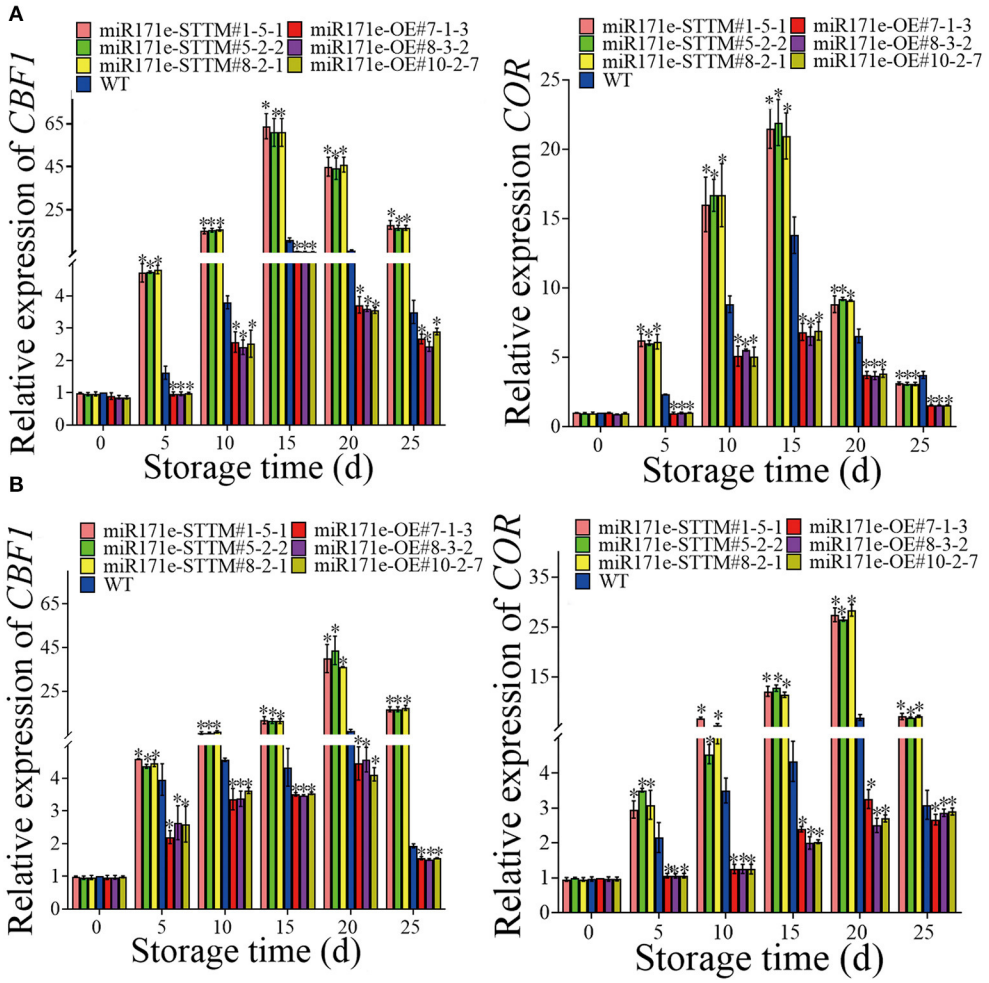


FIGURE 6
The relative expression of *CBF1* and *COR* in tomato fruit at MR **(A)** and MG stage **(B)**. Vertical bars represent standard deviations of the means, $n = 3$. Asterisks indicate the statistical difference in the significance at $^*p < 0.05$.

Discussion

The structural damage of the cell membrane is the leading cause of CI in refrigerated fruit (2). At low temperature, the cell membrane structure changed between a liquid crystal and a gel state, accompanied by an increase in the membrane lipid unsaturated fatty acid content, which led to a rise in membrane permeability and ion leakage, and finally caused CI (25–27). In the CI study, compared with the WT and overexpression of Sly-miR171e, silencing Sly-miR171e improved the hardness of tomato fruit, reduced its electrolyte leakage, and improved cold tolerance in the fruit. At low temperatures, plants will produce oxidative stress, that is, excessive production of ROS, including H_2O_2 and superoxide anion (O^{2-}) (28, 29). H_2O_2 is a second messenger of defense response (30–32) and is a substance that causes damage to cell membranes. Excessive accumulation of H_2O_2 under CI conditions results in degradation of membrane fatty acids and deepening of CI. In the present study, the H_2O_2 content of miR171e-OE fruit was induced and accumulated to the CI phenotype at 20 and 25 days of MG stage and at 15 days of MR stage, respectively. Compared with WT and miR171e-OE, the H_2O_2 content of miR171e-STTM tomato fruit decreased and showed less sensitivity to CI.

GA, an important plant hormone, is widely distributed in higher plants. It participates in the growth and development of plants, and also plays a vital role in the adaptability of plants at low temperatures (25, 33–35). Low temperature stress could up-regulate the expression of the *GA2ox* gene in *A. thaliana* seedlings, reduce the content of active GA *in vivo*, increase the content of the DELLA protein, and inhibit the growth of seedlings (25). Postharvest GA3 treatment could reduce fruit softening and cold damage during storage, and prolong their

shelf life (35). The GA3 (0.5 mM) treatment could effectively maintain the stability of the tomato fruit membrane, maintain the integrity of the cell wall and reduce the activity of antioxidant enzymes under low temperature storage, thus alleviating the CI of postharvest tomato fruit (34). Under low temperature, GA3 treatment reduced CI, electrolyte leakage, and MDA content and alleviated the CI of the cherry tomato fruit (35). *GA20ox*, *GA3ox*, and *GA2ox* are essential regulators of GA participation in abiotic stress (36, 37). In *A. thaliana*, when the bioactivity of GA was at a low level, the expression of three *GA20ox* genes (*GA20ox1*, *GA20ox2*, and *GA20ox3*) and one *GA3ox1* gene (*GA3ox1*) is up-regulated; in contrast, the expression of *GA2oxs*, an enzyme related to GA inactivation, was down-regulated and resulted in GA biosynthesis and accumulation. However, after treatment with exogenous GA, the expression of these genes was just the opposite (38, 39). The DELLA protein is located in the nucleus and mainly inhibits plant growth and development by repressing gene transcription. Exogenous GA3 treatment can down-regulate the expression of the DELLA protein gene (*GAI)* of tomato fruit and abiotic stress resistance (33). In *A. thaliana*, the expression of GA synthase genes *GA20ox* and *GA3ox* was up-regulated when GA content was low (high DELLA protein content) (36). GA steady-state positive feedback regulates the *GA2ox* gene, and with the increase of GA signal output, the transcription level of *GA2ox* is up-regulated in the plant (40). In the present study, contrasted with the WT, the content of GA3 increased and the expression level of the *GAI* gene decreased in miR171e-STTM tomato fruit, which might inhibit the ripening and senescence of the fruit in cold storage and delay the CI of the fruit. In addition, contrasted with the WT, the expression levels of *GA20ox1* and *GA3ox1* decreased and the expression level of *GA2ox1* increased in miR171e-STTM fruit, which might be related to the improvement of postharvest cold tolerance in tomato fruit under low temperature storage.

CBF gene plays an essential role in plant cold response (23, 41). Cold tolerance of plants was increased by overexpressing *CBF2*, *CBF3*, and *CBF1*, and then CBF transcription factors led to the expression of the *COR* gene (42). Cold stress can only effectively induce the *CBF1* gene in tomatoes, and the expression of *CBF* could increase rapidly and reach the maximum under transient cold stress treatment (43, 44). The correlation between the expression level of *CBF1* and the cold resistance of tomato can be used to measure the cold resistance of tomato fruit (23). Plant hormone assays and qRT-PCR analyses showed that overexpression of *CBF1* in *A. thaliana* induced the expression of the *GA2ox* and GA metabolism in response to low temperature (45). Results of microarray analysis and qRT-PCR showed that the expression levels of *CBF1* and *GA2ox1* in tobacco plants were consistent under low-temperature stress, indicating that *CBF1* and GA signals have a synergistic response at low temperature (46, 47). In the present results, the expression of *CBF1* was the same as that of *GA2ox1*. Therefore, we believed that the *CBF1* gene might have participated in the GA-induced cold resistance by regulating the expression of the *GA2ox1*. Expression analysis showed that higher GA content in overexpressed *GRAS24* than that in the control group, indicating that overexpression of *GRAS24* affected various agronomic traits by regulating GA (16). In the present study, Sly-miR171e might regulate the expression of *GRAS24*, which influenced the GA signaling and CBF pathway and improved the cold tolerance of postharvest fruit (Figure 7).

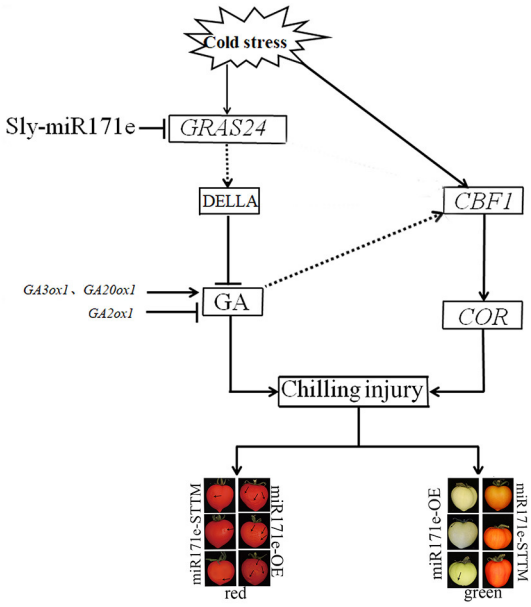


FIGURE 7
Regulation mechanism of Sly-miR171e on cold tolerance in tomato fruit stored at low temperature.

Conclusion

The silencing of Sly-miR171e enhanced the expression of *GRAS24*, increased the GA content and the expression of *CBF1* and *COR*, and by which CI of tomato fruit was alleviated.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary materials, further inquiries can be directed to the corresponding author.

Author contributions

KZ: writing—original draft. RC, WD, QW, HS, LM, and JL: availability of resources. KZ and XX: analysis of data.

XX: writing—the revision of the manuscript. HS: funding. All authors have edited and approved the manuscript.

Funding

This study was supported by the National Natural Science Foundation of China (31872160) and the Hainan Provincial Natural Science Foundation of China (321RC1025 and 2019RC127).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.906227/full#supplementary-material

References

1. Zhang B, Tieman DM, Jiao C, Xu Y, Chen K, Fei Z, et al. Chilling-induced tomato flavor loss is associated with altered volatile synthesis and transient changes in DNA methylation. *Proc Natl Acad Sci USA.* (2016) 113:12580–5. doi: 10.1073/pnas.1613910113

2. Sevillano L, Sanchez-Ballesta MT, Romojaro F, Flores FB. Physiological, hormonal and molecular mechanisms regulating chilling injury in horticultural species. Postharvest technologies applied to reduce its impact. *J Sci Food Agric.* (2009) 89:555–73. doi: 10.1002/jsfa.3468

3. Luengwilai K, Beckles DM, Saltveit ME. Chilling-injury of harvested tomato (*Solanum lycopersicum* L.) cv. Micro-Tom fruit is reduced by temperature pre-treatments. *Postharvest Biol Technol.* (2012) 63:123–8. doi: 10.1016/j.postharvbio.2011.06.017

4. Dong CH, Pei H. Over-expression of miR397, improves plant tolerance to cold stress in *Arabidopsis thaliana*. *J Plant Biol.* (2014) 57:209–17. doi: 10.1007/s12374-013-0490-y

5. Wang PF, Yang Y, Shi HM, Wang YM, Ren FS. Small RNA and degradome deep sequencing reveal respective roles of cold-related microRNAs across Chinese wild grapevine and cultivated grapevine. *BMC Genomics.* (2019) 20:740. doi: 10.1186/s12864-019-6111-5

6. Zhao KY, Song HM, Wang ZQ, Xing ZT, Tian JX, Wang Q, et al. Knockdown of Sly-miR164a by short tandem target mimic (STTM) enhanced postharvest chilling tolerance of tomato fruit under low temperature storage. *Postharvest Biol Technol.* (2022) 187:11872. doi: 10.1016/j.postharvbio.2022.111872

7. Xu XB, Ma XY, Lei HH, Yin LL, Shi XQ, Song HM, et al. MicroRNAs play an important role in the regulation of strawberry fruit senescence in low temperature. *Postharvest Biol Technol.* (2015) 108:39–47. doi: 10.1016/j.postharvbio.2015.05.006

8. Xu XB, Song HM, Lai TF, Li JK. MiR164 is involved in delaying senescence of strawberry (*Fragaria ananassa*) Fruit by Negatively Regulating NAC Transcription Factor Genes under Low Temperature. *Russ J Plant Physiol.* (2017) 64:250–8. doi: 10.1134/S102144371702008X

9. Tang W, Thompson WA. OsmiR528 enhances cold stress tolerance by repressing expression of stress response-related transcription factor genes in plant cells. *Curr Genomics.* (2019) 20:100–14. doi: 10.2174/1389202920666190129145439

10. Shi XP, Jiang FL, Wen JQ, Wu Z. Overexpression of Solanum habrochaites microRNA319d (sha-miR319d) confers chilling heat stress tolerance in tomato (S. *lycopersicum*). *BMC Plant Biology.* (2019) 214:2637. doi: 10.1186/s12870-019-1823-x

11. Wang GD, Xu XP, Wang H, Liu Q, Yang XT, Liao LX, et al. A tomato transcription factor, SlDREB3 enhances the tolerance to chilling in transgenic tomato. *Plant Physiol Biochem.* (2019) 142:254–62. doi: 10.1016/j.plaphy.2019.07.017

12. Luo XY, Shi T, Sun HL, Zhao JS, Zhi JN, Gao H, et al. Selection of suitable inner reference genes for normalisation of microRNA expression response to abiotic stresses by RT-qPCR in leaves, flowers and young stems of peach. *Sci Hortic.* (2014) 165:281–7. doi: 10.1016/j.scienta.2013.10.030

13. Feng QJ, Wang F, Li MY, Tan HW, Ma J, Xiong S, et al. High-throughput analysis of small RNAs and characterization of novel microRNAs affected by abiotic stress in a local celery cultivar. *Sci Hortic.* (2014) 169:36–43. doi: 10.1016/j.scienta.2014.02.007

14. Lee MH, Kim B, Song SK, Heo JO, Yu NI, Lee SA, et al. Large-scale analysis of the GRAS gene family in *Arabidopsis thaliana*. *Plant Mol Biol.* (2018) 67:659–70. doi: 10.1007/s11103-008-9345-1

15. Huang W, Xian ZQ, Kang X, Tang N, Li ZG. Genome-wide identification, phylogeny and expression analysis of GRAS gene family in tomato. *BMC Plant Biol.* (2015) 15:209. doi: 10.1186/s12870-015-0590-6

16. Huang W, Peng SY, Xian ZQ, Lin DB, Hu GJ, Ren MZ, et al. Overexpression of a tomato miR171 target gene *SlGRAS24* impacts multiple agronomical traits via regulating gibberellin and auxin homeostasis. *Plant Biotechnol J.* (2017) 14:472–88. doi: 10.1111/pbi.12646

17. Achard P, Renou JP, Berthome R, Harberd NP, Genschik P. Plant DELLAs restrain growth and promote survival of adversity by reducing the levels of reactive oxygen species. *Curr Biol.* (2008) 18:656–60. doi: 10.1016/j.cub.2008.04.034

18. Liu YD, Shi Y, Zhu N, Zhong S, Bouzayen M, Li G, et al. SlGRAS4 mediates a novel regulatory pathway promoting chilling tolerance in tomato. *Plant Biotechnol J.* (2020) 18:1620–33. doi: 10.1111/pbi.13328

19. Kravchik M, Stav R, Belausov E, Arazi T. Functional characterization of microRNA171 family in tomato. *Plants.* (2019) 8:10. doi: 10.3390/plants8010010

20. Lauressergues D, Delaux PM, Formey D, Lelandais-Brière C, Fort S, Cottaz S, et al. The microRNA miR171h modulates arbuscular mycorrhizal colonization of Medicago truncatula by targeting NSP2. *Plant J.* (2012) 72:512–22. doi: 10.1111/j.1365-313X.2012.05099.x

21. Yan J, Gu JY, Jia XY, Kang WJ, Pan SJ, Tang XQ, et al. Effective small RNA destruction by the expression of a short tandem target mimic in *Arabidopsis*. *Plant Cell.* (2012) 24:415–27. doi: 10.1105/tpc.111.094144

22. Qin GZ, Zhu Z, Wang WH, Cai JH, Chen Y, Li L, et al. A tomato vacuolar invertase inhibitor mediates sucrose metabolism and influences fruit ripening. *Plant Physiol.* (2016) 172:1596–611. doi: 10.1104/pp.16.01269

23. Zhao RR, Sheng JP, Lv SN, Zheng Y, Zhang J, Yu MM, et al. Nitric oxide participates in the regulation of *LeCBF1* gene expression and improves cold tolerance in harvested tomato fruit. *Postharvest Biol Technol.* (2011) 62:121–6. doi: 10.1016/j.postharvbio.2011.05.013

24. Zhao DY, Shen L, Fan B, Liu KL, Yu MM, Zheng Y, et al. Physiological and genetic properties of tomato fruits from 2 cultivars differing in chilling tolerance at cold storage. *J Food Sci.* (2009) 74:C348–52. doi: 10.1111/j.1750-3841.2009.01156.x

25. Achard P, Gong F, Cheminant S, Alioua S, Hedden P, Genschik P, et al. The cold-inducible CBF1 factor-dependent signaling pathway modulates the

accumulation of the growth-repressing DELLA proteins via its effect on gibberellin metabolism. *Plant Cell.* (2005) 20:2117–29. doi: 10.1105/tpc.108.058941

26. Tian JX, Xie SY, Zhang P, Wang Q, Xu XB. Attenuation of postharvest peel browning and chilling injury of banana fruit by Astragalus polysaccharides. *Postharvest Biol Technol.* (2022) 184:111783. doi: 10.1016/j.postharvbio.2021.111783

27. Wang ZQ, Pu HL, Shan SS, Zhang P, Li JK, Song HM, et al. Melatonin enhanced chilling tolerance and alleviated peel browning of banana fruit under low temperature storage. *Postharvest Biol Technol.* (2021) 179:111571. doi: 10.1016/j.postharvbio.2021.111571

28. Zhang L, Cao X, Wang ZQ, Zhang ZK, Li JK, Wang Q, et al. Brassinolide alleviated chilling injury of banana fruit by regulating unsaturated fatty acids and phenolic compounds. *Sci Hortic.* (2022) 297:110922. doi: 10.1016/j.scienta.2022.110922

29. Duan WH, Mekontso FN, Li W, Tian JX, Li JK, Wang Q, et al. Alleviation of postharvest rib-edge darkening and chilling injury of carambola fruit by brassinolide under low temperature storage. *Sci Hortic.* (2022) 299:111015. doi: 10.1016/j.scienta.2022.111015

30. Camp W, van Montagu M, van Inze D. H_2O_2 and NO: redox signals in disease resistance. *Trends Plant Sci.* (1998) 3:330–4. doi: 10.1016/S1360-1385(98)01297-7

31. Orozco-Cárdenas ML, Narveáz-Vásquez J, Ryan CA. Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. *Plant Cell.* (2001) 13:179–91. doi: 10.1105/tpc.13.1.179

32. Ngaffo Mekontso F, Duan WH, Cisse EHM, Chen TY, Xu XB. Alleviation of postharvest chilling injury of carambola fruit by γ-aminobutyric acid: physiological, biochemical, and structural characterization. *Front Nutr.* (2021) 752:538. doi: 10.3389/fnut.2021.752583

33. Gang C, Li J, Chen Y, Wang YJ, Li H, Pan B, et al. Synergistic effect of chemical treatments on storage quality and chilling injury of honey peaches. *J Food Process Preserv.* (2015) 39:1108–17. doi: 10.1111/jfpp.12325

34. Zhu Z, Ding Y, Zhao JH, Nie Y, Zhang Y, Sheng JP, et al. Effects of postharvest gibberellic acid treatment on chilling tolerance in cold-stored tomato (*Solanum lycopersicum* L.) fruit. *Food Bioproc Tech.* (2016) 9:1202–9. doi: 10.1007/s11947-016-1712-3

35. Ding Y, Sheng JP, Li SY, Nie Y, Zhao JH, Zhu Z, et al. The role of gibberellins in the mitigation of chilling injury in cherry tomato (*Solanum lycopersicum* L.) fruit. *Postharvest Biol Technol.* (2015) 101:88–95. doi: 10.1016/j.postharvbio.2014.12.001

36. Rieu I, Ruiz-Rivero O, Fernandez-Garcia N, Griffiths J, Powers SJ, Gong F, et al. The gibberellin biosynthetic genes *AtGA20ox1* and *AtGA20ox2* act, partially redundantly, to promote growth and development throughout the Arabidopsis life cycle. *Plant J.* (2008) 53:488–504. doi: 10.1111/j.1365-313X.2007.03356.x

37. Yamaguchi S, Sun T, Kawaide H, Kamiya, Y. The *GA2* locus of *Arabidopsis thalianaen* codes ent-kaurene synthase of gibberellin biosynthesis. *Plant Physiol.* (1998) 116:1271–8. doi: 10.1104/pp.116.4.1271

38. Elliott RC, Ross JJ, Smith JJ, Lester DR, Reid JB. Feed-forward regulation of gibberellin deactivation in pea. *J Plant Growth Regul.* (2001) 20:87–94. doi: 10.1007/s003440010004

39. Sakamoto T, Miura K, Itoh H, Tatsumi T, Ueguchi-Tanaka M, Ishiyama K, et al. An overview of gibberellin metabolism enzyme genes and their related mutants in rice. *Plant Physiol.* (2004) 134:1642–53. doi: 10.1104/pp.103.033696

40. Hedden P, Thomas SG. Gibberellin biosynthesis and its regulation. *Biochem J.* (2012) 444:11–25. doi: 10.1042/BJ20120245

41. Zhang T, Zhao X, Wang W, Pan Y, Huang L, Liu X, et al. Comparative transcriptome profiling of chilling stress responsiveness in two contrasting rice genotypes. *PLoS ONE.* (2012) 7:e43274. doi: 10.1371/journal.pone.0043274

42. Gilmour SJ, Zarka DG, Stockinger EJ, Salazar MP, Houghton JM, Thomashow F, et al. Low temperature regulation of the *Arabidopsis CBF* family of AP2 transcriptional activators as an early step in cold-induced *COR* gene expression. *Plant J.* (1998) 16:433–42. doi: 10.1046/j.1365-313x.1998.00310.x

43. Zhang X, Fowler SG, Cheng HM, Lou YG, Rhee SY, Stockinger EJ, et al. Freezing-sensitive tomato has a functional CBF cold response pathway. *Plant J.* (2004) 39:905–19. doi: 10.1111/j.1365-313X.2004.02176.x

44. Akhtar M, Jaiswal A, Taj G, Jaiswal JP, Qureshi MI, Singh K, et al. DREB1/CBF transcription factors: their structure, function and role in abiotic stress tolerance in plants. *J Genet.* (2012) 91:385–95. doi: 10.1007/s12041-012-0201-3

45. Kendall SL, Hellwege A, Marriot P, Whalley C, Graham LA, Penfield S, et al. Induction of dormancy in Arabidopsis summer annuals requires parallel regulation of DOG1 and hormone metabolism by low temperature and CBF transcription factors. *Plant Cell.* (2011) 23:2568. doi: 10.1105/tpc.111.087643

46. Niu S, Gao Q, Li ZX, Chen XY, Li W. The role of gibberellin in the CBF1-mediated stress-response pathway. *Plant Mol Biol Rep.* (2004) 32:852–63. doi: 10.1007/s11105-013-0693-x

47. Li YL, Li L, Ding WJ, Li HY, Shi TT, Yang XL, et al. Genome-wide identification of osmanthus fragrans bHLH transcription factors and their expression analysis in response to abiotic stress. *Environ Exp Bot.* (2020) 172:103990. doi: 10.1016/j.envexpbot.2020.103990

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership