NATURAL COMPOUNDS IN FOOD SAFETY AND PRESERVATION

EDITED BY: Susana Ferreira, Gloria Sánchez Moragas, Maria João Fraqueza and Maria José Gonçalves Alves

PUBLISHED IN: Frontiers in Nutrition

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88971-589-3
DOI 10.3389/978-2-88971-589-3

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

NATURAL COMPOUNDS IN FOOD SAFETY AND PRESERVATION

Topic Editors:
Susana Ferreira, University of Beira Interior, Portugal
Gloria Sánchez Moragas, Institute of Agrochemistry and Food Technology (IATA), Spain
Maria João Fraqueza, University of Lisbon, Portugal
Maria José Gonçalves Alves, Centro de Investigação de Montanha (CIMO), Portugal

Citation: Ferreira, S., Moragas, G. S., Fraqueza, M. J., Alves, M. J. G., eds. (2021). Natural Compounds in Food Safety and Preservation. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88971-589-3

Table of Contents

frontiers in Nutrition

EDITORIAL
published: 16 September 2021
doi: 10.3389/fnut.2021.759594

Editorial: Natural Compounds in Food Safety and Preservation

Susana Ferreira[1]*, *Gloria Sanchez*[2], *Maria J. Alves*[3] and *Maria J. Fraqueza*[4]

[1] CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal, [2] Department of Preservation and Food Safety Technologies, Instituto de Agroquímica y Tecnología de Alimentos – Consejo Superior de Investigaciones Científicas (IATA-CSIC), Valencia, Spain, [3] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Bragança, Portugal, [4] CIISA-Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal

Keywords: editorial, natural compounds, food safety, food preservation, antimicrobial

Editorial on the Research Topic

Natural Compounds in Food Safety and Preservation

Food safety is a global challenge, with foodborne diseases posed as a relevant concern for human health, and food microbial spoilage being a problem for agri-food companies (1). Considerable research has been dedicated to diverse approaches that can be applied to control foodborne pathogens and microbial spoilage, among which the potential use of natural compounds has been highlighted as a strategy for improving food safety, but also quality and extending self-life (1–3). Furthermore, the negative consumer perception of the synthetic preservatives used in food industry, associated with an increasing demand for maintenance of nutritional and quality properties, has encouraged the pursue for the use of natural-based preservatives in food production (1–3).

In this context, in this Research Topic, natural antimicrobial compounds have been highlighted by their activity against *Chronobacter* spp. in infant powdered formula by Yemiş and Delaquis. The authors reviewed the potential of natural compounds from plants, microbial and animal sources as alternatives to synthetic chemical preservatives, addressing nutritional, toxicological, and regulatory issues. In fact, the use of natural antimicrobial compounds needs to be guided considering the regulatory framework, and so the authors suggest the use of well-studied single compounds over multiple-component preparations.

Among the natural compounds, essential oils have been pointed as promising antimicrobial mixtures. Yousefi et al. reviewed the potential application of essential oils with antilisterial activity in meat and poultry products, since contaminated meat products are recognized as one main source for *Listeria monocytogenes* infection. The authors described the efficiency of several essential oils in the control of *L. monocytogenes*, whilst addressing the mechanism of action of some selected compounds and the major drawbacks associated with the application of essential oils in food products. The activity of natural compounds in food is dependent of various factors, namely on the complexity and composition of the product, this highlights the need of the validation of antimicrobial activity in food matrixes. Kiprotich et al. explored the use of thyme oil combined with *Yucca schidigera* extract to marinate raw chicken breast meat in lemon juice. The authors considered the potential of antimicrobial marinade formulations as an approach to reduce enteric pathogens. Based on their results, thyme oil showed to be an enhancer of the inactivation of *Salmonella enterica* on raw chicken breast, increasing the antimicrobial efficacy of lemon juice marinade containing yucca extract to emulsify the thyme oil.

Besides, the perspective of the use of natural antimicrobial compounds for controlling foodborne pathogens, research has also been carried out to elucidate its use against microbial spoilage.

OPEN ACCESS

Edited and reviewed by:
Elena Ibañez,
Institute of Food Science Research (CIAL), Spain

***Correspondence:**
Susana Ferreira
susana.ferreira@fcsaude.ubi.pt

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

Received: *16 August 2021*
Accepted: *23 August 2021*
Published: *16 September 2021*

Citation:
Ferreira S, Sanchez G, Alves MJ and Fraqueza MJ (2021) Editorial: Natural Compounds in Food Safety and Preservation. Front. Nutr. 8:759594. doi: 10.3389/fnut.2021.759594

Shen et al. reported the antifungal activity of Loquat leaves extract against citrus postharvest pathogens, providing a mechanistic overview of the anti-*Penicillium digitatum* activity. The antifungal activity of this extract against *P. digitatum* was attributed to the derangement of cell membrane permeability and the disordered energy metabolism.

Among the limitations of the use of essential oils are: off-flavors and odors that may result in an unacceptance of the food product by the consumer usually associated with the use of high concentrations of essential oils, the degradation of the components or the limited interaction with the microorganisms. The incorporation of these natural bioactive compounds, into edible coatings, food packaging materials, or other formulations may be presented as an approach to overcome these problems. Asensio et al. reported the use of nanoemulsions as an approach to encapsulate, protect, and deliver Argentinean oregano essential oil. The authors optimized the physical stability of the nanoemulsion and characterized it, showing that the formulation may even increase the antimicrobial activity and inhibition of quorum sensing when comparing with the pure essential oil.

The application of these natural compounds may be accomplished alone or in combination with already existing preservatives or even processing methods for the development of a food preservation system, providing mechanisms to ensure food safety. This subject was approached by Barroug et al. who reviewed the use of natural compounds with non-thermal strategies on poultry products addressing the effects on the microbiological and physicochemical characteristics. This paper gives a well-balanced overview of the use of non-thermal technologies, natural compounds and their combination as an intervention for safer poultry products.

In conclusion, the present Research Topic provides several examples of natural antimicrobial compounds and their application in different contexts.

AUTHOR CONTRIBUTIONS

SF drafted the manuscript. GS, MA, and MF provided critical review and insight and revised the final version of the editorial. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The editors of this topic would like to thank all authors and reviewers for their contributions to the present collection.

REFERENCES

1. Chibane LB, Degraeve P, Ferhout H, Bouajila J, Oulahal N. Plant antimicrobial polyphenols as potential natural food preservatives. *J Sci Food Agric.* (2019) 99:1457–74. doi: 10.1002/jsfa.9357
2. Tiwari BK, Valdramidis VP, O'Donnell CP, Muthukumarappan K, Bourke P, Cullen PJ. Application of natural antimicrobials for food preservation. *J Agric Food Chem.* (2009) 57:5987–6000. doi: 10.1021/jf900668n
3. Calo JR, Crandall PG, O'Bryan CA, Ricke SC. Essential oils as antimicrobials in food systems – a review. *Food Control.* (2015) 54:111–9. doi: 10.1016/j.foodcont.2014.12.040

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.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 09 October 2020
doi: 10.3389/fnut.2020.569913

Rheological Behavior, Antimicrobial and Quorum Sensig Inhibition Study of an Argentinean Oregano Essential Oil Nanoemulsion

Claudia Mariana Asensio[1*†], *Patricia Raquel Quiroga*[2], *Ammar Al-Gburi*[1], *Quingron Huang*[1] *and Nelson Rubén Grosso*[2]

[1] *Department of Food Science, School of Environmental and Biological Sciences, Rutgers, State University of New Jersey, New Brunswick, NJ, United States,* [2] *Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba (UNC), Instituto Multidisciplinario de Biología Vegetal (IMBIV), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Córdoba, Argentina*

In this study, Argentinean oregano essential oil (OEO) nanoemulsions (NEs) were developed. Four NEs were prepared: a control (CNE), EONE1 (10.6 mg EO/g NE), EONE2 (106 mg EO/ g NE), and EONE3 (160 mg EO/g NE) and tested for antimicrobial activity against *Staphylococcus aureus* ATCC 13565, *Listeria monocytogenes* Scott A, *Pseudomonas aeruginosa* ATCC 14213, and *Escherichia coli* O157:H7 using a broth microdilution assay and quorum sensing inhibition in a model using *Chromobacterium violaceum* ATCC 12472, where the production of violacein was quantified. The chemical composition of the EO was determined by gas chromatography–mass spectrometry. The average particle size (nm) and polydispersity index were monitored over 14 days at two different storage temperatures (4 and 23°C). A rheological behavior study was carried out using a dynamic shear rheometer, and flow curves, as well as viscoelastic properties, were determined. *E. coli* and *L. monocytogenes* were the most sensitive microorganisms to EONE (MIC of 2 and 5 mg/ml for EOEN3). Sub-MICs for NE were found at lower concentrations than those for pure EO. A significant reduction in violet pigment intensity and colorless coloration ($p < 0.05$) were observed at different NE concentrations concerning the control sample. The flow behavior index (n) decreased, and the consistency index (k) increased when the EO concentration was increased. CNE, EONE1, and EONE2 showed liquid-like behavior ($G' < G''$) in the low-frequency region, whereas a solid-like behavior ($G' > G''$) was observed in the high-frequency region, presenting a viscoelastic behavior, appearing as a wormlike micellar solution. For EONE3, a strong increase in both moduli was observed with increasing OEO concentration. The G' was about one order of magnitude higher than the G'' over the whole frequency range, indicating the presence of a gel-like structure. The incorporation of EOs into an NE increased their stability, lowering the particle size, leading to a wormlike micelle with higher viscosity. Moreover, this NE had good antimicrobial activity and novel quorum-sensing inhibition activity. The results of this study indicated that Argentinean OEO NE could be used in a food system as a natural and stable antimicrobial agent.

Keywords: oregano, nanoemulsion, quorum sensing, viscosity, viscoelasticity, oregano (*Origanum vulgare* L.)

OPEN ACCESS

Edited by:
Susana Ferreira,
University of Beira Interior, Portugal

Reviewed by:
Fohad Mabood Husain,
King Saud University, Saudi Arabia
Dan Cristian Vodnar,
University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania
Filippo Fratini,
University of Pisa, Italy
Dejan S. Stojkovic,
University of Belgrade, Serbia

****Correspondence:***
Claudia Mariana Asensio
cmasensio@agro.unc.edu.ar

†Present address:
Claudia Mariana Asensio, Química Biológica, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba (UNC), Instituto Multidisciplinario de Biología Vegetal (IMBIV), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Córdoba, Argentina

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

Received: *05 June 2020*
Accepted: *31 August 2020*
Published: *09 October 2020*

Citation:
Asensio CM, Quiroga PR, Al-Gburi A, Huang Q and Grosso NR (2020) Rheological Behavior, Antimicrobial and Quorum Sensig Inhibition Study of an Argentinean Oregano Essential Oil Nanoemulsion. Front. Nutr. 7:569913. doi: 10.3389/fnut.2020.569913

INTRODUCTION

Foodborne illnesses are a major concern for consumers, the food industry, and food safety authorities. In years past, an increase in the occurrence of disease outbreaks caused by pathogenic and spoilage microorganisms in foods has occurred (1). The misuse and mishandling of chemical antimicrobials have resulted not only in more tolerant and resistant viruses, bacteria, and parasites to chemical agents but also in hazards to human being's health, including respiratory allergies, and a rise in carcinogens and toxic substances. Moreover, consumers are concerned about the adverse effects of using synthetic antimicrobials and would prefer foods treated with safe and natural antibacterial agents (2).

Essential oils (EOs) are well-recognized as natural antimicrobial preservatives, are widely used as flavoring compounds, antimicrobial agents, and functional ingredients in food, and are classified by the US Food and Drug Administration as generally recognized as safe (3–5). Argentinian oregano essential oils (OEOs) were found to preserve the chemical, sensory, and microbiological qualities of ricotta cheese, cottage cheese, olive oil, roasted peanuts, fried peanuts, sunflower kernels, and hake burgers, among others (3, 6–10). However, their direct incorporation into foods is limited because of the hydrophobicity of these compounds and their difficulty interacting with microorganisms in aqueous media (11, 12). Moreover, due to their volatile nature, they can easily suffer degradation upon exposure to heat, pressure, light, and oxygen (13, 14).

In this context, nanoemulsions (NEs) are being used increasingly often to encapsulate, protect, and deliver lipophilic ingredients into liquid foods or minimally processed fruits and vegetables (12, 15). NE, owing to their subcellular size (20 and 200 nm), offers high thermodynamic stability, flocculation, and coalescence and increased distribution of the antimicrobial agent in food matrices, protecting it from deleterious interactions with food components and the environment (16). Furthermore, pH-, temperature-, and ionic-strength-sensitive compounds can be incorporated conveniently into food systems after nanoencapsulation (17).

The formation of NEs using medium-chain triacylglyceride (MCT) oils is often challenging due to their relatively low polarity, high interfacial tension, and high viscosity. It is difficult to prepare NEs from these oils using high-pressure homogenization methods because their high viscosity limits droplet disruption within the homogenizer. On the other hand, EOs have a relatively higher polarity, lower interfacial tension, and lower viscosity than MCT, which facilitates the formation of very small droplets by high-pressure homogenization (18, 19). This system is an effective method to achieve a constant release of bioactive compounds (10, 20) and allows to obtain NEs with smaller droplet sizes. Alexandre et al. (21) observed that increasing ginger EO concentration decreases the droplet size. The same behavior was found by Walker et al. (22) in NEs made with thyme EO, and these NEs were stable during storage. Preservation of food products with EO NE was reported. The addition of NEs with oregano EO protected hake (*Merluccius hubbsi*) burgers from deterioration, extending their shelf life (23). NE prepared with thyme EO and thymol effectively extended the shelf life of fresh pork (24). The antimicrobial activity of NEs based on different EOs was also investigated. Yazgan (25) demonstrated that sage EO and its nanoemulsified form could be used as a natural antimicrobial agent against food-related pathogens. NEs can be designed to form highly viscous or gel-like systems at much lower droplet concentrations than conventional emulsions.

Concentrated micellar solutions find applications in various industries (paint, chemical, pharmaceutical, drug delivery, and nanobiotechnology) and consumer products (home and personal care: detergents, hard surface cleaning, drain opening, perfumes, hair bleaching, skin cosmetics, shower gels, and sunscreens). The formation of entangled wormlike micelles (WLM) increases the viscosity of a solution and may even confer a certain viscoelastic property on it (26–29). The phenomenon of viscoelasticity can be induced by the addition of specific additives to some surfactants. Shear-thinning flow behavior often occurs at an increased surfactant concentration and in the presence of cosurfactants, additives, salts, or appropriate counterions (27, 28). Additives can compress the diffused electric double layer of the micellar interface, screen electrostatic repulsion between charged headgroups, and finally allow closer packing of the surfactant monomers into aggregates, resulting in the formation of a weak gel (27, 30).

Quorum sensing (QS) is a communication system that allows bacteria to monitor their population density through the production and sensing of small signaling molecules (31–33). *Chromobacterium violaceum* is a gram-negative bacterium that synthesizes a violet pigment (violacein) as a result of QS (34). Food deterioration, at least in part, is regulated by a mechanism of cell-to-cell communication (such as QS). Until now, only a few studies have developed food preservation techniques based on the anti-QS property of EOs (32). The search for an efficient QS inhibitor that can inhibit the spoilage of food products is a promising alternative (33). A stable NE with OEO that has antimicrobial and QS-inhibitory properties could be useful in food systems to avoid spoilage.

This study was performed with the following objectives: (i) to study the stability and to characterize the rheological properties of a stable NE with OEO; (ii) to determine the antimicrobial activity against food pathogens; and (iii) to evaluate the QS-inhibitory effects of OEO NE.

MATERIALS AND METHODS

Materials

Leaves and flowers of *Origanum vulgare* ssp. *hirtum* (clone Criollo) were purchased (crop 2015) on a farm located in Villa Dolores (Córdoba, Argentina).

Neobee 1053 MCT was a gift from Stepan Co. (Northfield, USA). Alcolec PC75 (phosphatidylcholine enriched) soy lecithin was obtained from American Lecithin Co. (Oxford, USA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, USA). Deionized water was obtained from a Milli-Q water purification system (Millipore Co., Bedford, USA) and used in

all experiments. Culture media were purchased from BD Bacto™ (New Yersey, USA).

Essential Oil Extraction and Analysis

Dried leaves and flowers were hydro-distilled for 2 h in a Clevenger-type apparatus with a separated extraction chamber. The EOs were kept in dark flasks at −18°C in a freezer. The EO was analyzed with a Perkin Elmer Clarus 600 gas chromatography–mass spectrometry (Shelton, Connecticut, USA) coupled with an ion trap mass detector (MS) and non-polar capillary column Elite-5 MS (methylpolysiloxane, 5% phenyl, 30 m, 0.25 mm id, and 0.25 mm coating thickness). The compounds were identified by comparing their mass spectra with those from the literature (35) and the National Institute of Standards and Technology (2.0) library (NIST 2.0). The main components were further identified by the co-injection of authentic standards (Sigma® St. Louis, MO, USA). The quantitative composition was obtained by peak area normalization, and the response factor for each component was considered equal (5).

Nanoemulsion Preparation and Physical Stability Characterization

An aqueous solution of soy lecithin was prepared to disperse the dried lecithin powder in deionized water at room temperature and stirring for 30 min. The oil phase consisted of MCT and *Origanum* EO mixed at different ratios (15:1, 1.5:1, and 1:1 w/w). The final NE composition was 81% water, 3% soy lecithin, and 16% oil phase. Both phases were premixed with a high-speed homogenizer (Ultra-Turrax T25 IKA Works Inc., Wilmington, NC, USA), equipped with an S25 N18 G rotor operated at 12,000 rpm per 3 min at room temperature. These coarse emulsions were finely homogenized with a high-pressure homogenizer (EmulsiFlex-C3, Avestin Inc., Ottawa, Canada) for six cycles at a pressure of 150 MPa. Four NEs were prepared: CNE, EONE1, EONE2, and EONE3. After homogenization, samples were stored in 20-ml glass vials (Supelco Analytical) with a screw cap (PTFE/silicone septum, Supelco Analytical) covered with aluminum foil in two different storage conditions: room temperature (RT) (23°C) and fridge (F) (4°C), and stored for 14 days.

Particle Size and Polydispersity Index

The average particle size (mean diameters, nm) and polydispersity index (PDI) of NEs were monitored over 14 days using a dynamic light scattering instrument (Brookhaven BIC 90 plus) equipped with a Brookhaven BI-9000AT digital correlator (Brookhaven Instrument Corp, New York, NY) to evaluate the stability. Samples were diluted 1:100 using deionized water to prevent multiple scattering effects. All measurements were performed in triplicate at a fixed scattering angle at 25°C. The light source of the particle size analyzer was a solid-state laser operating at 658 nm with 30 mW power. The mean diameters of emulsions were determined by cumulant analysis of the intensity–intensity autocorrelation function, G (q, t).

Rheological Behavior Study

The rheological characteristic study provides information about the behavior of the fluid, shear-thinning or shear-thickening nature. Viscosities of formulated NEs from MCT surfactants were measured with a dynamic shear rheometer (Discovery Hybrid Rheometer, TA Instruments, DE, USA) equipped with a cone-plate geometry with a cone diameter of 60 mm. Flow curves were determined at 25°C with two consecutive continuous shear rate ramps from 0.50 to 150 s^{-1}. The apparent shear viscosity at a fixed shear rate (100 s^{-1}) was reported. The viscosity curves were analyzed using the power-law mathematical model. The value of n defines the nature of the fluids. If $n < 1\rightarrow$ shear-thinning nature, $n = 1\rightarrow$ Newtonian fluid nature, and $n > 1\rightarrow$ shear-thickening nature (36).

Pseudo Plastic Nature

The following equation describes the pseudoplastic behavior:

$$\tau = k \times \dot{\gamma}^{n} \quad (1)$$

where τ is the shear stress, k is the consistency index, n is the flow behavior index, and $\dot{\gamma}$ is the shear rate. Values of n and k were calculated, taking the log on both sides of Equation (1) and plotting log τ vs. log $\dot{\gamma}$. The apparent or effective viscosity (μ) along with n and k values was determined by fitting the experimental data to the following equation:

$$\mu = k\dot{\gamma}^{n-1} \quad (2)$$

although the power-law model is a good descriptor of fluid behavior across a shear rate range up to which coefficients are fitted, due to its simplicity with more versatility than the Bingham plastic model, it is widely practiced (37).

Viscoelastic Properties Analysis

Viscoelastic property study provides information about the elastic and viscous nature of fluids and involves the determination of real, storage, or elastic modulus (G′), and imaginary, loss or viscous modulus (G″) as a function of angular frequency (ω) (37). Strain sweep experiments (data not shown) were performed to determine the linear viscoelastic regions of the samples at 25°C and a constant frequency of 1 Hz, with a strain % in the range of 0.1–100%. Frequency sweep tests were performed using a strain amplitude of 0.4 (within the linear viscoelastic regions) over an angular frequency range of 0.1–100 rad s^{-1}. Finally, G′ and G″ moduli curves were plotted against angular frequency. If G′ > G″, fluid behaves as solid or gel, and for G′ < G," fluid acts as liquid or viscous (36, 37).

Antimicrobial Activity

Microbial Strains

The antimicrobial activity of the EO and its NEs was tested against: *S. ureus* ATCC 13565, *Listeria monocytogenes* Scott A (*L. monocytogenes*), *Pseudomonas aeruginosa* ATCC 14213, and *Escherichia coli* O157:H7. The cultures were obtained from the Department of Food Science, Rutgers University, culture collection (New Brunswick, NJ, USA). From a frozen stock (−80°C), bacteria were inoculated into trypticase soy agar

plates (TSA, Becton Dickson and Co., Cockeysville, MD, USA) and propagated under aerobic conditions at 37°C for 24 h. After the incubation, one colony of each bacterial strain was transferred separately to test tubes with trypticase soy broth (TSB) and incubated at 37°C with agitation for 18–24 h. For broth microdilution assay, the bacterial growth suspensions were further diluted in fresh TSB medium to achieve 10^6 colony forming units (CFU)/ml (38).

C. violaceum ATCC 12472 was grown in Luria–Bertani (LB) broth (ACROS, Miller, NJ) at 26°C for 48 h aerobically. *P. aeruginosa* ATCC 14213 was aerobically grown in LB broth at 37°C for 24 h and used as a positive control for QS inhibition in gram-negative bacteria (39).

Determination of Antimicrobial Activity

Antimicrobial activity was performed using a broth microdilution assay (40). Briefly, EO (first diluted in dimethyl sulfoxide) and NEs were 2-fold diluted with fresh TSB in a 96-well tissue culture plate (Falcon, Corning Incorporated, Corning, NY, USA). The final volume of the antimicrobial diluted into the broth was 100 µl in each well. The overnight cell culture was diluted in TSB to the final 5×10^6 CFU ml^{-1} (the number of bacterial cells was confirmed by the spot-plate method). From the diluted bacterial cells, 100 µl was transferred in the wells containing predetermined concentrations of antimicrobials. Plates were incubated under aerobic conditions at 37°C for 24 h. Mineral oil (Sigma-Aldrich chemical, St. Louis, MO, USA) was added (75 µl) to each well to avoid evaporation. The optical density readings of the microorganism at 595 nm were tracked using a microplate reader (Model 550, Bio-Rad Laboratories, Hercules, CA). The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antimicrobial agent that produced no visible growth after overnight incubation (38, 39). Each experiment was performed three times in duplicates.

Quorum Sensing Inhibition Assay

This assay was performed according to Algburi et al. (39). Briefly, the overnight-grown cells of *C. violaceum* were diluted in fresh LB broth to achieve 10^6 CFU/ml. NE-containing EOs were serially 2-fold diluted with LB into a 48-well microplate (BD, Franklin Lakes, NJ). A bacterial suspension (500 µl) (10^6 CFU/ml) was mixed with 500 µl of LB broth and 500 µl of the NE dilution. Once the samples were prepared, the plate was aerobically incubated at 26°C without shaking for 48 h. The cell-free supernatant (CFS) of *P. aeruginosa* grown in LB was used as a control, preventing violacein production by *C. violaceum*.

Quantification of Violacein Production

After incubation, 750 µl from each well (test and control wells) was transferred to a 1-ml centrifuge tube and centrifuged at 8,000 g for 5 to collect violacein and the producer cells. The supernatants were discarded, and the pellets were vigorously vortexed with 750 µl of 100% dimethyl sulfoxide to dissolve the insoluble violacein. The samples were centrifuged again at 8,000 g for 5 min to precipitate *C. violaceum* cells. To evaluate violacein production, 200 µl of violacein-containing supernatant was added into a 96-well microplate (Falcon, Corning Inc., Corning, NY) in triplicate, and the optical density at 585 nm (OD585) was measured using a plate reader (Model 550, Bio-Rad Laboratories, Hercules, CA). To ensure that the QS inhibition occurred without NE killing the targeted microorganisms by sub-NE MICs, the precipitated bacterial cells were resuspended in 750 µl of DW (pH 7.0), and the absorbance was measured at OD600. The ODs of cells treated with sub-MICs of NE were compared against the non-treated cells (positive control). The control value was set to 100% violacein production (39).

Violacein inhibition (%) = 100-((OD_{585} Sample - OD_{585} PFSC Control) * 100/OD_{585} Control).

The 95 (%) violacein inhibition concentration was calculated considering the regression equations ($R^2 \geq 0.8$) of each treatment obtained by plotting violacein inhibition (%) against samples' concentrations.

Statistical Analysis

The experiments were carried out three times, and results were expressed as mean ± standard deviations. Normal distribution

TABLE 1 | Oregano clone Criollo essential oil chemical composition analyzed by gas chromatography–mass spectrometry.

	RT	Compound	EO‡	
		(g/100 g)	$\bar{X}$	SD
1	8.41	α-Phellandrene	1.02	0.04
2	8.53	α-Pinene	0.76	0.01
3	8.72	Camphene	0.34	0.01
4	8.90	β-Pinene	3.8	0.02
5	8.97	3-Octanone	1.65	0.02
6	9.06	β-Myrcene	0.28	0.01
7	9.34	α-Terpinene	4.00	0.05
8	**9.41**	**0rto-cymene**	**11.08**	**0.16**
9	9.51	β-trans-Ocimene	1.02	0.04
10	**9.74**	**γ-Terpinen**	**15.08**	**0.03**
11	9.88	cis Sabinene hidrate	2.52	0.16
12	10.02	β-cis-Ocimene	0.88	0.03
13	**10.20**	**Terpinolene**	**18.62**	**0.53**
14	11.00	Borneol	0.51	0.4
15	**11.12**	**4-Terpineol**	**9.28**	**0.22**
16	11.47	α-Terpineol	2.28	0.1
17	11.51	Thymol methyl ether	1.02	0.03
18	11.98	Carvacrol methyl ether	0.31	0.05
19	**12.08**	**Thymol**	**16.87**	**0.78**
20	13.06	Carvacrol	0.83	0.09
21	14.03	Caryophyllene	1.77	0.03
22	14.33	Germacrene D	0.85	0.01
23	15.01	γ-Gurjunene	1.14	0.03
24	15.09	δ-Cadinene	1.01	0.01
25	15.12	Aromadendrene, dehydro-	2.29	0.02
26	15.19	Lanceol, cis	1.14	0.02

‡*Only those compounds with amount higher than 0.3 g/100 g are presented. Letters and numbers in bold indicate main compounds.*

TABLE 2 | Means and standard deviations ($n = 3$) of effective diameter (ED) and polydispersity index (PDI) of control (CNE) and essential oil-containing nanoemulsions (EONE1, EONE2, and EONE3).

		Day 0					Day 14				
Sample	**Temp**				†	‡				†	†
ED											
CNE	F	150.77	±	0.06	D	1	148.00	±	2.90	F	1
	RT	150.77	±	0.06	D	2	111.75	±	2.65	D	1
EONE1	F	131.70	±	0.20	C	1	127.57	±	3.02	E	1
	RT	131.70	±	0.20	C	2	98.10	±	5.87	C	1
EONE2	F	89.17	±	5.71	B	2	73.83	±	5.84	B	1
	RT	89.17	±	5.71	B	2	64.17	±	2.25	A	1
EONE3	F	42.80	±	3.00	A	1	62.60	±	0.20	A	2
	RT	42.80	±	3.00	A	1	62.90	±	0.20	A	2
PDI											
CNE	F	0.307	±	0.002	A	1	0.314	±	0.01	A	1
	RT	0.307	±	0.002	A	1	0.356	±	0.01	B	2
EONE1	F	0.292	±	0.003	A	1	0.332	±	0.02	A	2
	RT	0.292	±	0.003	A	1	0.334	±	0.01	A	2
EONE2	F	0.288	±	0.049	A	1	0.322	±	0.03	A	1
	RT	0.288	±	0.049	A	1	0.320	±	0.03	A	1
EONE3	F	0.278	±	0.007	A	1	0.316	±	0.01	A	2
	RT	0.278	±	0.007	A	1	0.310	±	0.02	A	1

†*Different letters in each column indicate significant differences between samples (ANOVA, DCG test, alpha 0.05).*
‡*Different numbers in each row indicate significant differences between storage temperatures for each sample (ANOVA, DCG test, a = 0.05).*

was tested with a Shapiro–Wilk test. Analysis of variance (ANOVA, $\alpha = 0.05$) and Fisher's least significant difference multiple range test were performed to determine significant differences between means. Data were analyzed using the InfoStat software, version 2019 (41).

RESULTS AND DISCUSSION

Essential Oil Composition

The chemical composition of EO from *O. vulgare* ssp. *hirtum* clone Criollo is shown in **Table 1**. The major compounds were terpinolene (18.62 g/100 g), thymol (16.87 g/100 g), γ-terpinene (15.08 g/100 g), and ortho-cymene (11.08 g/100 g). A similar composition was reported previously (5, 23, 42). This clone was shown to have higher levels of the bioactive phenol thymol than any other OEO from Argentina (5, 43). Moreover, previous studies reported that EOs of both *O. vulgare* ssp. *hirtum* clones (Criollo and Cordobes) were more active in *in vitro* antimicrobial tests than other tested OEOs (3, 5).

Nanoemulsion Characterization

Physical Stability of Nanoemulsions

The mean particle sizes after homogenization were 150.77, 131.7, 89.17, and 74.7 for CNE, EONE1 (15:1 10.6 mg EO/g NE), EONE2 (1.5:1 106 mg EO/g NE), and EONE3 (1:1 160 mg EO/g NE), respectively. From these data, it was concluded that EONE3 had the smallest droplets and the lowest PDI values. Therefore, EONE3 was the most stable. These results were confirmed after 14 days in storage; droplet sizes and PDI values remained the lowest for EONE3 (**Table 2**).

The droplet size decreased, as the proportion of OEO in the NE increased. Similar behavior was found for NEs prepared with ginger EO (21) and lemon EO (22). The size of the droplets produced during high-pressure homogenization typically decreases, as the oil phase viscosity and interfacial tension decrease; this facilitates droplet disruption (44).

It is difficult to prepare NEs from MCT oils (18, 19). With the addition of EOs as cosurfactants, it is expected the formation of very small droplets during homogenization when the EO concentration increases, as in this case. The droplet size of the EONE stored at 4 and 23°C tended to decrease during the storage, except for EONE3. Similar behavior was found by Pongsumpun et al. (45). They observed that the droplet size of the cinnamon EO NE stored at 4 and 30°C decreased during the first 60 and 30 days of storage, respectively. Afterward, the size of the droplets tended to increase, probably due to the coalescence of the emulsion droplets. It could be possible that droplet sizes of EONEs would increase if storage time increases.

The EONEs had no visible creaming or phase separation (data not shown) during the storage at both tested temperatures. Therefore, the NEs had good stability in terms of droplet size under the tested temperatures and storage time.

Rheological Behavior Study

Viscosity

Table 3 presents the values of flow behavior (n) and consistency (k) indexes for the prepared NE samples. The power-law model

TABLE 3 | Consistency coefficient (k) and flow behavior index (*n*) of tested nanoemulsions.

	CNE†	EONE1†	EONE2†	EONE3†
k (Pa. s)	0.0013	0.0037	0.0186	0.0537
N	1.1553	1.0545	0.8375	0.7542
R^2	0.9992	0.9998	0.9998	0.9994

† *Treatments: control (CNE) and essential oil-containing nanoemulsions at different concentrations (EONE1, EONE2, and EONE3).*

describes with high accuracy the flow curves of prepared NEs. As seen from **Table 3**, the values of *n* for samples EONE2 (0.8375) and EONE3 (0.7542) were <1; these NEs showed non-Newtonian or shear thinning behavior. EONE1 (1.0545) showed Newtonian behavior, and CNE (1.1553) demonstrated shear thickening behavior. The values of *n* decreased, and those of *k* increased, as the EO concentration increased.

The viscosity of a micellar solution with a hydrophilic surfactant increases gradually when a lipophilic surfactant is added and increases steeply above a certain level due to the formation of WLM (28). Mitrinova et al. (26) found that some terpenes increased the solution viscosity and led to shear thinning behavior. Such non-Newtonian solution behavior was associated with the formation of entangled WLM in the solutions (26). Polar terpenes could solubilize in the palisade layer, changing the curvature of the micellar surface and thus increasing the solution viscosity (26, 28, 46). Terpenes from the oregano clone Criollo EO could act as cosurfactants, solubilizing in the palisade layer. Depending on their molecular structure and polarity, they could increase NE viscosity, leading to shear thinning behavior and probably forming WLM.

Viscoelastic Property Analysis

The variation in dynamic moduli (G′ and G″) and complex viscosity (μ*) as a function of the angular frequency (ω) and the variation in apparent viscosity (μ_a) as a function of the shear rate ($\dot{\gamma}$) for NE samples are shown in **Figure 1**.

From dynamic rheological tests in the linear viscoelastic range, the storage modulus G′ and the loss modulus G″ were obtained. G′ value is a measure of the deformation energy stored in the sample during the shear process, representing the elastic behavior of a sample. In contrast, the G″ value is a measure of the deformation energy used up in the sample during the shear and lost to the sample afterward, representing the viscous behavior of a sample (36). CNE, EONE1, and EONE2 showed liquid-like behavior (G′ < G″) (**Figures 1A–C**) in low-frequency regions, whereas solid-like behavior (G′ > G″) was observed in high-frequency regions. This is a typical viscoelastic behavior shown by WLM solutions (28).

In EONE3, the storage modulus (G′) was above the loss modulus (G″) over the whole frequency range analyzed (**Figure 1D**). When the G′ and G″ curves do not cross over the whole frequency range, this indicates that a gel-like structure is

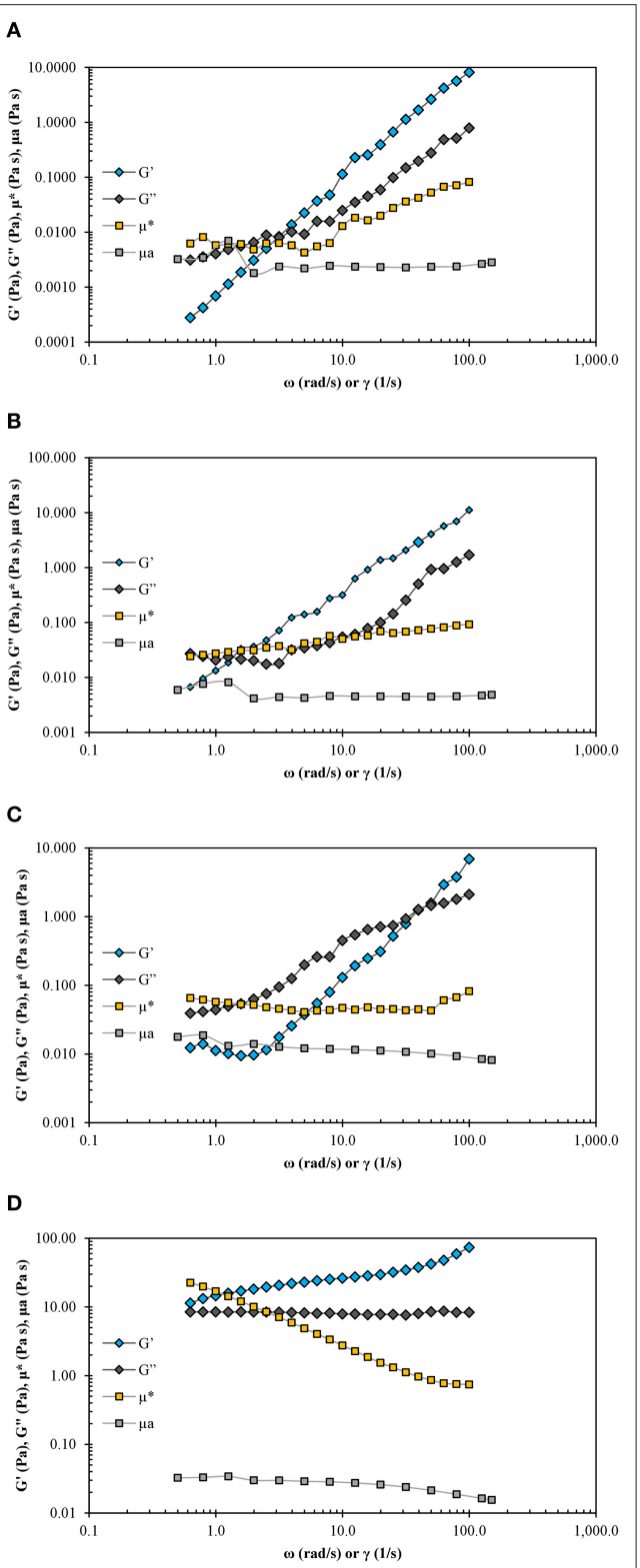

FIGURE 1 | Storage (G′) and loss (G″) modulus, complex viscosity (μ*), and apparent viscosity (μ_a) of nanoemulsion: **(A)** CNE, **(B)** EONE1, **(C)** EONE2, and **(D)** EONE3 as a function of the angular frequency (ω) or shear rate ($\dot{\gamma}$).

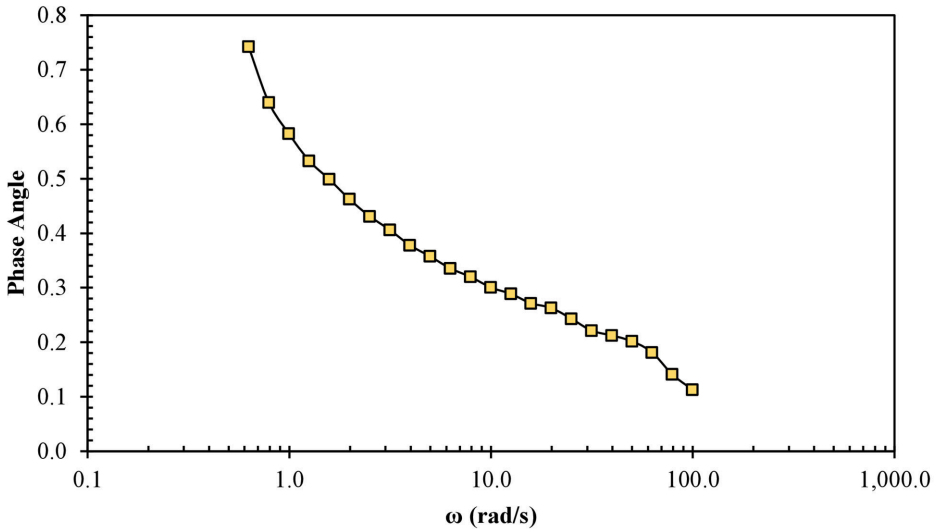

FIGURE 2 | Gel network behavior of EONE3: changes of phase angle as a function of the angular frequency.

present (36, 47), as in this case. The decrease in μ* (complex viscosity) in EONE3 (**Figure 1D**) confirmed the shear-thinning nature of this NE. Moreover, values of μ* were greater than μ_a for all magnitudes of shear rates and oscillatory frequencies; this NE sample did not behave as a true gel. In **Figure 1D**, complex viscosity as a function of angular frequency lay above the curve of apparent viscosity as a function of the shear rate. This behavior is typical for a weak gel. Such systems do not obey the Cox–Merz rule, which states that the frequency dependence of complex viscosity (μ*) and the shear rate dependence of apparent viscosity (μ_a) are similar at the same corresponding values of frequency (measured in radians s^{-1}) and shear rate (s^{-1}) (30).

In the plot of frequency dependence as a function of the phase angle of EONE3 (**Figure 2**), it was observed that the phase angle decreased, as the frequency increased over the whole range of frequencies, which indicates that in EONE3, there was no damage to the gel network. Moreover, this suggested that EONE3 did not behave as a true gel, as described by Mezger (36). Terpenes, when solubilized in the palisade layer, could decrease the repulsive forces on the micellar surface and finally allow closer packing of the surfactant monomers in the aggregates, resulting in the formation of a weak gel, as in this NE (26, 28, 36).

Antimicrobial Activity

The broth microdilution method was used to determine the MICs and sub-MICs of OEO and its NE against *P. aeruginosa, S. aureus, L. monocytogenes,* and *E. coli* (**Figures 3A–D**). The sub-MICs of OEO NEs were identified for use in the quorum-sensing inhibition assay. EO concentrations as high as 5 mg/ml could not inhibit the growth of *P. aeruginosa* and *S. aureus* (**Figures 3A,B**). However, EONE3 had a greater inhibitory effect against these microorganisms than the EO, and sub-MICs were also identified.

The antimicrobial or other biological activities of OEOs has been deeply studied; there is a direct correlation between chemical composition and biological properties (3, 5, 23). The antimicrobial effects are related to phenolic compounds, monoterpenes hydrocarbon, total monoterpenes, and sesquiterpenes. Argentinean OEOs are rich in acyclic compounds and sesquiterpenoids. In previous studies, terpenes as p-cymene and thymol were mainly responsible for the antimicrobial activity. Those results revealed that not only gram-positive bacteria but also gram-negative bacteria showed sensitivity to these oils (3, 6). Thymol acts as a transmembrane carrier of monovalent cations by exchanging their hydroxyl proton for another ion. Cyemene does not have this property but acts synergistically, expanding the membrane. Terpineol has a hydroxyl group, but it does not possess high antimicrobial activity, probably because of the absence of delocalized electron system of double bonds (48, 49). Moreover, the synergistic action taking place among the components of an EOs has greater antimicrobial activity than the major components alone (5, 49, 50).

NEs interact with the lipids of microorganisms to cause cell death (51). The electrostatic attraction can improve their chances of combining with charges on the pathogen surface. When NEs combine with the microorganism, they discharge some portion of their interior contents resulting in cell lysis (52). Emulsification enhanced the dispersibility of EO in aqueous solution, and its physicochemical stability, therefore, increased its antimicrobial activity (53).

The obtained results show that *E. coli* (MIC of 5 and 2 mg/ml EO and EONE3) and *L. monocytogenes* (MIC 5 mg EO/ml) (**Figures 3C,D**) were the most sensitive to both OEO and NE. The concentration of EO in the NE was 160 mg/G, 6.25 times lower than that of pure EO. Moreover, sub-MICs of the NE were found for both microorganisms at lower

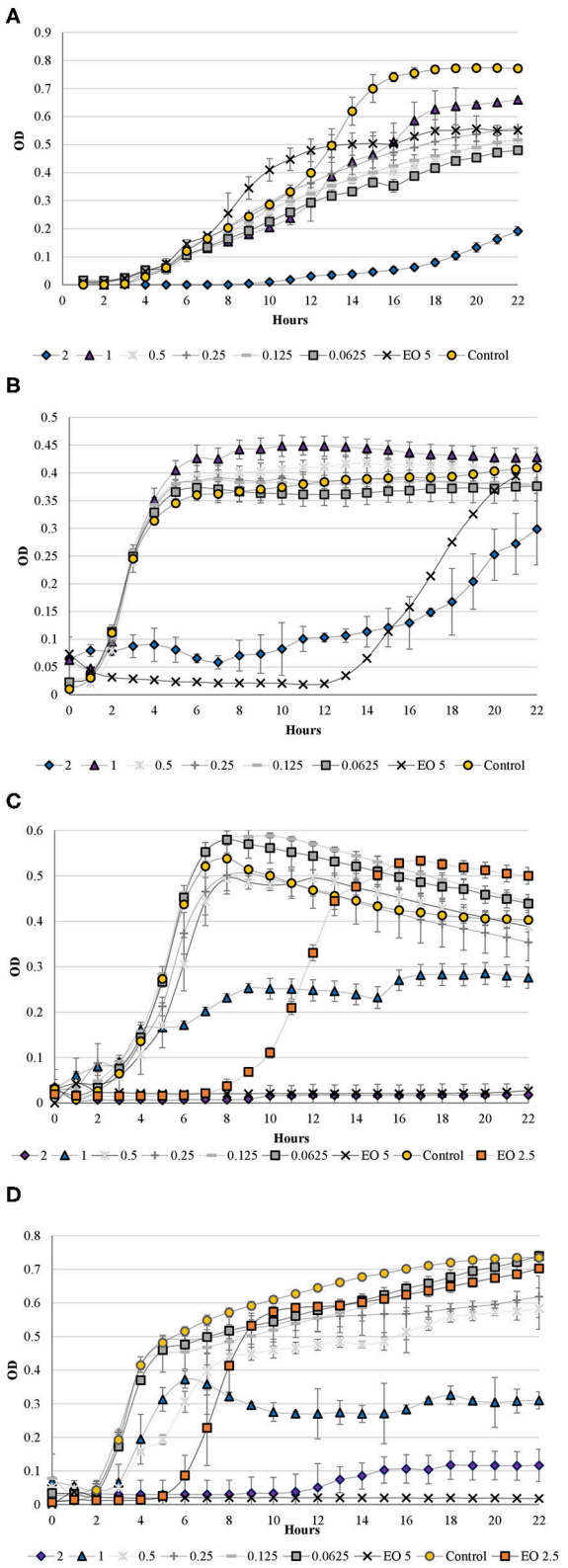

FIGURE 3 | Antimicrobial activity of oregano essential oil (EO) (5 and 2.5 mg/ml) and oregano nanoemulsion (concentrations: 0.0625 to 2 mg/ml) against: **(A)** *P. aeruginosa,* **(B)** *S. aureus,* **(C)** *E. coli,* and **(D)** *L. monocytogenes*.

concentrations than for pure EO. It was observed for *E.coli* that 5 mg/ml of OEO and 2 mg/ml EONE3 inhibited the growth; in the same way, 2.5-mg/ml EO inhibited the growth of bacteria for 8 h, and 1 mg/ml of EONE3 allowed the growth of bacteria but at one half the concentration than the control sample. Regarding the gram-negative *P. aeruginosa,* no MIC was found. However, 2-mg/ml EONE3 inhibited the growth of cells for 9 h, and an OD much lower than the OD registered for the control sample was observed at the end of the study. *P. aeruginosa* has various virulence mechanisms and a diverse metabolic capacity, which makes it resistant to antibiotics because of its impermeable outer membrane, efflux capabilities, tendency to colonize surfaces in a biofilm form, and ability to acquire and maintain antibiotic plasmids (54). Gram-negative bacteria have a complex structure of membrane phospholipids, proteins, and lipid-based peptidoglycan. The presence of an outer hydrophilic membrane embedded with lipopolysaccharide molecules on gram-negative bacteria serves as an effective protective barrier toward macromolecules and hydrophobic compounds (55, 56). However, it was demonstrated that highly lipophilic compounds penetrate easily through the outer membrane of several bacteria (49). The peptidoglycan layer of the gram-positive *L. monocytogenes* is not as effective as a barrier against antimicrobial agents. In this research, the antimicrobial influence of pure EO (MIC 5 mg/ml) was observed. However, a complete inhibitory effect was also observed for EONE3 (2 mg/ml) for 12 h of incubation, and substantially lower growth of cells was observed in the treatment with 1-mg/ml EONE3. In a different study, the MIC and MBC of an OEO for *L. monocytogenes* and *E. coli* were 50 μl/ml, whereas those of the OEO NE were 10 and 15 μl/ml for *L. monocytogenes* and *E. coli*, respectively. EO NEs were observed to be more effective against *E. coli* and *L. monocytogenes* than the non-encapsulated EOs applied directly (57), as it was found in this study. Nanoencapsulation was observed to reduce the MIC and MBC of oregano, rosemary, and cinnamon EOs by an average of 50% (57). In a different study, a blended clove/cinnamon EO NE showed higher antimicrobial activity against *E. coli*, *Bacillus subtilis*, *S. typhimurium*, and *S. aureus* than their individual non-NE counterparts, even at far lower concentrations (58). The antimicrobial effects of thyme NEs against foodborne pathogens were significantly higher ($P < 0.05$) than the pure EO (59). On the contrary, in a different study where sage EO and its NEs were tested as antimicrobials against fish spoilage bacteria, it was found that pure sage EO had more effectiveness than the NE form (25). This confirms that the bioactivity of NEs based on EO varies with droplet size, emulsion formulation, EO chemical composition, viscosity, and microbial strain (60).

Quorum-Sensing Inhibitory Effects of Essential Oil Nanoemulsions

Inhibition of violacein production of *C. violaceum* is commonly used as an indicator of QS inhibition in gram-negative bacteria (31, 32). The QS mechanism in *C. violaceum* ATCC 12472 is controlled by the CviI/CviR system (LuxI/LuxR homologs),

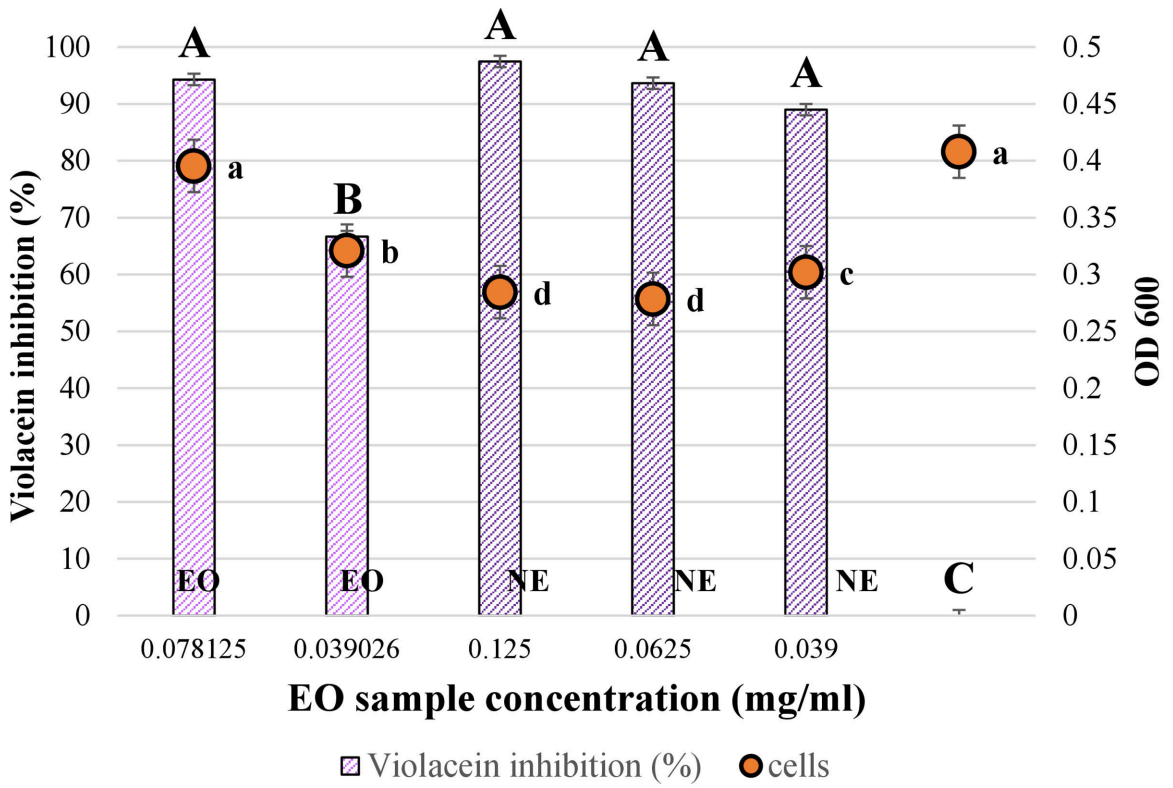

FIGURE 4 | Quorum sensing inhibition assay of oregano essential oil (EO) and oregano nanoemulsion (NE) against *Chromobacterium violaceum*. Percentage of violacein inhibition (%) by at different concentrations and evaluation of microbial viability (OD600) after 36-h incubation in the presence of the OE and NE. Bars and points labeled with different letters indicate significant differences ($p < 0.05$).

correlated with the production of the purple pigment violacein in response to threshold concentrations of the autoinducer N-hexanoyl homoserine lactone (61). EONEs at sub-MICs were tested. A significant reduction in the violet pigment and colorless colorations ($p < 0.05$) were observed at different NE concentrations. The concentrations of EO in the NE that inhibited 97.4–88.9% of violacein production ranged from 0.125 to 0.039 mg EO/ml. Similar concentrations of pure EO (0.078 mg/ml and 0.039 mg EO/ml) inhibited 94.3 and 66.67% violacein coloration. These results demonstrated that when the concentration of EO decreases, violacein inhibition also decreases but differently depending on if the EO is pure or in the NE. No significant differences in violacein inhibition were registered between the tested NE concentrations ($p > 0.05$); all the tested concentrations produced a similar effect on violacein coloration. Similar results were observed when thyme EO, carvacrol, and thymol caused 90, 80, and 78% inhibition of violacein synthesis, respectively, after 72 h of culturing (62). The inhibition of violacein production by *C. violaceum* ATCC 12472 by carvacrol was 40% at 0.7 mM (equal to 0.105 mg/ml) (63). In a different study, cumin oil NE exhibited 42.2% inhibition of violacein production at 40 µl/ml, whereas pepper oil showed 15.8% inhibition at 50 µl/ml. In that case, cumin EO NE showed higher bioactivity than pepper EO NE (64).

In a different study, where cumin and fennel oil emulsions were tested as QS inhibitors by disc diffusion method, 50 µl exhibited anti-QS activity through violacein inhibition around the discz. Those emulsions showed the immediate zone of clearance, causing bactericidal effect followed by the opaque, halo zone of clearance, which indicated the inhibition of violacein production (65). Comparable results were observed in this research, where a slight decrease in the cell viability was observed when the NE was applied as a QS inhibitor ($p < 0.05$). However, a concentration as low as 0.039 mg/ml reduced cell growth but inhibited nearly 90% cell communication (**Figure 4**). These data suggested that AHL synthesis was probably altered and violacein inhibited by the presence of EONE3 in a dose-dependent manner. Contrary, the cell viability of *C. violaceum* showed no significant difference among control and cultures treated with carvacrol ($P \geq 0.05$). Carvacrol inhibited the production of violacein product of QS, indicating its interference with QS systems (63). The QS inhibition with original systems like this NE can be an innovative, fresh technique to control food spoilage and to reduce the number of antimicrobials in food.

CONCLUSION

Physically stable oil-in-water NEs can be produced using OEO and MCT at different MCT/OEO concentrations. As the OEO

concentration increases, the NE droplet size and PDI decreases. EONE3 (160 mg/G NE) is the most stable NE, which shows the smallest droplet size and PDI value after storage. The viscosity of the NE increases, as the concentration of OEO increases, leading to the shear-thinning behavior of the NEs. This effect can be attributed to the presence of OEO terpenes that may solubilize in the palisade layer and change the curvature of the micellar surface, leading to the formation of a WLM structure. Increasing the OEO concentration induces a predominantly solid-like viscoelastic behavior. For EONE3, a weak gel structure can be prepared. EONE presented higher antimicrobial activity than pure EO. Furthermore, a reduction in the intensity of violet pigment produced by *C. violaceum* and no effect on cell growth at concentrations lower than 0.125 mg EO/ml was produced, suggesting that QS in this gram-negative model might be inhibited.

This study provides information about the stability and viscosity and helps to understand the viscoelastic behavior of a NE when the EO concentration varies. These OEO NEs can be used as a novel food preservation technique, reducing cell-to-cell communication (QS) of gram-negative bacteria to lessen food deterioration.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

CA designed the experiments, did the experimental part, data analyzing, and manuscript writing. PQ did data analyzing and manuscript writing of the rheological behavior study section. AA-G taught and collaborated with CA in the antimicrobial activity and quorum sensing inhibition experiments. QH advised and taught CA during the interneship where experiments where carried out. NG supported the study and revised and corrected the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

We thank CONICET (PIP 11220170101065CO) that supported this study and the internship at the University of Rutgers. This work was also supported by SECYT-UNC (411/2018), MinCyT (PIODO Resol. 58/18), and FONCYT (PICT 2017-2134, Res. 310-18).

ACKNOWLEDGMENTS

Thank you to Dr. Maria Corrandini (Department of Food Science, University of Guelph) and Dr. Hiujuan Zheng, who kindly advised me.

REFERENCES

1. Hu K, Renly S, Edlund S, Davis M, Kaufman J. A modeling framework to accelerate food-borne outbreak investigations. *Food Control.* (2016) 59:53–8. doi: 10.1016/j.foodcont.2015.05.017
2. Moghimi R, Ghaderi L, Rafati H, Aliahmadi A, Mcclements DJ. Superior antibacterial activity of nanoemulsion of thymus daenensis essential oil against *E. coli. Food Chem.* (2016) 194:410–5. doi: 10.1016/j.foodchem.2015.07.139
3. Asensio CM, Gallucci N, De Las Oliva M, Demo MS, Grosso NR. Sensory and bio-chemical preservation of ricotta cheese using natural products. *Int J Food Sci Technol.* (2014) 49:2692–702. doi: 10.1111/ijfs.12604
4. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – a review. *Food Chem Toxicol.* (2008) 46:446–75. doi: 10.1016/j.fct.2007.09.106
5. Asensio CM, Grosso NR, Juliani HR. Quality characters, chemical composition and biological activities of oregano (*Origanum* Spp.) essential oils from central and southern Argentina. *Indus Crops Products.* (2015) 63:203–13. doi: 10.1016/j.indcrop.2014.09.056
6. Asensio CM, Grosso NR, Juliani HR. Quality preservation of organic cottage cheese using oregano essential oils. *LWT Food Sci Technol.* (2015) 60:664–71. doi: 10.1016/j.lwt.2014.10.054
7. Grosso AL, Asensio CM, Nepote V, Grosso NR. Antioxidant activity displayed by phenolic compounds obtained from walnut oil-cake used for walnut oil preservation. *J Am Oil Chem Soc.* (2018) 95:1409-19. doi: 10.1002/aocs.12145
8. Olmedo R, Nepote V, Grosso NR. Antioxidant activity of fractions from oregano essential oils obtained by molecular distillation. *Food Chem.* (2014) 156:212–9. doi: 10.1016/j.foodchem.2014.01.087
9. Riveros CG, Nepote V, Grosso NR. Thyme and basil essential oils included in edible coatings as a natural preserving method of oilseed kernels. *J Sci Food Agric.* (2016) 96:183–91. doi: 10.1002/jsfa.7080
10. Asensio CM, Paredes AJ, Martin MP, Allemandi DA, Nepote V, Grosso NR. Antioxidant stability study of oregano essential oil microcapsules prepared by spray-drying. *J Food Sci.* (2017) 82:2864–72. doi: 10.1111/1750-3841.13951
11. Silva LM, Hill LE, Figueiredo E, Gomes CL. Delivery of phytochemicals of tropical fruit by-products using poly (Dl-lactide-co-glycolide) (PLGA) nanoparticles: synthesis, characterization, and antimicrobial activity. *Food Chem.* (2014) 165:362–70. doi: 10.1016/j.foodchem.2014.05.118
12. Almadiy A, Gomah A, Nenaah E, Basma A, Al Assiuty E, Moussa A, et al. Chemical composition and antibacterial activity of essential oils and major fractions of four achillea species and their nanoemulsions against foodborne bacteria. *LWT Food Sci Technol.* (2016) 69:529–37. doi: 10.1016/j.lwt.2016.02.009
13. Mohammadi A, Hashemi M, Hosseini SM. Nanoencapsulation of zataria multiflora essential oil preparation and characterization with enhanced antifungal activity for controlling botrytis cinerea, the causal agent of gray mould disease. *Innovative Food Sci Emerg Technol.* (2015) 28:73–80. doi: 10.1016/j.ifset.2014.12.011
14. Alonso D, Miquel G, Sepúlveda-Sánchez JD, Keiko S. Chitosan-based microcapsules containing grapefruit seed extract grafted onto cellulose fibers by a non-toxic procedure. *Carbohydrate Res.* (2010) 345:854–9. doi: 10.1016/j.carres.2010.01.018
15. Acevedo-fani A, Salvia-trujillo L, Rojas-graü MA, Martín-belloso O. Edible films from essential-oil-loaded nanoemulsions: physicochemical characterization and antimicrobial properties. *Food Hydrocolloids.* (2015) 47:168–77. doi: 10.1016/j.foodhyd.2015.01.032
16. Ezhilarasi PN, Karthik P, Chhanwal N, Anandharamakrishnan C. Nanoencapsulation techniques for food bioactive components: a review. *Food Bioprocess Technol.* (2013) 6:628–47. doi: 10.1007/s11947-012-0944-0
17. Herculano ED, de Paula HCB, de Figueiredo ET, Dias FGB, de Pereira VA. Physicochemical and antimicrobial properties of nanoencapsulated eucalyptus staigeriana essential oil. *LWT Food Sci Technol.* (2015) 61:484–91. doi: 10.1016/j.lwt.2014.12.001

18. McClements DJ, Rao J. Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Crit Rev Food Sci Nutr.* (2011) 51:285–330. doi: 10.1080/10408398.2011.559558
19. Rao J, McClements DJ. Food-grade microemulsions, nanoemulsions and emulsions: fabrication from sucrose monopalmitate & lemon oil. *Food Hydrocolloids.* (2011) 25:1413–23. doi: 10.1016/j.foodhyd.2011.02.004
20. Syed I, Banerjee P, Sarkar P. Oil-in-water emulsions of geraniol and carvacrol improve the antibacterial activity of these compounds on raw goat meat surface during extended storage at 4 °C. *Food Control.* (2020) 107:106757. doi: 10.1016/j.foodcont.2019.106757
21. Alexandre EMC, Lourenço RV, Quinta Barbosa Bittante AM, Freitas Moraes IC, do Amaral Sobral PJ. Gelatin-based films reinforced with montmorillonite and activated with nanoemulsion of ginger essential oil for food packaging applications. *Food Packaging Shelf Life.* (2016) 10:87–96. doi: 10.1016/j.fpsl.2016.10.004
22. Walker RM, Gumus CE, Decker EA, McClements DJ. Improvements in the formation and stability of fish oil-in-water nanoemulsions using carrier oils: MCT, thyme oil, & lemon oil. *J Food Eng.* (2017) 211:60–8. doi: 10.1016/j.jfoodeng.2017.05.004
23. Asensio CM, Quiroga PR, Huang Q, Nepote V, Grosso NR. Fatty Acids, Volatile Compounds and Microbial Quality Preservation With an Oregano Nanoemulsion to Extend the Shelf Life of Hake *(Merluccius hubbsi)* Burgers. *Instit. Food Sci. Technol.* (2019) 54:149–60. doi: 10.1111/ijfs.13919
24. Liu T, Liu L. Fabrication and characterization of chitosan nanoemulsions loading thymol or thyme essential oil for the preservation of refrigerated pork. *Int J Biol Macromol.* (2020) 162:1509-15. doi: 10.1016/j.ijbiomac.2020.07.207
25. Yazgan H. Investigation of antimicrobial properties of sage essential oil and its nanoemulsion as antimicrobial agent. *Lwt.* (2020) 130:109669. doi: 10.1016/j.lwt.2020.109669
26. Mitrinova Z, Tcholakova S, Denkov N. Control of surfactant solution rheology using medium-chain cosurfactants. *Coll Surf A Physicochem Eng Aspects.* (2018) 537:173–84. doi: 10.1016/j.colsurfa.2017.10.018
27. Geng XF, Hu XQ, Jia XC, Luo LJ. Effects of sodium salicylate on the microstructure of a novel zwitterionic gemini surfactant and its rheological responses. *Coll Polymer Sci.* (2014) 292:915–21. doi: 10.1007/s00396-013-3137-0
28. Kamada M, Shimizu S, Aramaki K. Manipulation of the viscosity behavior of wormlike micellar gels by changing the molecular structure of added perfumes. *Coll Surf A Physicochem Eng Aspects.* (2014) 458:110–6. doi: 10.1016/j.colsurfa.2014.01.003
29. Zana R, Kaler WE. *Giant Micelles*. Boca Raton, FL: CRC Press (2007). doi: 10.1201/9781420007121
30. Mleko S, Foegeding EA. PH induced aggregation and weak gel formation of whey protein polymers. *J Food Sci.* (2000) 65:139–43. doi: 10.1111/j.1365-2621.2000.tb15969.x
31. Truchado P, Castro-Ibañez I, Allende A. Plant food extracts and phytochemicals: their role as quorum sensing inhibitors. *Trends Food Sci Technol.* (2015) 43:189–204. doi: 10.1016/j.tifs.2015.02.009
32. Zhang Y, Kong J, Xie Y, Guo Y, Cheng Y, Qian H. Essential oil components inhibit bio film formation in erwinia carotovora and pseudomonas fluorescens via anti-quorum sensing activity. *LWT Food Sci Technol.* (2018) 92:133–9. doi: 10.1016/j.lwt.2018.02.027
33. Hou H, Wang Y, Zhang G, Zhu Y, Xu L, Hao H, et al. Effects of sulfide flavors on AHL-mediated quorum sensing and biofilm formation of hafnia alvei. *J Food Sci.* (2018) 83:2550–9. doi: 10.1111/1750-3841.14345
34. Lamberte LE, Cabrera EC, Rivera WL. Activity of the ethanolic extract of propolis (EEP) as a potential inhibitor of quorum sensing-mediated pigment production in *Chromobacterium violaceum* and virulence factor production in *Pseudomonas aeruginosa*. *Philippine Agric Sci.* (2011) 94:14–22.
35. Adams RP. *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy.* Carol Stream, IL: Allured Pub. Corporation (1995).
36. Mezger TG. *The Rheology Handbook 2nd Revised Edition. European Coatings and Tech Files.* Hanover (2006).
37. Kumar N, Mandal A. Oil-in-water nanoemulsion stabilized by polymeric surfactant: characterization and properties evaluation for enhanced oil recovery. *Eur Polymer J.* (2018) 109:265–76. doi: 10.1016/j.eurpolymj.2018.09.058
38. Zhang Y, Algburi A, Wang N, Kholodovych V, Oh DO, Chikindas M, et al. Self-assembled cationic amphiphiles as antimicrobial peptides mimics: role of hydrophobicity, linkage type, and assembly state. *Nanomed Nanotechnol Biol Med.* (2017) 13:343–52. doi: 10.1016/j.nano.2016.07.018
39. Algburi A, Zehm S, Netrebov V, Bren AB, Chistyakov V, Chikindas ML. Subtilosin prevents biofilm formation by inhibiting bacterial quorum sensing. *Probiotics Antimicrobial Proteins.* (2017) 9:81–90. doi: 10.1007/s12602-016-9242-x
40. Algburi A, Volski A, Chikindas ML. Natural antimicrobials subtilosin and lauramide arginine ethyl ester synergize with conventional antibiotics clindamycin and metronidazole against biofilms of *Gardnerella vaginalis* but not against biofilms of healthy vaginal lactobacilli. *Pathog Dis.* (2015) 73:1–12. doi: 10.1093/femspd/ftv018
41. Di Rienzo JA, Casanoves F, Balzarini MG, Gonzales L, Tablada M, Robledo CW. *InfoStat*. Cordoba: Centro de Transferencia InfoStat; FCA; Universidad Nacional de Córdoba (2019). Available online at: www.infostat.com.ar
42. Prieto MC, Lapaz MI, Lucini EI, Pianzzola MJ, Grosso NR, Asensio CM. Thyme and suico essential oils: promising natural tools for potato common scab control. *Plant Biol.* (2019) 22:81–9. doi: 10.1111/plb.13048
43. Dambolena JS, Zunino MP, Lucini EI, Olmedo R, Banchio E, Bima PJ, et al. Total phenolic content, radical scavenging properties, and essential oil composition of origanum species from different populations. *J Agric Food Chem.* (2010) 58:1115–20. doi: 10.1021/jf903203n
44. Qian C, McClements DJ. Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: factors affecting particle size. *Food Hydrocolloids.* (2011) 25:1000–8. doi: 10.1016/j.foodhyd.2010.09.017
45. Pongsumpun P, Iwamoto S, Siripatrawan U. Response surface methodology for optimization of cinnamon essential oil nanoemulsion with improved stability and antifungal activity. *Ultrasonics Sonochem.* (2020) 60:104604. doi: 10.1016/j.ultsonch.2019.05.021
46. Rodriguez-Abreu C, Aramaki K, Tanaka Y, Lopez-Quintela MA, Ishitobi M, Kunieda H. Wormlike micelles and microemulsions in aqueous mixtures of sucrose esters and nonionic cosurfactants. *J Coll Interface Sci.* (2005) 291:560–9. doi: 10.1016/j.jcis.2005.05.018
47. Talukdar MM, Vinckier I, Moldenaers P, Kinget R. Rheological characterization of xanthan gum and hydroxypropylmethyl cellulose with respect to controlled-release drug delivery. *J Pharm Sci.* (1996) 85:537–40. doi: 10.1021/js950476u
48. Ultee A, Bennik MHJ, Moezelaar R. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen bacillus cereus. *Appl Environ Microbiol.* (2002) 68:1561–8. doi: 10.1128/AEM.68.4.1561-1568.2002
49. Gallucci MN, Oliva M, Casero C, Dambolena J, Luna A, Zygadlo J, et al. Antimicrobial combined action of terpenes against the food-borne microorganisms *Escherichia coli*, *Staphylococcus aureus* and bacillus cereus. *Flavour Fragrance J.* (2009) 24:348–54. doi: 10.1002/ffj.1948
50. Camiletti BX, Asensio CM, Gadban LC, Giménez Pecci MP, Conles MY, Lucini E. I. Essential oils and their combinations with iprodione fungicide as potential antifungal agents against withe rot (*Sclerotium cepivorum* Berk) in garlic (*Allium sativum* L) crops. *Indus Crops Products*. (2016) 85:117–24. doi: 10.1016/j.indcrop.2016.02.053
51. Pathania R, Khan H, Kaushik R, Khan MA. Essential oil nanoemulsions and their antimicrobial and food applications. *Curr Res Nutr Food Sci.* (2018) 6:626–43. doi: 10.12944/CRNFSJ.6.3.05
52. Severino R, Ferrari G, Vu KD, Donsì F, Salmieri S, Lacroix M. Antimicrobial effects of modified chitosan based coating containing nanoemulsion of essential oils, modified atmosphere packaging and gamma irradiation against *Escherichia coli* O157:H7 and *Salmonella typhimurium* on green beans. *Food Control.* (2015) 50:215–22. doi: 10.1016/j.foodcont.2014.08.029
53. Li Z, Cai M, Liu Ys, Sun Pl. Development of finger citron (*Citrus medica* L. *Var. Sarcodactylis*) essential oil loaded nanoemulsion and its antimicrobial activity. *Food Control.* (2018) 94:317–23. doi: 10.1016/j.foodcont.2018.07.009
54. Letsididi KS, Lou Z, Letsididi R, Mohammed K, Maguy BL. Antimicrobial and antibiofilm effects of trans-cinnamic acid nanoemulsion and its potential application on lettuce. *Lwt.* (2018) 94:25–32. doi: 10.1016/j.lwt.2018.04.018
55. Liew SN, Utra U, Alias AK, Tan TB, Tan CP, Yussof NS. Physical, morphological and antibacterial properties of lime essential oil

nanoemulsions prepared via spontaneous emulsification method. *Lwt.* (2020) 128:109388. doi: 10.1016/j.lwt.2020.109388
56. Burt S. Essential oils: their antibacterial properties and potential applications in foods–a review. *Int J Food Microbiol.* (2004) 94:223–53. doi: 10.1016/j.ijfoodmicro.2004.03.022
57. Dávila-Rodríguez M, López-Malo A, Palou E, Ramírez-Corona N, Jiménez-Munguía MT. Antimicrobial activity of nanoemulsions of cinnamon, rosemary, and oregano essential oils on fresh celery. *Lwt.* (2019) 112:108247. doi: 10.1016/j.lwt.2019.06.014
58. Zhang S, Zhang M, Fang Z, Liu Y. Preparation and characterization of blended cloves/cinnamon essential oil nanoemulsions. *LWT Food Sci Technol.* (2017) 75:316–22. doi: 10.1016/j.lwt.2016.08.046
59. Ozogul Y, Boga EK, Akyol I, Durmus M, Ucar Y, Regenstein JM, et al. Antimicrobial activity of thyme essential oil nanoemulsions on spoilage bacteria of fish and food-borne pathogens. *Food Biosci.* (2020) 36:100635. doi: 10.1016/j.fbio.2020.100635
60. Donsì F, Ferrari G. Essential oil nanoemulsions as antimicrobial agents in food. *J Biotechnol.* (2016) 233:106–20. doi: 10.1016/j.jbiotec.2016.07.005
61. Almeida RED, Molina RDI, Viola CM, Luciardi MC, Nieto Peñalver C, Bardón A, et al. Comparison of seven structurally related coumarins on the inhibition of quorum sensing of *Pseudomonas aeruginosa* and *Chromobacterium violaceum*. *Bioorganic Chem.* (2017) 73:37–42. doi: 10.1016/j.bioorg.2017.05.011
62. Myszka K, Schmidt MT, Juzwa W, Olkowicz M, Czaczyk K. International biodeterioration & biodegradation inhibition of quorum sensing -related bio film of pseudomonas fluorescens KM121 by thymus vulgare essential oil and its major bioactive compounds. *Int Biodeterioration Biodegrad.* (2016) 114:252–9. doi: 10.1016/j.ibiod.2016.07.006
63. Tapia-Rodriguez MR, Hernandez-Mendoza A, Gonzalez-Aguilar GA, Martinez-Tellez MA, Martins CM, Ayala-Zavala JF. Carvacrol as potential quorum sensing inhibitor of *Pseudomonas aeruginosa* and biofilm production on stainless steel surfaces. *Food Control.* (2017) 75:255–61. doi: 10.1016/j.foodcont.2016.12.014
64. Amrutha B, Sundar K, Shetty PH. Spice oil nanoemulsions: potential natural inhibitors against pathogenic *E. coli* and *Salmonella* spp. from fresh fruits and vegetables. *LWT Food Sci Technol.* (2017) 79:152–9. doi: 10.1016/j.lwt.2017.01.031
65. Venkadesaperumal G, Rucha S, Sundar K, Shetty PH. Anti-quorum sensing activity of spice oil nanoemulsions against food borne pathogens. *LWT Food Sci Technol.* (2016) 66:225–31. doi: 10.1016/j.lwt.2015.10.044

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.

frontiers in Nutrition

REVIEW
published: 23 November 2020
doi: 10.3389/fnut.2020.595964

Natural Compounds With Antibacterial Activity Against *Cronobacter* spp. in Powdered Infant Formula: A Review

Gökçe Polat Yemiş[1] *and Pascal Delaquis*[2]*

[1] Department of Food Engineering, Sakarya University, Serdivan, Turkey, [2] Summerland Research and Development Research Centre, Agriculture and AgriFood Canada, Summerland, BC, Canada

Bacteria from the genus *Cronobacter* are opportunistic foodborne pathogens capable of causing severe infections in neonates, the elderly and immunocompromised adults. The majority of neonatal infections have been linked epidemiologically to dehydrated powdered infant formulas (PIFs), the majority of which are manufactured using processes that do not ensure commercial sterility. Unfortunately, the osmotolerance, desiccation resistance, mild thermotolerance and wide-ranging minimum, optimum and maximum growth temperatures of *Cronobacter* spp. are conducive to survival and/or growth during the processing, reconstitution and storage of reconstituted PIFs. Consequently, considerable research has been directed at the development of alternative strategies for the control of *Cronobacter* spp. in PIFs, including approaches that employ antimicrobial compounds derived from natural sources. The latter include a range of phytochemicals ranging from crude extracts or essential oils derived from various plants (e.g., thyme, cinnamon, clove, marjoram, cumin, mint, fennel), to complex polyphenolic extracts (e.g., muscadine seed, pomegranate peel, olive oil, and cocoa powder extracts), purified simple phenolic compounds (e.g., carvacrol, citral, thymol, eugenol, diacetyl, vanillin, cinnamic acid, trans-cinnamaldehyde, ferulic acid), and medium chain fatty acids (monocaprylin, caprylic acid). Antimicrobials derived from microbial sources (e.g., nisin, other antibacterial peptides, organic acids, coenzyme Q_0) and animal sources (e.g., chitosan, lactoferrin, antibacterial peptides from milk) have also been shown to exhibit antibacterial activity against the species. The selection of antimicrobials for the control of *Cronobacter* spp. requires an understanding of activity at different temperatures, knowledge about their mode of action, and careful consideration for toxicological and nutritional effects on neonates. Consequently, the purpose of the present review is to provide a comprehensive summary of currently available data pertaining to the antibacterial effects of natural antimicrobial compounds against *Cronobacter* spp. with a view to provide information needed to inform the selection of compounds suitable for control of the pathogen during the manufacture or preparation of PIFs by end users.

Keywords: natural, antimicrobials, *Cronobacter*, safety, powdered infant formula

OPEN ACCESS

Edited by:
Maria João Fraqueza, University of Lisbon, Portugal

Reviewed by:
Ben Davies Tall, United States Food and Drug Administration, United States
Dejan S. Stojkovic, University of Belgrade, Serbia

***Correspondence:**
Pascal Delaquis
pascal.delaquis@canada.ca

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

Received: *18 August 2020*
Accepted: *08 October 2020*
Published: *23 November 2020*

Citation:
Polat Yemiş G and Delaquis P (2020) Natural Compounds With Antibacterial Activity Against Cronobacter spp. in Powdered Infant Formula: A Review. Front. Nutr. 7:595964. doi: 10.3389/fnut.2020.595964

INTRODUCTION

Fatal bacterial infections in neonates caused by "yellow-pigmented coliforms" were first reported in the early 1960s (1). Early clinical isolates were classified as strains of *Enterobacter cloacae* until comparative analysis by DNA–DNA hybridization showed they belong to a distinct species that was initially named *Enterobacter sakazakii* (2). Further genomic analysis by ribotyping, amplified fragment length polymorphism and 16S rDNA sequencing eventually provided evidence to support assignment of *E. sakazakii* and other closely related *Enterobacter* species to the novel genus *Cronobacter* (3). The List of Prokaryotic names with Standing in Nomenclature (LPSN) presently includes seven species of *Cronobacter* with a validly published and correct name, including *Cronobacter sakazakii*, *Cronobacter malonaticus*, *Cronobacter universalis*, *Cronobacter turicensis*, *Cronobacter muytjensii, Cronobacter dublinensis* and *Cronobacter condimenti* (https://www.bacterio.net/genus/cronobacter). *C. sakazakii* is the most frequently reported clinical isolate and is considered to be the prototype species for the genus (4, 5). However, all *Cronobacter* spp. with the exception of *C. condimenti* have been recovered from clinical specimens (6). *C. sakazakii* and *C. malonaticus* are currently the major species of public health concern, followed by *C. turicensis, C. universalis*, *C. muytjensii*, and *C. dublinensis* (5). *Cronobacter* infections typically affect hosts with immature or compromised immune systems, primarily neonates and infants, and to a lesser extent the elderly or individuals from all age groups with underlying chronic disease. Consequently, *Cronobacter* spp. are considered opportunistic human pathogens. Infections in neonates can lead to septicemia, necrotizing enterocolitis or severe meningitis with estimated case-fatality rates of 10, 20, and 42%, respectively, and to severe neurological sequelae upon recovery (7–9). Symptoms of infection in adults include wound and urinary tract infections, gastroenteritis, appendicitis, conjunctivitis, biliary sepsis, pneumonia, septicemia, and osteomyelitis (10). *Cronobacter* infections were considered exceptional and sporadic occurrences until the late 1980s when several clusters were reported in neonatal care units (11). Clinical investigation of a landmark incident in a US hospital showed that neonates were infected by enteral administration of reconstituted powdered infant formula (PIF) (12). Epidemiological investigations of similar incidents in other jurisdictions have confirmed that PIFs can serve as a vehicle for the foodborne transmission of *C. sakazakii* to neonates (13–15). Only one suspected foodborne outbreak involving ostensibly healthy and older individuals has been reported to date. Yong et al. (16) presented evidence that food contaminated with *Cronobacter* spp. consumed in a senior high school canteen led to an outbreak of acute gastroenteritis that resulted in 124 suspected, 12 probable, and 20 confirmed cases. Molecular analysis of isolates recovered from clinical, food, or environmental samples revealed the presence of both *C. sakazakii* (four isolates from two sequence types determined by multilocus sequence typing) and *C. malonaticus* (two isolates from one sequence type). However, the whole genome sequences of two *C. sakazakii* isolates recovered from a food sample and a clinical specimen differed by only five single nucleotide polymorphisms, which was highly suggestive of an epidemiological link.

While *Cronobacter* infections remain uncommon, alarmingly high case-fatality rates in neonates and uncertainty about transmission outside hospital care settings have prompted considerable research to determine the origin, distribution and fate of this emerging foodborne pathogen in food chains. Despite these efforts, the primary habitats of *Cronobacter* spp. remain unknown. Infrequent isolation from livestock and limited survival in the animal gut are indicative of a non-zoonotic nature, although contamination of meat and milk have been reported (17–19). A review and meta-analysis of data published between 2008–14 revealed a prevalence of 5.7% in meat products and 19.0% in plant based foods or food ingredients, which is suggestive of a stronger association with plants or environments in which they are grown (20). Irrespective of their primary habitat, *Cronobacter* spp. have been isolated from diverse dehydrated food products (PIFs, infant cereals, dairy-based preparations, flours, pasta, candies, spices, herbs, and nuts), fresh or frozen vegetables, and both natural (soil, water, insects) and man-made (hospitals, households, food storage and processing facilities) environments (20–23).

Current understanding about the fate of *Cronobacter* spp. in food systems is primarily derived from research concerned with the role of PIFs in foodborne transmission. PIFs intended to serve as complete or partial substitutes for human milk at birth or after the introduction of solid food (follow-up formulas) contain mixtures of protein, fat, carbohydrates, vitamins, minerals, and other functional ingredients (e.g., essential fatty acids, nucleotides) in proportions needed to achieve nutrient contents mandated by national or international regulatory standards. Intact bovine milk powder is the most common source of protein, although specialized formulas containing hydrolyzed casein or proteins derived from plant sources such as soy bean are used for feeding of neonates with underlying pathologies or to accommodate cultural or religious practices (24). Manufacture of PIF products is accomplished by spray-drying of the mixed ingredients solubilized in water (wet processes), by mixing of heat-labile ingredients with a previously spray-dried base powder (dry processes), or by a combination of both approaches (25). Despite the application of heat at one or more stages of these processes PIF is not a sterile food; *Cronobacter* spp. are routinely detected in microbiological analysis of commercial products (26, 27). For example, a recent survey of 128 products in Latin American markets revealed a prevalence rate of 4.7% (28). Microbiological assessments of milk powder and PIF manufacturing environments and processes have shown that *Cronobacter* spp. may derive from extrinsic sources, notably dry ingredients, or intrinsically contaminated sites where specific strains may persist over long periods of time (29–33). Moreover, most strains examined to date have shown higher resistance to potentially lethal osmotic and dessication stresses than other human pathogen belonging to the family Enterobacteriaceae. Dessication resistance likely contributes to the environmental persistence of *Cronobacter* spp. in some niches within manufacturing plants, and to long term survival in powdered milk and PIF (34–36).

The suspected role of PIF in the transmission of infections has prompted examination of *Cronobacter* behavior during reconstitution in water and subsequent handling, including storage for later use. Incipient work by Nazarowec-White and Farber (37) showed that *C. sakazakii* could survive reconstitution with water heated to 52–60°C. Data from additional studies conducted with numerous strains over a wider range of temperatures and in different substrates suggests that most *Cronobacter* spp. are mildly thermotolerant, although strain-associated stress tolerance or prior heat adaptation can enhance thermal stability (38–41). In response to the risk implied by latent contamination of PIFs with infectious bacteria the WHO recommends reconstitution in water at a minimum temperature of 70°C, conditions which have been shown to reduce *C. sakazakii* by >5 $\log_{10}$ cycles (42, 43). Reconstitution at ≥70°C is often impractical, however, as high temperatures can lead to curdling or other undesirable organoleptic changes, cause depletion of heat sensitive nutrients, and introduce scald or burn hazards particularly in home settings (27, 44). Consequently, lower temperatures are endorsed in some jurisdictions despite experimental evidence of limited thermal inactivation at lower temperatures. Moreover, growth of *C. sakazakii* has been reported to occur in reconstituted PIF stored between 5.5 and 47°C, conditions that can occur when feeding is delayed or during storage (15, 21, 41). Additionally, recent work has shown that *C. sakazakii* is readily transferred from caregiver hands and utensils to reconstituted PIF, thereby highlighting the significance of contact surfaces as reservoirs of contamination (45). Adherence to and biofilm formation have been demonstrated on a wide range of materials used in the manufacture of equipment, tools and utensils used in hospital and home settings (46–48). Biofilm formation also contributes to the persistence of *Cronobacter* on surfaces by enhancing resistance to adverse environmental stresses, including chemicals used in cleaning and sanitation of food processing facilities and equipment (49).

The risk of contamination with *Cronobacter* spp. is an enduring food safety challenge for the PIF industry, public health authorities and consumers worldwide. Manufacturers have adopted risk mitigation strategies that primarily rely on rigorous microbiological analysis of raw materials, improved cleaning and sanitation of the manufacturing environment, and enhanced testing of finished products. Despite these efforts, levels of contamination detected through recent surveys clearly show that the risk persists (28). Because the modification of existing industrial processes is constrained by the heat lability of PIF ingredients, alternative non-thermal physical treatments meant to inactivate microbial contaminants without affecting ingredient stability have been investigated or are under study (reviewed in (50, 51)). To date, none have been adapted to the production of PIF on an commercial scale. The use of synthetic preservatives is likewise impractical due to regulatory restrictions and enduring concerns about the negative effects of man-made food additives on human health. Accordingly, natural antibacterial compounds (NACs) derived from plant, microbial or animal sources are under consideration as alternatives to synthetic chemical preservatives for the control of *Cronobacter* spp. in PIF. This approach is aligned with increasing consumer willingness to accept food additives and preservatives of natural origin over synthetic products (52). The present work is intended to provide a summary of current knowledge about NACs with antibacterial activity against *Cronobacter* spp., with a view to inform their application in the development of improved PIF manufacturing processes or the formulation of safer products. For example, NACs that increase the lethality of mild heat may find value in the development of alternative processes to enhance bacterial inactivation in manufacture or during reconstitution by end-users (53). Where possible, the mode of action, toxicological data, regulatory status and potential health benefits of specific NACs are provided.

NACs FROM PLANTS WITH ANTIBACTERIAL ACTIVITY AGAINST *CRONOBACTER* SPP.

The scope of research on plants as sources of natural antimicrobials and their applications in food preservation or safety has expanded significantly in recent years (54). Plant components and extractives thereof are attractive alternative food additives because many have a long history of use, are likely to have received regulatory consent or benefit from the increasing availability of toxicological data needed to support requests for approval (55). Recognition of PIF as a vector for foodborne transmission has led to the assessment of numerous crude extracts, essential oils recovered by distillation of whole plants or their parts, and purified phytochemicals for the control of *Cronobacter* spp. (**Table 1**). Crude extracts obtained in water or an alcoholic solvent are generally not modified after evaporation of the liquid phase and contain several bioactive constituents. For example, a tea extract examined by Li et al. (61) was reported to contain catechins, flavonoids, phenolic acids, anthocyanins, malic acid, and citric acid. The latter is typical of crude plant extracts which tend to consist of complex mixtures of compounds from different classes, notably large and diverse fractions of phenolic compounds referred to collectively as polyphenolics. Most crude extracts listed in **Table 1** contain polyphenolics known to have antibacterial properties, although additive or synergistic effects due to co-extracted plant constituents, notably organic acids, likely contribute to overall antibacterial activity (57). The mode of action of crude extracts is accordingly complex, however damage to the bacterial membrane leading to loss of function and cellular integrity induced by polyphenolics contributes significantly to overall antibacterial effects (83). Two extracts (cocoa powder and polyphenolic tea extract) were shown to exert bacteriostatic effects and reduce the growth of *Cronobacter* spp. in reconstituted PIF. Crude plant extracts are economically attractive food additives due to their low cost of production in comparison purified phytochemicals. However, variability in antibacterial activity due to differences in composition resulting from varietal, agronomic or production factors tend to limit practical applications in foods. Concentrations of compounds with antibacterial activity may also be lower than those of co-extracted compounds

TABLE 1 | Plant-derived crude extracts, essential oils, and purified phytochemicals with antibacterial activity against *Cronobacter* spp.

	Agents with *in vitro* antibacterial activity	Composition	Demonstrated or suggested mode of action	Antibacterial activity in PIF	References
Crude extracts	Aqueous extracts of red muscadine seed; muscadine juice	Malic, tartaric, tannic acids; polyphenols (gallic acid, catechin, epicatechin, ellagic acid, resveratrol)	Not determined. Suggested synergy between components	Not determined	(56, 57)
	Cocoa powder	Polyphenol rich	Not determined	Bacteriostatic in reconstituted PIF incubated at 37°C	(58)
	Proanthocyanidin- rich methanolic blueberry extract; blueberry juice	Not determined	Loss of membrane integrity, altered fatty acid profile, disruption of metabolism	Not determined	(59)
	Methanolic extracts of black pepper and cinnamon bark	Not determined	Inhibition of quorum sensing and biofilm formation at sub-inhibitory concentrations	Not determined	(60)
	Polyphenolic tea extract	Not determined	Membrane damage leading to cytoplasmic leakage; pH effects	Bacteriostatic effect in reconstituted PIF incubated at 37°C; enhanced at pH ≤4	(61)
	Polyphenolic olive oil extract	Hydroxytyrosol, tyrosol, phenolic acids	Reduction of intracellular ATP, membrane depolarization, decreased protein synthesis	Not determined	(62)
	Polyphenolic rich pomegranate peel extract	Elligitannins, α, β-punicalagin, ellagic acid and derivatives, punicalin	Not determined	Not determined	(63)
Essential oils	Cinnamon, clove, lemongrass, laurel, oregano essential oils	Not Determined	Not determined	Not determined	(64)
	Cinnamon, fir essential oils	Nor determined	Not determined. Suggested synergy between phenolic compounds, organic acids and other components	Bacteriostatic effects in reconstituted PIF incubated at 37°C with fir and cinnamon oils; bactericidal effects with fir + cinnamon oil	(65)
	Thyme, clove, ginger extracts obtained by hydrodistillation	Not determined	Not determined	Not determined	(66)
	Thyme, cinnamon, marjoram essential oils	Not determined	Not determined. Suggested synergy between phenolic compounds	Not determined	(67)
Purified phytochemicals	Carvacrol, thymol, eugenol, diacetyl, cinnamic acid	NA	Not determined	Not determined	(68)
	Trans-cinnamaldehyde	NA	No determined. Suggested disruption of bacterial cell membrane	Bactericidal effects in reconstituted PIF incubated at 37 and 8°C; Reduced resistance to acid and heat in reconstituted PIF	(69, 70)
	Vanillin, vanillic acid	NA	Disruption of bacterial cell membrane	Reduced heat resistance during reconstitution; Bactericidal effects in reconstituted PIF incubated at 21 and 10°C	(71, 72)
	Caprylic acid	NA	Disruption of bacterial cell membrane; synergistic effects when used in combination with citric acid or vanillin	Reduced heat resistance during reconstitution; Bacteriostatic effects in reconstituted PIF incubated at 40°C	(73, 74)
	Citral	NA	Reduction of intracellular ATP, cell membrane hyperpolarization, reduction in cytoplasmic pH.	Not determined	(75)
	Syringic acid	NA	Reduction of intracellular ATP, cell membrane hyperpolarization, reduction in cytoplasmic pH.	Not determined	(76)
	Ferulic acid	NA	Disruption of bacterial cell membrane	Not determined	(77)

(Continued)

TABLE 1 | Continued

Agents with *in vitro* antibacterial activity	Composition	Demonstrated or suggested mode of action	Antibacterial activity in PIF	References
Thymoquinone	NA	Disruption of bacterial cell membrane; Reduced stress tolerance; Decreased motility, quorum sensing, endotoxin production, biofilm formation at sub-inhibitory concentrations	Reduced resistance to heat during reconstitution; Reduced resistance to acid, heat, osmotic stress in reconstituted PIF	(78–80)
Carvacrol + citral	NA	Disruption of energy maintenance, membrane repair and proton motive force at sub-inhibitory concentrations	Not determined	(81)
Coenzyme Q_0	NA	Reduction of intracellular ATP; Disruption of bacterial cell membrane; inhibition of biofilm formation	Reduced resistance to heat during PIF reconstitution;	(82)

that can lend undesirable physico-chemical or organoleptic properties to target food products. Similar constraints hamper food applications for essential oils, complex mixtures of volatile lipophilic terpenoids, phenylpropanoids, or short-chain aliphatic hydrocarbon derivatives (84). Results from four scientific reports listed in **Table 1** show that essential oils recovered from several aromatic plant species show antibacterial activity against *Cronobacter* spp. Moreover, Al-Nabulsi et al. (65) found that growth of *C. sakazakii* in reconstituted PIF was inhibited by supplementation with cinnamon or fir essential oils and that mixtures of the two were bactericidal, reducing populations by >6 $\log_{10}$ after 3 h of incubation at 37°C. These results support the widely held view that the activity of essential oils stems from additive or synergistic antimicrobial effects involving multiple components, and underscores the need to determine concentrations of key active compounds needed to ensure consistent activity in foods (85).

Extraction and purification of NACs from plants circumvents challenges occasioned by variable crude extract or essential oil composition. Purified preparations of the phytochemicals listed in **Table 1** are readily available from commercial sources. Most are known to exert antimicrobial effects primarily by disruption of the bacterial cell membrane. Trans-cinnamaldehyde, vanillin, ethyl vanillin, vanillic acid, thymoquinone, and Coenzyme Q_0 have been shown to reduce the thermotolerance of *Cronobacter* spp. during reconstitution and to provide antibacterial effects during subsequent storage. Overall, the reported nature and magnitude of measured effects vary with type of compound, level of PIF supplementation and temperature. For example, Amalaradjou et al. (69) reported that trans-cinnamaldehyde exerts time- and temperature-dependent bactericidal activity against *C. sakazakii* in reconstituted PIF during storage at 4, 8, 23, and 37°C. In contrast, supplementation with caprylic acid could only elicit bacteriostatic effects against the same species in reconstituted PIF stored at 40°C (74). The effects of these compounds on the thermotolerance of *Cronobacter* are more consistent. Amalaradjou et al. (70) showed that complete thermal inactivation of *C. sakazakii* could be achieved by heating reconstituted PIF supplemented with 70 μM trans-cinnamaldehyde at 60°C for 10 min, but that longer heating times were required to achieved the same effect at lower supplementation levels or temperatures. Likewise, *C. sakazakii* was completely inactivated by heating reconstituted PIF supplemented with 30 mmol l^{-1} thymoquinone at 55°C for 10 min, and longer treatment times were needed to achieved the same effect at lower concentrations or temperatures (78). Caprylic acid (73), coenzyme Q_0 (82), vanillin, ethyl vanillin, and vanillic acid (72) have also been found to lower the thermotolerance of *C. sakazakii* during reconstitution. Moreover, the bactericidal activity of vanillin, ethyl vanillin, and vanillic acid were sustained during subsequent storage at 21 and 5°C, which illustrates that some phytochemical compounds can contribute antibacterial effects at multiple stages during the manufacture or preparation of PIF by end-users.

The volatility, susceptibility to conversion and degradation reactions, intense organoleptic character and poor solubility of many plant extracts, essentials oils or phytochemicals have long

TABLE 2 | Natural antimicrobials from microbial sources with antibacterial activity against *Cronobacter* spp.

Antibacterial activity *in vitro*	Demonstrated or suggested mode of action	Antibacterial activity in PIF	References
Cell-free extract of *Lactobacillus acidophilus* grown in caseinate, containing antimicrobial peptides caseicin A & B	Not determined	Bactericidal effect in reconstituted PIF incubated at 37 and 6°C	(87)
Heat-labile bacteriocins in cell-free extracts of *Lactobacillus casei* and *L. acidophilus*	Not determined	Bacteriostatic effect in reconstituted PIF incubated at 37°C	(88)
Cell-free extract of *Lactobacillus acidophilus*	Not determined. Suggested effect of organic acids released by *Lactobacillus acidophilus* during growth	Bactericidal effect in reconstituted PIF incubated at 37°C	(89)
Cell-free extracts of *Lactobacillus kefiri*	Disruption of bacterial cell membrane	Not determined	(90)
Cell-free extracts of *Lactobacillus acidophilus, L. bulgaricus L. casei rhamnosus, L. paracasei L. salivarius*	Disruption of bacterial cell membrane	Not determined	(91)
Nisin +citric acid	Not determined	Bactericidal effect in reconstituted PIF incubated at room temperature when mixed with citric acid	(92)

hindered wider use in food preservation. However, considerable progress has been achieved in the development of novel technologies for the delivery of food additives that are intended to overcome these limitations, notably encapsulation methods that stabilize active components and enable their release of over variable time periods, at specific temperatures or at different sites within food matrices (reviewed in (86)). To date, the merit of these technologies for the delivery of NACs for the control of *Cronobacter* in PIF remains unexplored. Encapsulation platforms that provide controlled release at temperatures applied during the manufacture, preparation or storage of reconstituted PIF could provide the means to ensure delivery when contaminants are most vulnerable to their effects or when the risk of proliferation is highest.

NACs FROM MICROBIAL SOURCES WITH ANTIBACTERIAL ACTIVITY AGAINST *CRONOBACTER* SPP.

Several NACs from microbial sources and their antibacterial activity against *Cronobacter* spp. are shown in **Table 2**. Probiotics, live microorganisms which when administered in adequate amounts confer a health benefit on the host, are presently used in some countries for pediatric care. Commercial PIFs products containing live preparations of *Lactobacillus* or *Bifidobacterium* spp. are available in the marketplace but the effect of probiotic supplementation on the behavior of *Cronobacter* spp. during or after reconstitution is unknown. The use probiotic bacteria for the explicit control of *Cronobacter* spp. infections was investigated by Collado et al. (93) who showed that species of *Lactobacillus, Bifidobacterium* and *Streptococcus* competitively excluded, inhibited and displaced *C. sakazakii* in a human intestinal model system. Despite evidence of their antibacterial properties, there have been few additional attempts to exploit the use of live probiotic bacteria for the control of human pathogens in PIF, likely in response to on-going controversy about the efficacy, safety, variability, quality, labeling, and lack of standards for the use of probiotic products destined for neonatal care (94, 95). NACs derived from microbial sources continue to attract interest, however, notably whole inactivated cells or crude cell extracts that retain bioactivity, which have been termed "parabiotics" (96), or purified soluble factors (products or metabolic byproducts) secreted by live microorganisms or released after lysis, which have been described as "postbiotics" (97). Hayes et al. (87) showed that addition of a crude cell-free extract prepared from a caseinate medium fermented by the common probiotic bacterium *Lactobacillus acidophilus* could inhibit *C. sakazakii* in reconstituted PIF. Antimicrobial effects were attributed to antimicrobial peptides (caseicin A & B) derived from the degradation of bovine casein by microbial enzymes. Charchoghlyan et al. (89) found that a purified heat inactivated aqueous extract of skim milk fermented with a commercial probiotic strain of *Lactobacillus acidophilus* used to supplement PIF also inactivated *C. sakazakii* in the reconstituted product stored at 37°C. The composition of the extract was not provided by the authors of the study, who offered that acids released during fermentation were likely responsible for antibacterial effects. A similar conclusion was reached by Kim et al. (90) in reference to the mechanism responsible for inhibition of *C. sakazakii* by cell free supernatants of laboratory grown cultures of *Lactobacillus kefiri* and *Lactobacillus kefiranofaciens* isolated from kefir, and a commercial probiotic strain of *Bifidobacterium longum*. It must be noted here that current international (e.g., CODEX STAN 72-1981) or national standards for ingredients or additives permissible in PIF do not include D-lactic acid, a metabolic by-product released during fermentation by *L. acidophilus, L., kefiri,* and *L. kefiranofaciens*. Probiotic bacteria such as *Bifidobacterium longum* that do not produce D-lactic acid could be used to circumvent the problem. However, uncertainty concerning the composition and variability in bioactive components in crude cell-free extracts

TABLE 3 | Natural antimicrobials from animal sources with antibacterial activity against *Cronobacter* spp.

Antibacterial activity *in vitro*	Demonstrated or suggested mode of action	Antibacterial activity in PIF	References
Lactoperoxidase	Not determined; Suggested result of oxidation of sulfhydryl groups on enzymes and proteins in cytoplasmic membranes.	Bactericidal effect in reconstituted PIF incubated at 21, 30, 37°C	(101)
Bovine lactoferrin	Not determined	Limited bacteriostatic effect when high concentrations were added to reconstituted PIF stored at 21°C; no effect at 10°C	(99)
Iron-saturated bovine lactoferrin	Not determined. Suggested destabilization of bacterial membrane	Not determined; Limited bacteriostatic activity in whey	(102)
Cationic peptides from enzymatic hydrolysis of lactoferrin	Not determined	Limited bacteriostatic effects in combination with native lactoferrin	(103)
Bicarinalin (cationic peptide from ants)	Disruption of bacterial cell membrane	Not determined	(104)
Camel milk	Not determined	Not determined	(105)

derived from any microbial species is a barrier to broader food applications. The problem can be avoided by the isolation, purification and characterization of antimicrobial compounds that accumulate in growth media. For example, the examination of cell-free supernatants from laboratory grown cultures of *L. acidophilus* and *L. casei* by Awaisheh et al. (88) revealed that both species produce heat-labile bacteriocins, proteinaceous or peptidic molecules with antibacterial activity against *C. sakazakii*. To date, nisin is the only antibacterial peptide to receive close scrutiny for enhancement of PIF safety. Nisin, a polycyclic peptide bacteriocin produced by *Lactococcus lactis*, is presently permitted as a food additive in over 50 countries, primarily for the extension of shelf-life or the prevention of quality defects in dairy products (98). The antibacterial activity of nisin is mainly due to depolarizing effects and consequent disruption of the cytoplasmic cell membrane. Gram-positive bacteria are more resistant to this effect than Gram-negative bacteria in which the cell membrane is surrounded by a lipopolysaccharide outer membrane. Consequently, means to destabilize and permeabilize the outer membrane by physical (mild heat, sonication) or chemical means (metal chelators, EDTA, disodium pyrophosphate, sodium hydrogen orthophosphate, citric acid, lactic acid) are often used in conjunction with nisin to improve antibacterial activity against Gram-negative bacteria such as *Cronobacter* spp. Al-Nabulsi et al. (99) found that reconstitution of PIF at 55°C did not significantly improve the antibacterial activity of nisin against 5 *Cronobacter* strains. In contrast, Campion et al. (92) observed strong bactericidal activity against *C. sakazakii* in reconstituted PIF supplemented with a commercial nisin preparation (Nisaplin) and citric acid, a food additive permitted in infant formula. This finding suggests that antibacterial strategies based on synergistic effects between nisin and additives compliant with regulatory standards for PIF merit further investigation. Corresponding efforts should be directed at the assessment of alternative sources of antimicrobial peptides, including other commercial bacteriocin products or the array of peptides derived from probiotics or bacterial starter cultures described in the scientific literature (100).

NACs FROM ANIMAL SOURCES WITH ANTIBACTERIAL ACTIVITY AGAINST *CRONOBACTER* SPP.

Selected NACs from animal sources and their antibacterial activity against *Cronobacter* spp. are shown in **Table 3**. The first natural antimicrobial derived from animal sources considered for the control of *Cronobacter* spp. in PIF was lactoperoxidase, an enzyme that occurs in milk, colostrum, tears, saliva, and other mammalian secretions (101). Lactoperoxidase catalyzes the oxidation of thiocyanate to hypothiocyanous acid and hypothiocyanate by H_2O_2 and generates intermediate products with antimicrobial properties. The "lactoperoxidase (LPO) system" relies on the interaction of all three components which must be present in sufficient amounts to initiate the reaction. Gurtler and Beuchat (101) showed that addition of bovine LPO to reconstituted milk-based PIF could inhibit the growth of *C. sakazakii* at temperatures >21°C. However, use of the LPO system in PIF is hindered by the need for addition of an exogenous source of thiocyanate, a known goitrogen (106). Another antibacterial protein also found in mammalian secretory fluids, lactoferrin, is not bound by this restriction. Lactoferrin, a small glycoprotein, inhibits bacteria indirectly by the sequestration of iron from the environment and through direct antibacterial effects resulting from disruption of the outer Gram-negative bacterial membrane, leading to alterations in cell permeability and loss of viability (107). Indirect antibacterial effects mediated by alteration of host innate immune functions have also been reported (108). Bovine milk is the most common source of lactoferrin and several manufacturers provide purified preparations for use in pharmaceutical, cosmetic and food applications, including the supplementation of PIF. Usage of lactoferrin supplemented PIF is common in some countries for the prevention of neonatal sepsis and necrotizing enterocolitis despite continued uncertainty about efficacy (109). Experimentation *in vitro* has shown that *Cronobacter* spp. are highly susceptible to the direct antibacterial effects of lactoferrin (99). However, the same authors found no evidence

TABLE 4 | Suggested health-promoting effects, toxicological data, safety assessments and regulatory status of selected natural antimicrobial compounds with antibacterial activity against *Cronobacter* spp. in PIF.

NAC	Reported Health Benefits	Toxicology/safety assessments	Regulatory status
Cocoa powder	Prevention of cardiovascular disease; improved blood pressure regulation, insulin resistance and vascular function; increased production of nitric oxide (NO) and antioxidant effects including delayed oxidation of low-density lipoprotein cholesterol, inhibition of ultraviolet-induced DNA oxidation (113).	Chronic dietary exposure not carcinogenic to rats (114); No evidence of toxic effects on the heart, liver, kidney, lungs, testis, and spleen of rats fed high oral doses (115).	Food ingredient.
Polyphenolic tea extracts	Black teas: prevention of cancer; obesity, antioxidant protective and anti-hyperglycemic effects (116). Green teas: prevention of cancer, obesity, metabolic syndrome, type 2 diabetes, cardiovascular diseases (117).	Suspected cytotoxicity of epigallocatechin 3-gallate, the major catechin present in green tea, in adults and children (117, 118).	Generally Recognized as Safe (GRAS) according to US Code of Federal Regulations (USCFR), Title 21, § 182.20, Essential oils, oleoresins (solvent-free), and natural extractives (including distillates (119). Listed as dietary supplements under the Health and Education Act of 1994 (120).
Cinnamon essential oil, trans-cinnamaldehyde	Antitumour, anti-inflammatory and analgesic, anti-diabetic and anti-obesity, antibacterial and antiviral, cardiovascular protective, cytoprotective, neuroprotective, and immunoregulatory effects (121); Treatment of high blood glucose and lipid levels and other symptoms of the metabolic syndrome, polycystic ovary syndrome (PCOS) and inflammatory disorders (122).	Occasional gastrointestinal disorders and allergic reactions reported (121); Potential nephrotoxicity and hepatotoxicity at higher than recommended daily dose (123).	Trans-cinnamaldehyde: USCFR GRAS, § 182.60.
Vanillin, ethyl vanillin, vanillic acid	Antioxidant, anti-inflammatory, antisickling, antimicrobial, and hypolipidemic effects; prevention of cancer, periodontal disease, and bone deterioration (124)	Lack of toxicity at approved levels of intake in foods; Vanillin may induce bronchoconstriction in asthmatics (125), contact dermatitis at high concentrations (126).	Vanilla extracts: USCFR GRAS, §182.20; Vanillin and ethyl vanillin: USCFR GRAS §182.60 (Synthetic flavoring substances and adjuvants, can be from natural sources); Vanillic acid is not listed in the US FDA Code of Federal Regulations; evaluation by the FAO/WHO Expert Committee on Food Additives (JECFA no. 959) yielded "no safety concern at current levels of intake when used as a flavoring agent" (127).
Caprylic acid	Role in the prevention of infection and inflammation as part of lipid emulsions used in parenteral feeding of neonates (128); Prevention of obesity by decreasing energy intake, possible effects on appetite (129).	No evidence of toxic effects at doses up to 10% in the diet (130).	USCFR GRAS §184.1025; Available as a dietary supplement.
Thymoquinone	Anti-inflammatory, antimicrobial, antiparasitic, antioxidant, antihyperglycemic, and anticancer properties (131).	Concentration dependant *in vitro* hepato-toxic effects (132); No evidence of cytotoxicity in rats (133); no evidence of toxicity in humans at daily oral doses up to 28 g/kg (134).	Source plant (*Nigella sativa* L., black seed or black cumin), is listed by USCFR GRAS in § 182.10 (Spices and other natural seasonings and flavorings); Source plant extracts listed as dietary supplements under the Health and Education Act of 1994 (120).
Coenzyme Q_0	Antitumor, anti-inflammatory and anti-angiogenic effects (82).	No evidence of toxicological effects from dietary supplements (135).	Not presently permitted as a food additive. Available as a dietary supplement.
Nisin	Prevention of dental caries (136); anticancer and antibacterial (137).	Effects on the cytoskeleton of keratinocytes derived from normal epithelium; increased blood cholesterol concentrations in rats (138).	USCFR GRAS, §184.1538, antimicrobial for specified uses which do not currently include PIF.
Lactoperoxidase	Inactivation of carcinogens (139); Contributions to cytotoxic effects against human cancer cells (140); Prevention of bone resorption through osteoclastogenesis (141).	Preparations derived from bovine milk could contain proteins which may be allergenic for sensitive individuals.	USCFR GRAS notice granted for lactoperoxidase system as a processing aid for dairy products pursuant to § 170.30 (Eligibility for classification as generally recognized as safe (GRAS).
Bovine lactoferrin	Contributions to cytotoxic effects against human cancer cells (139, 142); Contribution to gut health and immune development in neonates (143, 144); Prevention of acute gastrointestinal and respiratory symptoms in children aged 12–32 months (145).	No adverse effects in rats fed 2,000 mg/kg/day bovine lactoferrin for 13 days (146); Considered safe for human consumption (147).	USCFR GRAS notice granted for cow's milk-derived lactoferrin as an additive ingredient for PIF pursuant to §170.35 (Affirmation of generally recognized as safe (GRAS) status).

(Continued)

TABLE 4 | Continued

NAC	Reported Health Benefits	Toxicology/safety assessments	Regulatory status
Cell free extracts of *Lactobacillus* spp.	Variable, depending on species and nature of extracts; Management of intestinal, respiratory diseases (148); Cytotoxic effects against human cancer cells (149); Immunomodulation, anti-inflammatory, antiproliferative, hepatoprotective effects (97).	No evidence of adverse effects from oral use.	USCFR GRAS notices have been granted for some cell free extracts; Several are available as a dietary supplements.

of antibacterial effects in reconstituted PIF during storage at 10, 21, or 37°C, a result ascribed to interactions with food components that reduced the activity of lactoferrin, notably the divalent cations Ca^{2+}, Mg^{2+}, and Fe^{3+}. Harouna et al. (102) attempted to improve the activity of lactoferrin by saturation with iron cations but the saturated form of the protein had no measurable antibacterial effect against *C. sakzakii*. Numerous novel antimicrobial peptides with enhanced antimicrobial activity have been synthesized through chemical or enzymatic hydrolysis of lactoferrin (110). Harouna et al. (103) prepared lactoferrin hydrolysates using pepsin, chymosin and microbial rennet that exhibited enhanced antibacterial activity against *C. sakazakii* in a microbiological medium. None were effective in reconstituted PIF at 37°C, however, which provided further evidence that PIF ingredients interfere with the activity of proteic or peptidic antimicrobials. The majority of NACs from animal sources considered for the enhancement of PIF safety have been derived from bovine milk. Antimicrobial peptides have been detected in the milk of other food animal species including sheep and goats (111). A recent report that growth of *C. sakazakii* is inhibited by strong, inherent antibacterial factors in camel milk suggested they are likely derived from the protein component (105). A database assembled by Wang et al. (112) lists 1,972 known antimicrobial peptides from animal sources, in addition to 321 from plants and many from fungi, protists or other life forms. One antimicrobial peptide from a non-bovine source, bicarinalin from ants, exhibited stronger bactericidal effects against *C. sakazakii* than ampicillin and tetracycline (104). Clearly, animals are a rich and largely untapped source of NACs that could find value in the enhancement of PIF safety.

NUTRITIONAL, TOXICOLOGICAL, AND REGULATORY CONSIDERATIONS

Commercial PIF products are subject to regulations and regulatory oversight administered by national governments. All are based on the Codex Alimentarius "Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants (CODEX STAN 72-1981)," which provides recommendations regarding the essential composition, nutritional quality and additives in formula. CODEX recommendations are adopted by national governments in various ways, but there is general consistency in the application of compositional and nutritional quality standards for PIF across the globe. In contrast, some disparities exist with respect to the use of additives due to variable legislative, legal or regulatory frameworks within different jurisdictions. In the United States, food ingredients are subject to provisions in the Code of Federal Regulations. Ingredients that are not listed in the Code can obtain Generally Recognized as Safe (GRAS) designation through a notification program which requires petitioners to provide historical and scientific evidence that a substance added to food is considered safe and suitable for exemption from the food additive tolerance requirements of the United States Food and Drug Administration. The GRAS status of selected NACs with antibacterial activity against *Cronobacter* spp. in PIF is shown in **Table 4**, along with health benefits that have been ascribed to them and pertinent toxicological data. The authors recognize that lack of knowledge about the metabolism of food additives old or new, difficulties in determining accurate levels of exposure, and susceptibility to toxicity have historically hindered safety assessments in the neonatal context (150). Likewise, the purported health benefits noted in **Table 4** are largely derived from animal studies or human feeding trials conducted with children or adults, and extrapolation of results to the neonatal situation is challenging.

All NACs from plant sources with antibacterial activity in PIF listed in **Table 4**, with the exception of caprylic acid, are either purified phenolic compounds or contain high concentrations thereof. Vanillin, ethyl vanillin, and trans-cinnamaldehyde have GRAS status, and are widely consumed in food and nutritional supplements. Several vanilla-flavored PIF products containing natural and artificial flavors were once available in the marketplace but have since been withdrawn, although vanilla flavored "toddler" formula is still sold in North America. Whether the level of vanillin or ethyl vanillin supplementation used in these products was sufficient to achieve antibacterial effects against *Cronobacter* during PIF reconstitution or storage, as reported by Polat Yemiş et al. (72) is unknown. Supplementation of PIF with cocoa powder was also discontinued several years ago. The antibacterial activity of cocoa powder against *C. sakzakii* in reconstituted PIF described by Pina-Pérez et al. (58) was likely derived from polyphenols, many of which are known to interact with and damage the bacterial cell membrane (83). Polyphenols encompass several groups of compounds including phenolic acids and flavonoids, primarily flavanols present as monomeric epicatechin, catechin, and their oligomers referred to as proanthocyanidins. Research on the nutritional effects of moderate cocoa consumption suggests that the benefits likely outweigh the risks, and that beneficial effects on health are primarily derived from flavanol-mediated

protection against oxidative insult by the modulation of oxygen radical generation and antioxidant enzyme and non-enzyme defenses (151, 152). There is compelling evidence that most polyphenols are largely beneficial to human health, principally for the prevention and management of chronic diseases (153). On the other hand, the pharmacological properties of some polyphenols introduce concerns about their safety in products intended for use by infants. Isoflavones (genistein, daidzein, and glycitein) derived from soya beans are known activators of estrogen receptors with demonstrable effects on reproductive and endocrine functions in animal models (154). All soy-protein based PIFs contain isoflavones, mainly genistein, but no clear consensus has emerged regarding the short or long term implications of long-term dietary exposure on the development of infants (155, 156). In contrast, consumption of the medium length straight chain fatty acid caprylic acid (octanoic acid) is considered to be comparatively free of toxicological risk (130). Caprylic acid is found naturally in the milk of mammals including humans (157) and in infant formulas as part of the medium chain triglyceride component contributed by vegetable fat, or increasingly bovine milk fat (158). Choi et al. (74) showed that low concentrations of caprylic acid in conjunction with citric acid completely inactivated *C. sakazakii* in PIF during reconstitution at the relatively low temperature of 45°C. A GRAS compound, it is used as an additive in a range of foods as an adjuvant or, interestingly, as a flavoring agent despite having an odor described as "slightly unpleasant and rancid-like." Similarly, the monoterpene diketone thymoquinone, also a GRAS compound, could inactivate *C. sakazakii* during reconstitution (78) but it has a bitter taste and a "pencil-like" odor (159). The ubiquinone coenzyme Q_0 is the only ordorless and tasteless NAC from non-microbial or animal sources identified to date with antibacterial activity against *Cronobacter* spp. Coenzyme Q_0 extracted from the AC mushroom (*Antrodia cinnamomea),* a parasitic fungus that grows of the camphor tree, has a long history of use in traditional medicine but has only recently been considered for food applications. Toxicological assessment of supplements prepared from the fungus suggest they are safe for human consumption (132). However, toxicological assessments of the purified compound are lacking and coenzyme Q_0 does not currently have GRAS status.

The chemistry, biology, toxicology, pharmacokinetic properties, and functionality of nisin as a food preservative have been extensively investigated. A recent reassessment of toxicological data by the European Food Safety Authority (EFSA) reaffirmed the safety of nisin as a food additive (160). Activity against *Cronobacter* spp. in PIF relies on synergism with citric acid (92), but the latter is a permitted additive. Hence, there appear to be few regulatory impediments to the use of nisin in foods destined for infants. As noted above, antibacterial activity of lactoperoxidase, another GRAS food additive that is used worldwide for milk preservation, is dependent on a source of thiocyanate, which Gurtler and Beuchat (101) provided exogenously in the form of sodium thiocyanate. Thiocyanates are ubiquitous in food products, however, and it is unfortunate that no attempt was made to determine if endogenous levels could have sustained the reaction. Evidence in support of this presumption was provided by Banks and Board (161) who found that lactoperoxidase catalyzed degradation of endogenous thiocyanates reduced the growth of *Enterococcus*, *Pseudomonas* spp. and Enterobacteriaceae in reconstituted PIF stored at 30°C for 48 h, which coincided with the depletion of free SCN- ions. These observations suggest that the value of lactoperoxidase for the control of *Cronobacter* spp. in PIF merits further investigation. There are also few regulatory obstacles to the application of lactoferrin in PIF since it is already in use for therapeutic purposes, disease prevention or health promotion in neonates (82, 143, 162), and is available in highly purified forms safe for use in infant foods (163). Cell-free extracts derived from microbial cultures present greater regulatory challenges as noted above due to the multiplicity of bioactive compounds and variable composition of extracts which add complexity to toxicological assessments. In this context, the selection of candidate microorganisms for the production of cell free-extracts among those already permitted as probiotics in PIF or that are considered GRAS on the basis of historical, safe use in food fermentations is highly advisable.

CONCLUSIONS AND FUTURE PROSPECTS

Societal concerns and regulatory response to the risk of exposure to harmful food chemicals in early life provide strong impetus to pursue the search for alternatives. Research on NACs with antibacterial activity against *Cronobacter* spp. has shown that several could find value in the control of this hazardous pathogen in PIF. However, technological obstacles to practical applications persist and means to overcome them must be the focus of future research in the field. The delivery of NACs with strong antibacterial activity to food systems is often hampered by limited solubility in aqueous matrices, instability, reactions with other food components or adverse effects on sensory properties. For example, the low solubility, volatility, intense sensory characteristics, and reactivity of phenolic compounds and essential oils often hinders their incorporation in foods. Adverse effects on the sensory quality of PIF are a notable concern in light of evidence that exposure to flavors modulates neonatal feeding behavior and food acceptability and choice later in life (164, 165). Recent progress in the use of biopolymers from natural sources for the design of innovative encapsulation systems that provide means to deliver effective yet reduced doses of antibacterial agents, protect active ingredients from undesirable reactions, and provide controlled, quantitative release into food matrices will undoubtedly promote the development of delivery strategies that overcome constraints on both the choice and application of NACs (166, 167). The selection of suitable NACs must also be guided by careful consideration of the regulatory framework governing PIF composition, specifically the use of additives. In the near term, single compounds for which nutritional and toxicological data are available should take preference over preparations likely to contain multiple bioactive compounds. The latter remain eminently worthy of study, however, as possible sources of novel NACs. As a final note, it must be emphasized

that the NACs described in the present work were derived from a limited number of animal, plant and bacterial species. Future efforts should be directed at the assessment of additional sources of NACs for the control of *Cronobacter* spp., such as mushroom species known to contain compounds with antibacterial activity against other foodborne pathogens (168).

AUTHOR CONTRIBUTIONS

GP and PD equally contributed to a review of the scientific literature, collection of relevant references, writing, and editing of the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Urmenyi AM, White-Franklin, A. Neonatal death from pigmented coliform infection. *Lancet*. (1961) 1:313–15. doi: 10.1016/S0140-6736(61)91481-7
2. Farmer JJ III, Asbury MA, Hickman, FW, Brenner, DJ, the Enterobacteriaceae Study Group. *Enterobacter sakazakii:* a new species of "Enterobacteriaceae" isolated from clinical specimens. *Int J Syst Bacteriol.* (1980) 30:569–84. doi: 10.1099/00207713-30-3-569
3. Iversen C, Lehner A, Mullane N, Bidlasm E, Cleenwerck I, Marugg J., et al. The taxonomy of *Enterobacter sakazakii*: proposal of a new genus *Cronobacter* gen. nov. and descriptions of *Cronobacter sakazakii* comb. nov. *Cronobacter sakazakii* subsp. *sakazakii,* comb. nov., *Cronobacter sakazakii* subsp. *malonaticus* subsp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov. and *Cronobacter* genomospecies 1. *BMC Evol Biol.* (2007) 7:64. doi: 10.1186/1471-2148-7-64
4. Feeney A, Kropp KA, O'Connor R, Sleator RD. *Cronobacter sakazakii*: stress survival and virulence potential in an opportunistic foodborne pathogen. *Gut Microbes.* (2014) 5:711–18. doi: 10.4161/19490976.2014.983774
5. Henry M, Fouladkhah A. Outbreak history, biofilm formation, and preventive measures for control of *Cronobacter sakazakii* in infant formula and infant care settings. *Microorganisms.* (2019) 7:1–10. doi: 10.3390/microorganisms7030077
6. Jang H, Gopinath GR, Eshwar A, Srikumar S, Nguyen S, Gangiredla J. The secretion of toxins and other exoproteins of *Cronobacter*: role in virulence, adaption, and persistence. *Microorganisms*. (2020) 8:229. doi: 10.3390/microorganisms8020229
7. Healy B, Cooney S, O'Brien S, Iversen C, Whyte P, Nally J, Callanan JJ, Fanning S. *Cronobacter* (*Enterobacter sakazakii*): an opportunistic foodborne pathogen. *Foodborne Pathog Dis.* (2017) 7:339–50. doi: 10.1089/fpd.2009.0379
8. Friedemann M. Epidemiology of invasive neonatal *Cronobacter (Enterobacter sakazakii)* infections. *Eur J Clin Microbiol Infect Dis.* (2009) 28:1297–304. doi: 10.1007/s10096-009-0779-4
9. Forsythe SJ. Updates on the *Cronobacter* genus. *Annu Rev Food Sci Technol.* (2017) 9:23–44. doi: 10.1146/annurev-food-030117-012246
10. Lai KK. *Enterobacter sakazakii* infections among neonates, infants, children and adults: case reports and review of the literature. *Medicine*. (2001) 80:113–22. doi: 10.1097/00005792-200103000-00004
11. Nazarowec-White M, Farber JM. *Enterobacter sakazakii*: a review. *Int J Food Microbiol.* (1997) 34:103–13. doi: 10.1016/S0168-1605(96)01172-5
12. Simmons BP, Gelfand MS, Haas M, Metts L, Ferguson J. *Enterobacter sakazakii* infections in neonates associated with intrinsic contamination of a powdered infant formula. *Infect Control Hosp Epidemiol.* (1989) 10:398–401. doi: 10.1086/646060
13. Clark NC, Hill BC, O'Hara CM, Steingrimsson O, Cooksey RC. Epidemiologic typing of *Enterobacter sakazakii* in two neonatal nosocomial outbreaks. *Diagn Microbiol Infect Dis.* (1990) 13:467–72. doi: 10.1016/0732-8893(90)90078-A
14. Muytjens HL, Roelofs-Willemse H, Jaspar GH. Quality of powdered substitutes for breast milk with regard to members of the family Enterobacteriaceae. *J Clin Microbiol.* (1998) 26:743–46. doi: 10.1128/JCM.26.4.743-746.1988
15. Iversen C, Forsythe S. Isolation of *Enterobacter sakazakii* and other Enterobacteriaceae from powdered infant formula milk and related products. *Food Microbiol.* (2004) 21:771–6. doi: 10.1016/j.fm.2004.01.009
16. Yong W, Guo B, Shi X, Cheng T, Chen M, Jiang X, et al. An investigation of an acute gastroenteritis outbreak: *Cronobacter sakazakii,* a potential cause of food-borne illness. *Front Microbiol.* (2018) 9:2549. doi: 10.3389/fmicb.2018.02549
17. Molloy C, Cagney C, Fanning S, Duffy G. Survival characteristics of *Cronobacter* spp. in model bovine gut and in the environment. *Foodborne Pathog Dis.* (2010) 7:671–5. doi: 10.1089/fpd.2009.0449
18. Zeng H, Li C, Ling N, Zhang J, Chen M, Lei T, et al. Prevalence, genetic analysis and CRISPR typing of *Cronobacter* spp. isolated from meat and meat products in China. *Int J Food Microbiol.* (2020) 321:108549. doi: 10.1016/j.ijfoodmicro.2020.108549
19. Parra-Flores J, Cerda-Leal F, Contreras A, Valenzuela-Riffo N, Rodríguez A, Aguirre J. *Cronobacter sakazakii* and microbiological parameters in dairy formulas associated with a food alert in Chile. *Front Microbiol.* (2018) 9:1708. doi: 10.3389/fmicb.2018.01708
20. Sani NA, Odeyemi OA. Occurrence and prevalence of *Cronobacter* spp. in plant and animal derived food sources: a systematic review and meta-analysis. *SpringerPlus.* (2015) 4:545. doi: 10.1186/s40064-015-1324-9
21. Beuchat LR, Kim H, Gurtler JB, Lin LC, Ryu JH, Richards GM. *Cronobacter sakazakii* in foods and factors affecting its survival, growth, and inactivation. *Int J Food Microbiol.* (2009) 136:204–13. doi: 10.1016/j.ijfoodmicro.2009.02.029
22. Kandhai MC, Reij MW, Gorris LG, Guillaume-Gentil O, van Schothorst M. Occurrence of *Enterobacter sakazakii* in food production environments and households. *Lancet.* (2004) 363:39–40. doi: 10.1016/S0140-6736(03)15169-0
23. Lou X, Liu T, Zhang W, Yu H, Wan, H, Song S, et al. The occurrence and distribution characteristics of *Cronobacter* in diverse cereal kernels, flour, and flour-based products. *Food Microbiol.* (2019) 84:103269. doi: 10.1016/j.fm.2019.103269
24. Maldonado J, Gil A, Narbona E, Molina JA. Special formulas in infant nutrition: a review. *Early Hu Dev.* (1998) 53:23–32. doi: 10.1016/S0378-3782(98)00062-0
25. Masum AKM, Chandrapala J, Huppertz T, Adhikari B, Zisu B. Production and characterization of infant milk formula powders: a review. *Drying Technol.* (2020). doi: 10.1080/07373937.2020.1767645. [Epub ahead of print].
26. World Health Organization. *Safe Preparation, Storage and Handling of Powdered Infant Formula Guidelines*. Geneva: World Health Organization (2017). Available online at: https://www.who.int/foodsafety/publications/powdered-infant-formula/en/ (accessed October 26, 2020).
27. Kent RM, Fitzgerald GF, Hill C, Stanton C, Ross RP. Novel approaches to improve the intrinsic microbiological safety of powdered infant milk formula. *Nutrients.* (2015) 7:1217–44. doi: 10.3390/nu7021217
28. Parra-Flores J, Maury-Sintjago E, Rodriguez-Fernández A, Acuña S, Cerda F, Aguirre J, et al. Microbiological quality of powdered infant formula in Latin America. *J Food Prot.* (2020) 83:534–41. doi: 10.4315/0362-028X.JFP-19-399
29. Reich F, König R, Von Wiese W. Klein G. Prevalence of *Cronobacter* spp. in a powdered infant formula processing environment. *Int J Food Microbiol.* (2010) 140:214–7. doi: 10.1016/j.ijfoodmicro.2010.03.031
30. Jacobs C, Braun P, Hammer P. Reservoir and routes of transmission of *Enterobacter sakazakii* (*Cronobacter* spp.) in a milk powder-producing plant. *J Dairy Sci.* (2011) 94:3801–10. doi: 10.3168/jds.2011-4318
31. Yan Q, Power KA, Cooney S, Fox E, Gopinath G, Grim CJ, et al. Complete genome sequence and phenotype microarray analysis of *Cronobacter sakazakii* SP291: a persistent isolate cultured from a powdered infant formula production facility. *Front Microbiol.* (2013) 4:256. doi: 10.3389/fmicb.2013.00256
32. Pei X, Li Y, Zhang H, Zhan L, Yu X, Lan G, et al. Surveillance and characterisation of *Cronobacter* in powdered infant formula processing factories. *Food Control.* (2019) 96:318–23. doi: 10.1016/j.foodcont.2018.09.009

33. Lu Y, Liu P, Li C, Sha M, Fang J, Gao J, Xu X, Matthews KR. Prevalence and genetic diversity of *Cronobacter* species isolated from four infant formula production factories in China. *Front Microbiol.* (2019) 10:1938. doi: 10.3389/fmicb.2019.01938
34. Gurtler JB, Beuchat LR. Survival of *Enterobacter sakazakii* in powdered infant formula as affected by composition, water activity, and temperature. *J Food Prot.* (2007) 70:1579–86. doi: 10.4315/0362-028X-70.7.1579
35. Caubilla-Barron J, Forsythe S. Dry stress and survival time of *Enterobacter sakazakii* and other Enterobacteriaceae. *J Food Prot.* (2007) 70:2111–7. doi: 10.4315/0362-028X-70.9.2111
36. Breeuwer P, Lardeau A, Peterz M, Joosten HM. Desiccation and heat tolerance of *Enterobacter sakazakii*. *J Appl Microbiol.* (2010) 95:967–73. doi: 10.1046/j.1365-2672.2003.02067.x
37. Nazarowec-White M, Farber JM. Thermal resistance of *Enterobacter sakazakii* in reconstituted dried infant formula. *Lett Appl Microbiol.* (1997) 24:9–13. doi: 10.1046/j.1472-765X.1997.00328.x
38. Osaili TM, Shaker RR, Al-Haddaq MS, Al-Nabulsi AA, Holley RA. Heat resistance of *Cronobacter* species (*Enterobacter sakazakii*) in milk and special feeding formula. *J Appl Microbiol.* (1989) 107:928–35. doi: 10.1111/j.1365-2672.2009.04271.x
39. Arroyo C, Condon S, Pagan R. Thermobacteriological characterization of *Enterobacter sakazakii*. *Int J Food Microbiol.* (2009) 136:110–8. doi: 10.1016/j.ijfoodmicro.2009.09.013
40. Arku B, Fanning S, Jordan K. Heat adaptation and survival of *Cronobacter* spp. (formerly *Enterobacter sakazakii*). *Foodborne Pathog Dis.* (2011) 8:975–81. doi: 10.1089/fpd.2010.0819
41. Huertas JP, Álvarez-Ordóñez A, Morrissey R, Ros-Chumillas M, Esteban MD, Maté J, et al. Heat resistance of *Cronobacter sakazakii* DPC 6529 and its behavior in reconstituted powdered infant formula. *Food Res Int.* (2015) 69:401–9. doi: 10.1016/j.foodres.2015.01.010
42. World Health Organization. *WHO/FAO Guidelines for the Safe Preparation, Storage and Handling of Powdered Infant Formula.* (2006). Available online at: www.ennonline.net/infantformulaguidelines (accessed October 26, 2020).
43. Osaili TM, Al-Nabulsi AA, Shaker RR, Ayyash MM, Olaimat AN, Abu Al-Hasan AS, et al. Effects of extended dry storage of powdered infant milk formula on susceptibility of *Enterobacter sakazakii* to hot water and ionizing irradiation. *J Food Prot.* (2008) 71:934–9. doi: 10.4315/0362-028X-71.5.934
44. Turck D. Safety aspects in preparation and handling of infant food. *Ann Nutr Metab.* (2012) 60:211–4. doi: 10.1159/000338215
45. Cho TJ, Hwang JY, Kim HW, Kim YK, Kwon JI, Kim YJ, et al. Underestimated risks of infantile infectious disease from the caregiver's typical handling practices of infant formula. *Sci Rep.* (2019) 9:9799. doi: 10.1038/s41598-019-46181-0
46. Iversen C, Lane M, Forsythe SJ. The growth profile, thermotolerance and biofilm formation of *Enterobacter sakazakii* grown in infant formula milk. *Lett Appl Microbiol.* (2004) 38:378–82. doi: 10.1111/j.1472-765X.2004.01507.x
47. Lehner A, Riedel K, Eberl L, Breeuwer P, Diep B. Biofilm formation, extracellular polysaccharide production, and cell-to-cell signaling in various *Enterobacter sakazakii* strains: aspects promoting environmental persistence. *J Food Prot.* (2005) 6:2287–94. doi: 10.4315/0362-028X-68.11.2287
48. Hurrell E, Kucerova E, Loughlin M, Caubilla-Barron J, Forsythe SJ. Biofilm formation on enteral feeding tubes by *Cronobacter sakazakii*, *Salmonella* serovars and other Enterobacteriaceae. *Int J Food Microbiol.* (2009) 136:227–31. doi: 10.1016/j.ijfoodmicro.2009.08.007
49. Ling N, Forsythe S, Wu Q, Ding Y, Zhang J, Zeng H. Insights into *Cronobacter sakazakii* biofilm formation and control strategies in the food industry. *Engineering.* (2020) 6:393–405. doi: 10.1016/j.eng.2020.02.007
50. Pina-Pérez MC, Rodrigo D, Martínez A. Nonthermal inactivation of *Cronobacter sakazakii* in infant formula milk: a review. *Crit Rev Food Sci Nutr.* (2016) 56:1620–29. doi: 10.1080/10408398.2013.781991
51. Ahern GJ, Hennessy AA, Ryan CA, Ross R., Stanton C. Advances in infant formula science. *Ann Rev Food Sci Technol.* (2019) 10:75–102. doi: 10.1146/annurev-food-081318-104308
52. Roman S, Sanchez-Siles LM, Siegrist M. The importance of food naturalness for consumers: results of a systematic review. *Trends Food Sci Technol.* (2017) 67:44–57. doi: 10.1016/j.tifs.2017.06.010
53. Gurtler JB, Fan X, Jin T, Niemira BA. Influence of antimicrobial agents on the thermal sensitivity of foodborne pathogens: a review. *J Food Prot.* (2019) 82:628–44. doi: 10.4315/0362-028X.JFP-18-441
54. Quinto EJ, Caro I, Villalobos-Delgado LH, Mateo J, De-Mateo-Silleras B, Redondo-Del-Río MP. Food safety through natural antimicrobials. *Antibiotics*. (2019) 8:208. doi: 10.3390/antibiotics8040208
55. Tajkarimi M, Ibrahim S, Cliver D. Antimicrobial herb and spice compounds in food. *Food Control.* (2010) 21:1199. doi: 10.1016/j.foodcont.2010.02.003
56. Silva JL, Weng WL, Chen WW, Corbitt M, Jung YS, Chen YS. Inactivation of *Enterobacter sakazakii* by water-soluble muscadine seed extracts. *Int J Food Microbiol.* (2009) 129:3295–9. doi: 10.1016/j.ijfoodmicro.2008.12.014
57. Kim TJ, Weng WL, Silva JL, Jung YS, Marshall D. Identification of natural antimicrobial substances in red muscadine juice against *Cronobacter sakazakii*. *J Food Sci.* (2010) 75:150–4. doi: 10.1111/j.1750-3841.2010.01531.x
58. Pina-Pérez MC, Rodrigo D, Martinez A. Bacteriostatic effect of cocoa powder rich in polyphenols to control *Cronobacter sakazakii* proliferation in infant milk formula. Science and Technology against Microbial Pathogens. Research, Development and Evaluation. In: *Proceedings of the International Conference on Antimicrobial research (ICAR 2010)*. Valladolid: World Scientific Publishing Co. Pte. Ltd (2010). p. 85–8.
59. Joshi SS, Howell AB, D'Souza DH. *Cronobacter sakazakii* reduction by blueberry proanthocyanidins. *Food Microbiol.* (2014) 39:127–31. doi: 10.1016/j.fm.2013.11.002
60. Singh N, Patil A, Prabhune A, Goel G. Inhibition of quorum-sensing mediated biofilm formation in *Cronobacter sakazakii* strains. *Microbiology.* (2016) 162:1708–14. doi: 10.1099/mic.0.000342
61. Li R, Fei P, Man CX, Lou BB, Niu, JT, Feng J, et al. Tea polyphenols inactivate *Cronobacter sakazakii* isolated from powdered infant formula. *J Dairy Sci.* (2016) 99:1019–28. doi: 10.3168/jds.2015-10039
62. Fei P, Ali MA, Gong S, Sun Q, Bi X, Liu S, et al. Antimicrobial activity and mechanism of action of olive oil polyphenols extract against *Cronobacter sakazakii*. *Food Control.* (2018) 94:289–94. doi: 10.1016/j.foodcont.2018.07.022
63. Polat Yemiş G, Bach S, Delaquis P. Antibacterial activity of polyphenol-rich pomegranate peel extract against *Cronobacter sakazakii*. *Int J Food Prop.* (2019) 22:985–93. doi: 10.1080/10942912.2019.1622564
64. Franková A, Marounek M, Mozrová V, Weber J, Klouček P, Lukešová D. Antibacterial activities of plant-derived compounds and essential oils toward *Cronobacter sakazakii* and *Cronobacter malonaticus*. *Foodborne Pathog Dis.* (2014). 11:795–7. doi: 10.1089/fpd.2014.1737
65. Al-Nabulsi AA, Awaisheh SS, Osaili TM, Olaimat AN, Rahahaleh RJ, Al-Dabbas FM, et al. Inactivation of *Cronobacter sakazakii* in reconstituted infant milk formula by plant essential oils. *J Appl Bot Food Qual.* (2015) 88:97–101. doi: 10.5073/JABFQ.2015.088.013
66. Abu-Ghazaleh BM. Antimicrobial activity of *Thymus vulgaris* extract, *Syzygium aromaticum* extract and *Zingiber officinale* extract on *Cronobacter* spp. as compared with common preservatives. *Pharmacologyonline Arch.* (2019) 2:1–11.
67. Berthold-Pluta A, Stasiak-Rózańska L, Pluta A, Garbowska M. Antibacterial activities of plant-derived compounds and essential oils against *Cronobacter strains*. *Eur Food Res Technol.* (2019) 245:1137–47. doi: 10.1007/s00217-018-3218-x
68. Lee SY, Jin HH. Inhibitory activity of natural antimicrobial compounds alone or in combination with nisin against *Enterobacter sakazakii*. *Lett Appl Microbiol.* (2008) 47:315–21. doi: 10.1111/j.1472-765X.2008.02432.x
69. Amalaradjou MA, Hoagland TA, Venkitanarayanan K. Inactivation of *Enterobacter sakazakii* in reconstituted infant formula by trans-cinnamaldehyde. *Int J Food Microbiol.* (2009) 129:146–9. doi: 10.1016/j.ijfoodmicro.2008.11.016
70. Amalaradjou MAR, Venkitanarayanan K. Effect of trans-cinnamaldehyde on reducing resistance to environmental stresses in *Cronobacter sakazakii*. *Foodborne Pathog Dis.* (2011) 8:403–9. doi: 10.1089/fpd.2010.0691
71. Polat Yemiş, GP, Pagotto F, Bach S, Delaquis P. Effect of vanillin, ethyl vanillin, and vanillic acid on the growth and heat resistance of *Cronobacter* species. *J Food Prot.* (2011) 74:2062–9. doi: 10.4315/0362-028X.JFP-11-230
72. Polat Yemiş GP, Pagotto F, Bach S, Delaquis P. Thermal tolerance and survival of *Cronobacter sakazakii* in powdered infant formula supplemented

with vanillin, ethyl vanillin, and vanillic acid. *J Food Sci.* (2012) 77:523–7. doi: 10.1111/j.1750-3841.2012.02834.x

73. Jang HI, Rhee MS. Inhibitory effect of caprylic acid and mild heat on *Cronobacter* spp. (*Enterobacter sakazakii*) in reconstituted infant formula and determination of injury by flow cytometry. *Int J Food Microbiol.* (2009) 133:113–20. doi: 10.1016/j.ijfoodmicro.2009.05.009
74. Choi MJ, Kim SA, Lee NY, Rhee MS. New decontamination method based on caprylic acid in combination with citric acid or vanillin for eliminating *Cronobacter sakazakii* and *Salmonella enterica* serovar Typhimurium in reconstituted infant formula. *Int J Food Microbiol*. (2013). 166:499–507. doi: 10.1016/j.ijfoodmicro.2013.08.016
75. Shi C, Song K, Zhang X, Sun Y, Sui Y, Chen Y, et al. Antimicrobial activity and possible mechanism of action of citral against *Cronobacter sakazakii*. *PLoS ONE*. (2016) 11:e0159006. doi: 10.1371/journal.pone.0159006
76. Shi C, Sun Y, Zheng Z, Zhang X, Song K. Jia Z, et al. Antimicrobial activity of syringic acid against *Cronobacter sakazakii* and its effect on cell membrane. *Food Chem.* (2016) 197:100–6. doi: 10.1016/j.foodchem.2015.10.100
77. Shi C, Zhang X, Sun Y, Yang M, Song K, Zheng Z, et al. Antimicrobial activity of ferulic acid against *Cronobacter sakazakii* and possible mechanism of action. *Foodborne Pathog Dis*. (2016) 13:196–204. doi: 10.1089/fpd.2015.1992
78. Shi C, Jia Z, Chen Y, Yang M, Liu X, Sun Y, et al. Inactivation of *Cronobacter sakazakii* in reconstituted infant formula by combination of thymoquinone and mild heat. *J Appl Microbiol.* (2015) 119:1700–6. doi: 10.1111/jam.12964
79. Shi C, Yan C, Sui Y, Sun Y, Guo D, Chen Y, et al. Thymoquinone inhibits virulence related traits of Cro*nobacter sakazakii* ATCC 29544 and has anti-biofilm formation potential. *Front Microbiol.* (2017) 8:2220. doi: 10.3389/fmicb.2017.02220
80. Chen Y, Wen Q, Chen S, Guo D, Xu Y, Liang S, et al. Effect of thymoquinone on the resistance of *Cronobacter sakazakii* to environmental stresses and antibiotics. *Food Control.* (2020) 109:106944. doi: 10.1016/j.foodcont.2019.106944
81. Cao Y, Zhoua A, Zhou D, Xiao X, Yua Y, Li X. *Cronobacter sakazakii* CICC 21544 responds to the combination of carvacrol and citral by regulating proton motive force. *LWT Food Sci Technol.* (2020) 122:109040. doi: 10.1016/j.lwt.2020.109040
82. Guo D, Wang S, Li J, Bai F, Yang Y, Xu Y, et al. The antimicrobial activity of coenzyme Q_0 against planktonic and biofilm forms of *Cronobacter sakazakii*. *Food Microbiol.* (2020) 86:103337. doi: 10.1016/j.fm.2019.103337
83. Bouarab-Chibane L, Forquet V, Lantéri P, Clément Y, Léonard-Akkari L, Oulahal N, et al. Antibacterial properties of polyphenols: characterization and QSAR (Quantitative Structure-Activity Relationship) models. *Front Microbiol.* (2019) 10:829. doi: 10.3389/fmicb.2019.00829
84. Turek C, Stintzing FC. Stability of essential oils: a review. *Compr Rev Food Sci Food Saf.* (2013) 12:40–53. doi: 10.1111/1541-4337.12006
85. Rao J, Chen B, McClements DJ. Improving the efficacy of essential oils as antimicrobials in foods: mechanisms of action. *Annu Rev Food Sci Technol.* (2019) 10:365–87. doi: 10.1146/annurev-food-032818-121727
86. Maes C, Bouquillon S, Fauconnier ML. Encapsulation of essential oils for the development of biosourced pesticides with controlled release: a review. *Molecules.* (2019) 24:2539. doi: 10.3390/molecules24142539
87. Hayes M, Barrett E, Ross RP, Fitzgerald GF, Hill C, Stanton C. Evaluation of an antimicrobial ingredient prepared from a *Lactobacillus acidophilus* casein fermentate against *Enterobacter sakazakii*. *J Food Prot.* (2009) 72:340. doi: 10.4315/0362-028X-72.2.340
88. Awaisheh SS, Al-Nabulsi AA, Osaili TM, Ibrahim S, Holley R. Inhibition of *Cronobacter sakazakii* by heat labile bacteriocins produced by probiotic LAB isolated from healthy infants. *J Food Sci.* (2013) 78:1416–20. doi: 10.1111/1750-3841.12209
89. Charchoghlyan H, Kwon H, Hwang DJ, Lee JS, Lee J, Kim M. Inhibition of *Cronobacter sakazakii* by *Lactobacillus acidophilus* n.v. Er2 317/402. *Korean J Food Sci Anim Resour*. (2016) 36:635–40. doi: 10.5851/kosfa.2016.36.5.635
90. Kim DH, Jeong D, Song KY, Kang IB, Kim H, Seo KH. Culture supernatant produced by *Lactobacillus kefiri* from kefir inhibits the growth of *Cronobacter sakazakii*. *J Dairy Res.* (2018) 85:98–103. doi: 10.1017/S0022029917000802
91. Campana R, Federici S, Ciandrini E, Mani A, Buffone W. *Lactobacillus* spp. inhibit the growth of *Cronobacter sakazakii* ATCC 29544 by altering its membrane integrity. *J Food Sci Technol.* (2019) 56:3962–7. doi: 10.1007/s13197-019-03928-x
92. Campion A, Morrissey R, Field D, Cotter PD, Hill C, Ross RP. Use of enhanced nisin derivatives in combination with food-grade oils or citric acid to control *Cronobacter sakazakii* and *Escherichia coli* O157: H7. *Food Microbiol.* (2017) 65:254–63. doi: 10.1016/j.fm.2017.01.020
93. Collado MC, Isolauri E, Salminen S. Specific probiotic strains and their combinations counteract adhesion of *Enterobacter sakazakii* to intestinal mucus. *FEMS Microbiol Lett.* (2008) 285:58–64. doi: 10.1111/j.1574-6968.2008.01211.x
94. Kapourchali FR, Cresci GAM. Early-life gut microbiome-the importance of maternal and infant factors in its establishment. *Nutr Clin Pract*. (2020) 35:386–405. doi: 10.1002/ncp.10490
95. Navarro-Tapia E, Sebastiani G, Sailer, S Almeida, Toledano L, Serra-Delgado M, et al. Probiotic supplementation during the perinatal and infant period: effects on gut dysbiosis and disease. *Nutrients.* (2020) 12:2243. doi: 10.3390/nu12082243
96. Deshpande G, Athalye-Jape G, Patole S. Para-probiotics for preterm neonates - the next frontier. *Nutrients.* (2018)10:871. doi: 10.3390/nu10070871
97. Aguilar-Toalá JE, Garcia-Varela R, Garcia HS, Mata-Haro V, González-Córdova AF, Vallejo-Cordoba B, et al. Postbiotics: an evolving term within the functional foods field. *Trends Food Sci Technol.* (2018) 75:105–14. doi: 10.1016/j.tifs.2018.03.009
98. Ibarra-Sánchez LA, El-Haddad N, Mahmoud D, Miller MJ, Karam L. Invited review: advances in nisin use for preservation of dairy products. *J Dairy Sci*. (2020) 103:2041–52. doi: 10.3168/jds.2019-17498
99. Al-Nabulsi A, Osaili TM, Al-Holy MA, Shaker RR, Ayyash MM, Olaimat AN, Holley, RA. Influence of desiccation on the sensitivity of *Cronobacter* spp. to lactoferrin or nisin in broth and powdered infant formula. *Int J Food Microbiol.* (2009) 136:221–6. doi: 10.1016/j.ijfoodmicro.2009.08.008
100. O'Connor PM, Kuniyoshi TM, Oliveira RP, Hill C, Ross RP, Cotter PD. Antimicrobials for food and feed; a bacteriocin perspective. *Curr Opin Biotechnol.* (2020) 61:160–7. doi: 10.1016/j.copbio.2019.12.023
101. Gurtler JB, Beuchat LR. Inhibition of growth of *Enterobacter sakazakii* in reconstituted infant formula by the lactoperoxidase system. *J Food Prot.* (2007) 70:2104–10. doi: 10.4315/0362-028X-70.9.2104
102. Harouna S, Carramiñana JJ, Navarro F, Pérez MD, Calvo M, Sánchez L. Antibacterial activity of bovine milk lactoferrin on the emerging foodborne pathogen *Cronobacter sakazakii*: effect of media and heat treatment. *Food Control.* (2015) 47:520–5. doi: 10.1016/j.foodcont.2014.07.061
103. Harouna S, Franco I, Carramiñana JJ, Blázquez A, Abad I, Pérez MD, et al. Effect of hydrolysis and microwave treatment on the antibacterial activity of native bovine milk lactoferrin against *Cronobacter sakazakii*. *Int J Food Microbiol.* (2020) 319:108495. doi: 10.1016/j.ijfoodmicro.2019.108495
104. Téné N, Roche-Chatain V, Rifflet A, Bonnafe E, Lefranc B, Leprince J, et al. Potent bactericidal effects of bicarinalin against strains of the *Enterobacter* and *Cronobacter* genera. *Food Control.* (2014) 42:202–6. doi: 10.1016/j.foodcont.2014.02.026
105. Abusheliabi A, Al-Rumaithi HO, Olaimat AN, Al-Nabulsi AA, Osaili T, Shaker R, et al. Inhibitory effect of camel milk on *Cronobacter sakazakii. J Food Saf.* (2017) 37:e12343. doi: 10.1111/jfs.12343
106. Willemin ME, Lumen A. Thiocyanate: a review and evaluation of the kinetics and the modes of action for thyroid hormone perturbations. *Crit Rev Toxicol.* (2017) 47:537–63. doi: 10.1080/10408444.2017.1281590
107. Ellison RT, Giehl TJ, LaForce FM. Damage of the outer membrane of enteric Gram-negative bacteria by lactoferrin and transferrin. *Infect Immunol.* (1988) 56:2774–81. doi: 10.1128/IAI.56.11.2774-2781.1988
108. Kell DB, Heyden EL, Pretorius E. The biology of lactoferrin, an iron-binding protein that can help defend against viruses and bacteria. *Front Immunol.* (2020)11:1221. doi: 10.3389/fimmu.2020.01221
109. Gao Y, Hou L, Lu C, Wang Q, Pan B, Wang Q, et al. Enteral lactoferrin supplementation for preventing sepsis and necrotizing enterocolitis in preterm infants: a meta analysis with trial sequential analysis of randomized controlled trials. *Front Pharmacol.* (2020) 11:1186. doi: 10.3389/fphar.2020.01186
110. Hoek KS, Milne J, Grieve PA, Dionysius DA, Smith R. Antibacterial activity of bovine lactoferrin-derived peptides. *Antimicrob Agents Chemother.* (1997) 41:54–9. doi: 10.1128/AAC.41.1.54

111. Nielsen SD, Beverly RL, Qu Y, Dallas DC. Milk bioactive peptide database: a comprehensive database of milk protein-derived bioactive peptides and novel visualization. *Food Chem.* (2017) 232:673–82. doi: 10.1016/j.foodchem.2017.04.056
112. Wang G, Li X, Wang Z. APD3: the antimicrobial peptide database as a tool for research and education. *Nucleic Acids Res.* (2016) 44:1087–93. doi: 10.1093/nar/gkv1278
113. Montagna MT, Diella G, Triggiano, F, Caponio GR, De Giglio O, Caggianon, G, et al. Chocolate, "food of the gods": history, science, and human health. *Int J Environ Res Public Health.* (2019) 16:4960. doi: 10.3390/ijerph16244960
114. Tarka SM, Morrissey RB, Apgar JL, Hostetler KA, Shively CA. Chronic toxicity/carcinogenicity studies of cocoa powder in rats. *Food Chem Toxic.* (1991) 29:7–19. doi: 10.1016/0278-6915(91)90057-E
115. Ballotey-babington L, Kwapong A, N'Guessan B, Amponsah S, Asiedu-Gyekye I. Unsweetened natural cocoa powder: a potent nutraceutical in perspective. *IntechOpen.* (2019). Available online at: https://www.intechopen.com/books/theobroma-cacao-deploying-science-for-sustainability-of-global-cocoa-economy/unsweetened-natural-cocoa-powder-a-potent-nutraceutical-in-perspective (accessed October 26, 2020).
116. Zhang H, Qi R, Mine Y. The impact of oolong and black tea polyphenols on human health. *Food Biosci.* (2019) 29:55–61. doi: 10.1016/j.fbio.2019.03.009
117. Yang CS, Zhang JS. Studies on the prevention of cancer and cardiometabolic diseases by tea: issues on mechanisms, effective doses, and toxicities. *J Agri. Food Chem.* (2019) 67:5446–56. doi: 10.1021/acs.jafc.8b05242
118. Sergi C. Epigallocatechin-3-gallate toxicity in children: a potential and current toxicological event in the differential diagnosis with virus-triggered fulminant hepatic failure. *Front Pharmacol.* (2020) 10:1563. doi: 10.3389/fphar.2019.01563
119. United States Food and Drug Administration (US FDA). *CFR – Code of Federal Regulations Title 21.* (2019). Available online at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=184&showFR=1 (accessed October 26, 2020).
120. United States Food and Drug Administration (USFDA). *Dietary Supplements.* (2019). Available online at: https://www.fda.gov/food/dietary-supplements (accessed October 26, 2020).
121. Zhang C, Fan L, Fan S, Wang J, Luo T, Tang Y, et al. *Cinnamomum cassia* Presl: a review of its traditional uses, phytochemistry, pharmacology and toxicology. *Molecules.* (2019) 24:3473. doi: 10.3390/molecules24193473
122. Singletary K. Cinnamon. Update of potential health benefits. *Nutr Today.* (2019) 54:42–52. doi: 10.1097/NT.0000000000000319
123. Hajimonfarednejad M, Ostovar M, Raee MJ, Hashempur, MH, Mayer JG, Heydari M. Cinnamon: a systematic review of adverse events. *Clin Nutr.* (2019) 38:594–602. doi: 10.1016/j.clnu.2018.03.013
124. Singletary K. Vanilla. Potential health benefits. *Nutr Today.* (2020) 55:186–96. doi: 10.1097/NT.0000000000000412
125. van Assendelft A. Bronchospasm induced by vanillin and lactose. *Eur J Respir Dis.* (1984) 65:468–72.
126. Wang XS, Xue YS, Jiang Y, Ni HL, Zhu H, Luo BG, et al. Occupational contact dermatitis in manufacture of vanillin. *Chin Med J.* (1987) 100:250–4.
127. World Health Organization (WHO). *Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA).* (2019). Available online at: https://apps.who.int/food-additives-contaminants-jecfa-database/search.aspx (accessed October 26, 2020).
128. Deshpande GC, Cai W. Use of lipids in neonates requiring parenteral nutrition. *J Parenter Enteral Nutr.* (2020) 44:45–54. doi: 10.1002/jpen.1759
129. Maher T, Clegg ME. A systematic review and meta-analysis of medium-chain triglycerides effects on acute satiety and food intake. *Crit Rev Food Sci Nutr.* (2020) 26:1–13. doi: 10.1080/10408398.2020.1742654
130. EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food), Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Dusemund B, Frutos MJ, et al. Scientific opinion on the re-evaluation of fatty acids (E 570) as a food additive. *EFSA J.* (2017) 15:4785. doi: 10.2903/j.efsa.2017.4785
131. Samarghandian S, Azimi-Nezhad M, Farkhondeh T. Thymoquinone-induced antitumor and apoptosis in human lung adenocarcinoma cells. *J Cell Physiol.* (2019) 234:10421–31. doi: 10.1002/jcp.27710
132. Khader M, Bresgen N, Eckl PM. *In vitro* toxicological properties of thymoquinone. *Food Chem Toxicol.* (2009) 47:129–33. doi: 10.1016/j.fct.2008.10.019
133. Dollah HMA, Parhizkar S, Latiff LA, Hassan MHB. Toxicity effect of *Nigella sativa* on the liver function of rats. *Adv Pharm Bull.* (2013) 3:97–102. doi: 10.5681/apb.2013.016
134. Rahmani AH, Alzohairy MA, Khan MA, Aly SM. Therapeutic implications of black seed and its constituent TQ in the prevention of cancer through inactivation and activation of molecular pathways. *Evid Based Complement Alternat Med.* (2014) 2014:724658. doi: 10.1155/2014/724658
135. Lin CC, Kumar KJS, Liao JW, Kuo YH, Wang SY. Genotoxic, teratotoxic and oral toxic assessments of *Antrodia cinnamomea* health food product [Leader Deluxe Antrodia cinnamomea (R)]. *Toxicol Rep.* (2015) 2:1409–17. doi: 10.1016/j.toxrep.2015.10.007
136. Nguyen T, Brody H, Lin G-H, Rangé H, Kuraji R, Ye C, et al. Probiotics, including nisin-based probiotics, improve clinical and microbial outcomes relevant to oral and systemic diseases. *Periodontol.* (2000) 82:173–85. doi: 10.1111/prd.12324
137. Rodrigues G, Silva GGO, Buccini DF, Duque HM, Dias SC, Franco OL. Bacterial proteinaceous compounds with multiple activities toward cancers and microbial infection. *Front Microbiol.* (2019) 10:1690. doi: 10.3389/fmicb.2019.01690
138. Kitagawa N, Otani T, Inai T. Nisin, a food preservative produced by *Lactococcus lactis,* affects the localization pattern of intermediate filament protein in HaCaT cells. *Anat Sci Int*. (2019) 94:163–71. doi: 10.1007/s12565-018-0462-x
139. Gorlewska-Roberts KM, Teitel CH, Lay JO Jr, Roberts DW, Kadlubar FF. Lactoperoxidase-catalyzed activation of carcinogenic aromatic and heterocyclic amines. *Chem Res Toxicol.* (2004) 17:1659–66. doi: 10.1021/tx049787n
140. Abu-Serie MM, El-Fakharany EM. Efficiency of novel nanocombinations of bovine milk proteins (lactoperoxidase and lactoferrin) for combating different human cancer cell lines. *Sci Rep.* (2017) 7:16769. doi: 10.1038/s41598-017-16962-6
141. Morita Y, Ono A, Serizawa A, Yogo K, Ishida-Kitagawa N, Takeya T, et al. Purification and identification of lactoperoxidase in milk basic proteins as an inhibitor of osteoclastogenesis. *J Dairy Sci*. (2011) 94:2270–9. doi: 10.3168/jds.2010-4039
142. Gibbons JA, Kanwar JR, Kanwar RK. Iron-free and iron-saturated bovine lactoferrin inhibit surviving expression and differentially modulate apoptosis in breast cancer. *BMC Cancer*. (2015) 15:1–16. doi: 10.1186/s12885-015-1441-4
143. Manzoni P. Clinical benefits of lactoferrin for infants and children. *J Pediatr.* (2016) 173:43–52. doi: 10.1016/j.jpeds.2016.02.075
144. Telang S. Lactoferrin: a critical player in neonatal host defense. *Nutrients*. (2018) 10:1228. doi: 10.3390/nu10091228
145. Motoki N, Mizuki M, Tsukahara T, Miyakawa M, Kubo S, Oda H, et al. Effects of lactoferrin-fortified formula on acute gastrointestinal symptoms in children aged 12-32 months: a randomized, double-blind, placebo-controlled trial. *Front Pediatr.* (2020) 8:233. doi: 10.3389/fped.2020.00233
146. Yamauchi K, Toida T, Nishimura S, Nagano E, Kusuoka O, Teraguchi S, et al. 13-week oral repeated administration toxicity study of bovine lactoferrin in rats. *Food Chem Toxicol.* (2000) 38:503–12. doi: 10.1016/S0278-6915(00)00036-3
147. Cutone, A, Rosa L, Ianiro, G, Lepanto MS, Bonaccorsi di Patti MC, Valenti P, et al. Lactoferrin's anti-cancer properties: safety, selectivity, and wide range of action. *Biomolecules.* (2020) 10:456. doi: 10.3390/biom10030456
148. Piqué N, Berlanga M, Miñana-Galbis D, Piqué N, Berlanga M, Miñana-Galbis D. Health benefits of heat-killed (Tyndallized) probiotics: an overview. *Int J Mol Sci*. (2019) 20:2534. doi: 10.3390/ijms20102534
149. Lee JE, Lee J, Kim JH, Cho N, Lee SH, Park SB, et al. Characterization of the anti-cancer activity of the probiotic bacterium *Lactobacillus fermentum* using 2D vs. 3D culture in colorectal cancer cells. *Biomolecules.* (2019) 9:557. doi: 10.3390/biom9100557
150. Constable A, Mahadevan B, Pressman P, Garthoff JA, Meunier L, Schrenk D, et al. An integrated approach to the safety assessment of food additives in early life. *Toxicol Res Appl.* (2017) 1:1–26. doi: 10.1177/2397847317707370

151. Katz DL, Doughty K, Ali A. Cocoa and chocolate in human health and disease. *Antioxid Redox Signal.* (2011)15:2779–811. doi: 10.1089/ars.2010.3697
152. Martins TF, Palomino OM, Álvarez-Cilleros D, Martín MA, Ramos S, Goya L. Cocoa flavanols protect human endothelial cells from oxidative stress. *Plant Foods Hum Nutr.* (2020)75:161–8. doi: 10.1007/s11130-020-00807-1
153. 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) 5:1–9. doi: 10.3389/fnut.2018.00087
154. Liu X, Li FF, Xie JH, Huang DF, Xie MY. Fetal and neonatal genistein exposure aggravates to interfere with ovarian follicle development of obese female mice induced by high-fat diet. *Food Chem Toxicol.* (2020) 135:110982. doi: 10.1016/j.fct.2019.110982
155. Adgent AA, Umbach DM, Zemel BS, Kelly A, Schall JI, Ford EG, et al. Longitudinal study of estrogen-responsive tissues and hormone concentrations in infants fed soy formula. *J Clin Endocrinol Metab.* (2018) 103:1899–909. doi: 10.1210/jc.2017-02249
156. Testa, I, Salvatori, C, Di Cara, G, Latini A, Frati F, Troiani S, et al. Soy-based infant formula: are phyto-oestrogens still in doubt? *Front Nutr.* (2018) 5:110. doi: 10.3389/fnut.2018.00110
157. Jensen RG, Clark RM, Ferris AM. Composition of the lipids in human milk: a review. *Lipids.* (1980) 15:345–55. doi: 10.1007/BF02533550
158. Hageman JH, Danielsen M, Nieuwenhuizen AG, Feitsma, AL, Dalsgaard TK. Comparison of bovine milk fat and vegetable fat for infant formula: implications for infant health. *Int Dairy J.* (2019) 92:37–49. doi: 10.1016/j.idairyj.2019.01.005
159. Schreiner L, Bauer J, Ortner E, Buettner A. Structure–odor activity studies on derivatives of aromatic and oxygenated monoterpenoids synthesized by modifying p-cymene. *J Nat Prod.* (2020) 83:834–42. doi: 10.1021/acs.jnatprod.9b00339
160. Younes M, Aggett P, Aguilar F, Crebelli R, Dusemund B, Filipič M, et al. Safety of nisin (E 234) as a food additive in the light of new toxicological data and the proposed extension of use. *EFSA J.* (2017) 15:5063. doi: 10.2903/j.efsa.2017.5063
161. Banks J G, Board RG. Preservation by the lactoperoxidase system (LP-S) of a contaminated infant milk formula. *Lett Appl Microbiol.* (1985) 1:81–5. doi: 10.1111/j.1472-765X.1985.tb01495.x
162. Kawaguchi S, Hayashi T, Masano H, Okuyama K, Suzuki T, Kawase K. Effect of lactoferrin-enriched infant formula on low birth weight infants. *Shuusankiigaku.* (1989)19:125–30.
163. Wakabayashi H, Yamauchi K, Abe F. Quality control of commercial bovine lactoferrin. *BioMetals.* (2018) 31:313–9. doi: 10.1007/s10534-018-0098-2
164. Mennella JA, Beauchamp GK. The human infants' responses to vanilla flavors in human milk and formula. *Infant Behav Dev.* (1996) 19:13–9. doi: 10.1016/S0163-6383(96)90040-5
165. Beauchamp GK, Mennella JA. Early flavor learning and its impact on later feeding behavior. *J Pediatr Gastr Nutr.* (2009) 48:25–30. doi: 10.1097/MPG.0b013e31819774a5
166. Valencia GA, Zare EN, Makvandi P, Gutiérrez TJ. Self-assembled carbohydrate polymers for food applications: a review. *Compr Rev Food Sci Food Saf.* (2019) 18:2009–24. doi: 10.1111/1541-4337.12499
167. Sampathkumar K, Tan KX, Loo SCJ. Developing nano-delivery systems for agriculture and food applications with nature-derived polymers. *iScience.* (2020) 23:101055. doi: 10.1016/j.isci.2020.101055
168. Shen HS, Shao S, Chen JC, Zhou T. Antimicrobials from mushrooms for assuring food safety. *Compr Rev Food Sci Food Saf.* (2017) 16:316–29. doi: 10.1111/1541-4337.12255

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.

frontiers in Nutrition

REVIEW
published: 24 November 2020
doi: 10.3389/fnut.2020.577287

Potential Application of Essential Oils for Mitigation of *Listeria monocytogenes* in Meat and Poultry Products

Mojtaba Yousefi[1], *Nasim Khorshidian*[1] *and Hedayat Hosseini*[2]*

[1] *Food Safety Research Center (Salt), Semnan University of Medical Sciences, Semnan, Iran,* [2] *Department of Food Science and Technology, Faculty of Nutrition Sciences and Food Technology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

One of the most important challenges in the food industry is to provide healthy and safe food. Therefore, it is not possible to achieve this without different processes and the use of various additives. In order to improve safety and extend the shelf life of food products, various synthetic preservatives have been widely utilized by the food industry to prevent growth of spoilage and pathogenic microorganisms. On the other hand, consumers' preference to consume food products with natural additives induced food industries to use natural-based preservatives in their production. It has been observed that herbal extracts and their essential oils could be potentially considered as a replacement for chemical antimicrobials. Antimicrobial properties of plant essential oils are derived from some main bioactive components such as phenolic acids, terpenes, aldehydes, and flavonoids that are present in essential oils. Various mechanisms such as changing the fatty acid profile and structure of cell membranes and increasing the cell permeability as well as affecting membrane proteins and inhibition of functional properties of the cell wall are effective in antimicrobial activity of essential oils. Therefore, our objective is to revise the effect of various essential oils and their bioactive components against *Listeria monocytogenes* in meat and poultry products.

Keywords: meat, essential oil, preservatives, natural, antimicrobial, *Listeria monocytogenes*

OPEN ACCESS

Edited by:
Susana Ferreira,
University of Beira Interior, Portugal

Reviewed by:
Slim Smaoui,
Centre of Biotechnology of Sfax, Tunisia
Mohamed Fawzy Ramadan Hassanien,
Zagazig University, Egypt
Maria João Fraqueza,
University of Lisbon, Portugal

***Correspondence:**
Hedayat Hosseini
hedayat@sbmu.ac.ir

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

Received: *29 June 2020*
Accepted: *21 October 2020*
Published: *24 November 2020*

Citation:
Yousefi M, Khorshidian N and Hosseini H (2020) Potential Application of Essential Oils for Mitigation of Listeria monocytogenes in Meat and Poultry Products. Front. Nutr. 7:577287. doi: 10.3389/fnut.2020.577287

INTRODUCTION

Food safety is one of the most important issues in the food industry. In fact, concerns about pathogenic microbes causing foodborne diseases are manifested by consumers, food manufacturers, and regulatory organizations (1, 2). Therefore, the food industry wants to produce high-quality and safe foodstuff (3, 4). Hence, part of the research activities has always been dedicated to increasing knowledge about the production of safe food and the development of new methods applied to improve their safety.

One of the foodstuff that must be safely produced and stored under hygienic conditions is meat and meat products. These are rich in essential nutrients and extremely prone to microbial and chemical deterioration if not well processed and preserved. Therefore, poor hygienic conditions of processing and storage lead to microbial contamination, which can lead to safety and spoilage problems (4–7).

Various microorganisms such as bacteria, mold, and yeast are involved in the spoilage of meat and meat products. Furthermore, inappropriate production and storage condition of meat and meat products lead to incidence of diseases, which is caused by various pathogens such as *Clostridium* spp., *Salmonella* spp., *Campylobacter jejuni*, *Escherichia coli*, O157:H7, *Aeromonas hydrophila*, and *Listeria monocytogenes*, among which, *L. monocytogenes* is considered as the major causative agent responsible for serious diseases in both humans and animals (8, 9).

L. monocytogenes is frequently isolated in various food products. This pathogen results in listeriosis, which remarkably affects pregnant women, newborns, and individuals with immunodeficiency (10, 11). Due to the ability of this microorganism to growth at low temperatures (2–4°C), there is a particular concern about the presence of *L. monocytogenes* in meat and poultry products (12, 13). Various thermal and non-thermal methods including heat treatment, high hydrostatic pressure, irradiation, and high-intensity pulsed electric field processing as well as different methods of packaging have been utilized to produce safe food (11, 14–17).

Also, various preservatives have been utilized to hinder contamination during production, distribution, and storage as well as to increase shelf life of raw and processed meat and poultry products. Although food-grade and Generally Recognized as Safe (GRAS) synthetic additives have been usually used in the food industry, in recent years, consumers have shown an increasing concern about the use of synthetic chemical preservatives (18–20). Therefore, there is an increasing tendency in using natural additives including antioxidants, antimicrobials, sweeteners, and coloring agents that originated from animals, plants, and microorganisms (21, 22). Various naturally occurring antimicrobial agents have been recognized. Lactoperoxidase, lactoferrin, lysozyme from animal sources, bacteriocins and natamycin from microbial sources, and essential oils (EOs) from plant sources are examples of natural preservatives (3, 21). EOs that are volatile and lipophilic liquids, obtained from diverse plant organs such as seeds, roots, stems, buds, flowers, and wood, exert antimicrobial and antioxidant properties (23–25). Various studies have reported that EOs from aromatic and medicinal plants have antimicrobial properties against *L. monocytogenes* (4, 20, 26–31). Due to the described antimicrobial activity of EOs against various microorganisms, this study aimed to review the effect of various EOs on *L. monocytogenes* when applied to meat and poultry products.

L. MONOCYTOGENES IN MEAT AND MEAT PRODUCTS

The presence of *Listeria* spp. in meat and meat products is a serious problem in the meat industry due to the ability of this organism to grow in both raw and cooked meat during refrigerated storage, and among the food products, contaminated meat products are known to be one of the main sources for *L. monocytogenes* infections (32–34).

L. monocytogenes is a pathogenic, Gram-positive, non-spore-forming, facultative anaerobic, highly mobile, rod-shaped bacterium (35–37). *L. monocytogenes* is a major causative agent of foodborne illness worldwide. The severe invasive disease caused by *L. monocytogenes* is listeriosis (38). It has been indicated that listeriosis carries high rates of hospitalization and mortality. Nearly 94% of confirmed cases of listeriosis need to be hospitalized, and 14% of them die (9, 39). Based on the somatic O antigen, *L. monocytogenes* can be subclassified into 13 serotypes. All the 13 serotypes can cause listeriosis; however, serotypes 1.2b, 1.2a, and 4b are more widespread (27, 40). Apart from food matrix diversity, capacity for pathogenicity, and geographical area, the most isolated serotypes from food products are 4c, 4b, 3b, 1.2a, and 1.2b (9, 41).

L. monocytogenes is a psychrotrophic bacterium and can grow over a wide range of temperatures (1–45°C) and pHs (4.3–9.4) and at water activity with a value of 0.92 and above (36, 42). In comparison to other foodborne pathogens, *L. monocytogenes* can tolerate undesirable environmental conditions such as low-oxygen conditions, nitrite, and high salt content. Furthermore, it can persist in the environment, processing plants, and food products at refrigerated temperatures for a long time (43, 44). The ability of *L. monocytogenes* in forming biofilms allows it to remain successfully in food processing establishments and retails (45). Due to the formation of biofilms and attachment of *L. monocytogenes* to various surfaces in food establishments, it is hard to eradicate this pathogen without the performance of precise sanitary protocols. Indeed, because biocides are often highly chemically reactive molecules, the presence of various organic compounds such as polysaccharides, nucleic acids, and proteins can remarkably weaken their efficiency. Furthermore, possible interactions between antimicrobials and biofilm components might explain the limitations of penetration into the biofilm (36, 44, 46).

Due to the ability of *L. monocytogenes* to survive and grow in dry, cold, and high-salt environments, it is widely distributed in different matrices such soil, water, and various food products including meat, fish products, vegetables, dairy products, and ready-to-eat (RTE) food (9, 47).

Since many listeriosis outbreaks have been linked to meat product consumption, prevention of meat and meat product contamination with *L. monocytogenes* is one of the major concerns of the meat processing industry (40). Thermal processing of meat products can easily eliminate *L. monocytogenes*. However, posterior contamination of meat products especially RTE meat products with this pathogen is frequent. Various post-package decontamination strategies such as in-package thermal pasteurization, irradiation, high-pressure processing, and use of antimicrobial additives in the formulation of meat products have been utilized to mitigate and control the growth of *L. monocytogenes* in meat and poultry products (48). Various food-grade synthetic preservatives and antimicrobial agents have been utilized to prevent the growth of *L. monocytogenes*; however, due to increasing awareness of consumers about the potential adverse effects of synthetic preservatives, more researches have been developed to determine the potential use of natural additives and antimicrobial

compounds in the food industry. Among these, there has been great attention in using EOs as natural antimicrobials and antioxidants in the formulation of meat and poultry products.

EOs COMPOSITION AND MECHANISMS OF THEIR ANTIMICROBIAL ACTIVITY

As aforementioned, herbal extracts and EOs from plants can be considered as potential alternatives to artificial preservatives to improve the shelf life and the safety of food products such as meat and poultry and RTE meat products (49). EOs, which also known as volatile or ethereal oils, are naturally aromatic components found in many plants. They can be obtained from various parts of plants including buds, flowers, seeds, leaves, roots, peels, fruits, barks, and woods through only physical extraction and isolation such as pressing and distillation (3, 49).

EOs are made of different compounds characterized by colorless to slightly yellowish liquid and poorly soluble or insoluble in water, but soluble in organic solvents (50). EOs mostly possess a pleasant odor and sometimes a specific taste, and they are utilized in considerable amount in the flavoring and perfume industries. Various fragrance extraction methods including cold pressing and extraction and distillation such as steam distillation are used in order to prepare and obtain EOs (51–53). In total, almost 3,000 EOs are known, among which 300 are commercially utilized in pharmaceutical, food, agronomic, sanitary, cosmetic, and perfume industries (54). The species of plant, plant geographic origin, climate, composition of soil, the vegetative stage of the plant, and the part of plant that is utilized for extraction of EO are the factors that affect the composition of EOs (55–57). EOs are usually secreted as secondary metabolites which exhibit antibacterial, antifungal, and antibiofilm properties (58). These various biological activities are directly related to the bioactive volatile components that are present in EOs (25, 52). Almost 90–95% of EOs are volatile components such as aliphatic aldehydes, alcohols, esters, monoterpenes, and sesquiterpene hydrocarbons and their oxygenated derivatives. The nonvolatile part, which makes up 5–10% of EOs, comprises hydrocarbons, fatty acids, sterols, carotenoids, waxes, coumarins, and flavonoids (3, 59).

Apart from the fact that EOs are mainly used as flavoring agents in the food industry, due to their antimicrobial properties, they can also be used in foodstuff to increase shelf life. The main drawback for the application of EOs as antimicrobial agents is the creation of strong aromas and off-flavors, limiting the use of high concentrations (44, 52). Therefore, it is essential to have information about the target microorganisms, properties of EOs, minimum inhibitory concentrations (MICs), mechanisms of action of EOs, and their interaction with the matrix and sensory properties of the food (60). The antimicrobial properties of EOs are ascribed to the action of various compounds that can be generally divided into terpene and phenolic compounds (44).

As aforementioned, EOs have antimicrobial activities against a wide range of microorganisms; however, the exact antimicrobial mechanism has not been completely elucidated, and it cannot be attributed to an individual mechanism. It seems that based on the chemical compounds contained in the EOs, several mechanisms are involved in the antimicrobial properties of EOs (50, 52). It has been mentioned that the antimicrobial activity of EOs may be due to the possible penetration of EOs through the bacterial cell wall and exertion of inhibitory effects on the functional properties of the cell (61, 62). The hydrophobicity of EOs lets them break down the lipid layer of the bacterial cell membrane and mitochondrion, making the structure more penetrable, and therefore, leakage of ions and other cell compounds occurs, and when the leakages are more than the limit, cell death occurs (52, 63). It has been indicated that disruption of the cell membrane by phenolic compounds of EOs causes exudation of the internal contents of the cell and inhibits functional properties of the cell (49). It has been proposed that phenolic compounds of EOs exert antimicrobial properties by changing the permeability of the microbial cell, damaging cytoplasmic membranes, intervening in the generation system of cellular energy (ATP), and disrupting the proton motive force (49, 53, 64, 65).

Generally, the interaction of EOs with cell membranes of bacteria can be effective in preventing bacterial growth. The hydrophilic or lipophilic properties of EO constituents, type of microorganism, and structure of the cell wall are the factors that affect the antimicrobial activity of EOs (52, 60, 66). Furthermore, the shape of the bacteria can be effective in EO activity, and it has been indicated that rod-shaped cells are more sensitive to EOs in comparison with coccoid-shaped cells (50). It is indicated that Gram-positive bacteria are more sensitive to EOs in comparison with Gram-negative ones.

It seems that the sensitivity of Gram-positive bacteria is related to the direct interaction of the hydrophobic components of the EOs with the cell wall (66–68). The cell wall of Gram-positive bacteria is made of a thick layer of peptidoglycans (90–95%), teichoic acid, and proteins (44, 69). Due to the hydrophobic nature of major parts of EOs, they can easily pass through it. On the other hand, Gram-negative bacteria have a more complex structure including a monolayer of peptidoglycans surrounded by an outer layer comprising proteins and lipopolysaccharides (LPS). This outer cell membrane is charged and possesses a hydrophilic nature, and therefore, diffusion of hydrophobic compound is limited through LPS (3, 50, 70). Therefore, due to structure variation in the outer layers of bacteria, Gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, and *L. monocytogenes* can be more easily inhibited by EOs than Gram-negative bacteria such as *E. coli* and *Salmonella enteritidis* (52, 53). The possible basic mechanisms of EO antimicrobial activity are shown in **Figure 1**.

APPLICATION OF EOs IN MEAT AND POULTRY PRODUCTS

As aforementioned, there has been a growing interest in replacing synthetics additives with natural antioxidants and antimicrobials. EOs are one of these natural additives that have been utilized as antioxidants, antimicrobials, and flavoring agents in meat and poultry products. As Gram-positive organisms are more susceptible to EOs, attention has been focused on utilizing EOs in

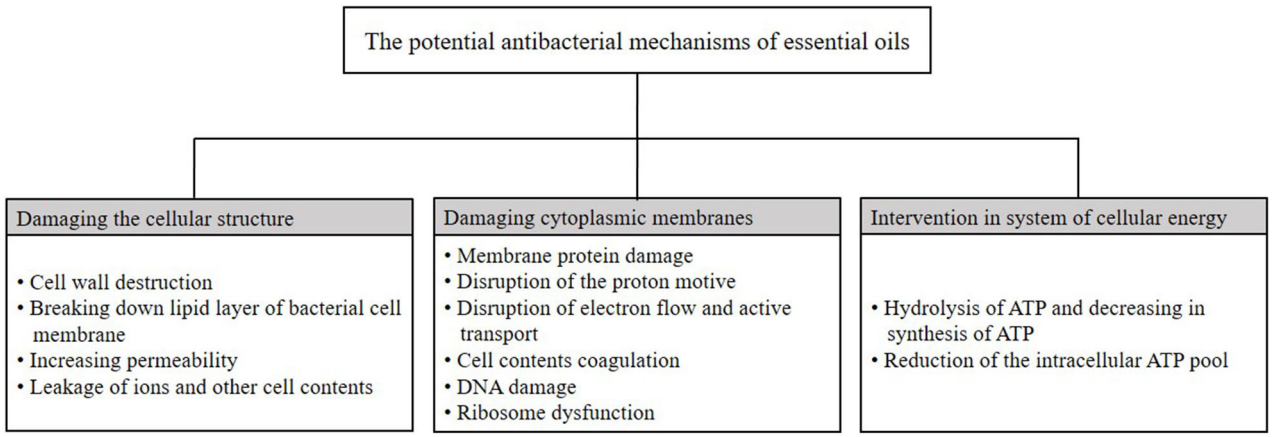

FIGURE 1 | The potential mechanisms of essential oils activity on microorganisms.

the inhibition of Gram-positive bacteria such as *L. monocytogenes* in meat and poultry products.

Upadhyay et al. studied the effect of GRAS plant-derived antimicrobial compounds against *L. monocytogenes* in frankfurters by applying them as post-processing dip treatments. The surface of frankfurters was inoculated with a mixture of five strains of *L. monocytogenes* (∼6.0 log CFU per frankfurter) and treated at 55°C (60 s) and 65°C (30 s) in in sterile deionized water or water with as β-resorcylic acid (1.5%), carvacrol (0.75%), and *trans*-cinnamaldehyde (0.75%) alone. After that, the samples were vacuum-packaged (VP) and kept at 4°C for 70 days. They found that the application of plant-derived compounds as antimicrobial dips was effective in preventing growth of *L. monocytogenes* on frankfurters during refrigerated storage. They also found that β-resorcylic acid had the highest activity against *L. monocytogenes* in comparison to other individual antimicrobial treatments. They concluded that plant-derived antimicrobial compounds could be efficiently utilized as post-processing dips to decrease *L. monocytogenes* on frankfurters (71). The antimicrobial activities of *Thymus capitata* EO against *L. monocytogenes* ATCC 19118 inoculated in minced beef meat were investigated by El Abed et al. They also investigated anti-*Listeria* activity of various concentrations [0.01, 0.05, 0.25, and 1.25% (v/w)] of *T. capitata* EO in minced beef meat and found that by increasing EO concentration, a gradual decrease in *L. monocytogenes* ATCC 19118 count occurred. They figured out that the *L. monocytogenes* population was significantly decreased by the application of 0.25 or 1.25% (v/w) of *T. capitata* EO to minced beef in comparison to control samples (18). Moon et al. studied the synergism effect of soy sauce and teriyaki sauce with carvacrol or thymol (0.3 and 0.5%) as common natural compounds in controlling *L. monocytogenes* in marinated beef stored at 4°C for 7 days. They figured out that *L. monocytogenes* was not inhibited by usage of Teriyaki sauce alone, while teriyaki sauce in combination with 0.5% carvacrol or thymol inactivated *L. monocytogenes* during 7 days of storage (72).

Giarratana et al. studied the effect of thyme and rosemary EOs (0.025 and 0.05%) against a mix of three strains of *L. monocytogenes* (*L. monocytogenes* ATCC 19111, ATCC 13932, and ATCC 19117) in Italian mortadella packaged in a modified atmosphere and kept at 4°C for 30 days. Their results revealed that the mixture of rosemary and thyme EOs had a bacteriostatic activity against *L. monocytogenes* and that both 0.025 and 0.05% of tested EOs significantly inhibited *L. monocytogenes* growth compared with the control sample. The *L. monocytogenes* population increased from approximately 2.50 log CFU/g to 5.31, 3.01, and 2.52 log CFU/g in control, 0.025% EO-treated, and 0.05% EO-treated samples, respectively. They indicated that heat treatment of mortadella at 80°C for 4 h could change the antimicrobial activity of tested EOs. Therefore, complementary preservation strategies such as modified atmosphere packaging (MAP) can improve the EO antimicrobial effect. They also studied the effect of lactic acid bacteria (LAB) growth and pH changes of mortadella on antimicrobial activity of tested EOs and understood that with the growth of LAB and decrease of pH values in mortadella, bacteriostatic activity of tested EOs against as *L. monocytogenes* increased (12). It has been indicated that the pH of the food matrix affects the activity of EOs and that the hydrophobicity of some EOs increased at low pH. Therefore, EOs can more easily penetrate the lipid part of the bacterial membrane and hence exert increased antimicrobial activities (12, 53, 73). Similarly, Gouveia et al. evaluated the antimicrobial effect of rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus vulgaris* L.) against *L. monocytogenes* ATCC 679 in sous vide cook–chill beef at 2 and 8°C during 28 days of storage. Their results showed that thyme and rosemary EOs at 3.9 and 62.5 μl/ml could inhibit *L. monocytogenes*. They also studied the effect of EOs at MIC values on the inhibition of *L. monocytogenes* in beef samples. The sample containing thyme EOs and the control sample had similar counts of *L. monocytogenes*. On the other hand, a 2-log CFU/g reduction occurred in the rosemary-treated samples stored at 2 and 8°C. They concluded that rosemary EO can be potentially used as a

natural preservative. They indicated that the lower antimicrobial effect of thyme EOs could be attributed to the low concentrations of thymol (phenolic compound) in the *T. vulgaris* chemotype that was utilized in their investigation (74). Conversely, a higher antimicrobial activity of thyme EO against *L. monocytogenes* in minced meat stored at 4°C was reported by Pesavento et al. They stated that *p*-cymene (47.9%) and thymol (43.1%) were the main antimicrobial constituents (75). In contrast, in the study carried out by Gouveia et al., the reduced antimicrobial activity of tested thymol EOs was associated with lower levels of *p*-cymene (4.91%) and thymol (7.48%). They also indicated that the decreased antimicrobial activity of thymol EO can be associated with the lower concentrations used (74). It is stated that *p*-cymene can be placed on the bacterial membrane and interfere with its function. Furthermore, the thymol compound, which is a phenolic monoterpenoid, has a phenolic ring and can cause functional and structural damage to the bacterial cytoplasmic membrane (60).

In a study by Mytle et al., the antimicrobial activity of clove (*Syzygium aromaticum*) EOs (1 and 2%, v/w) on RTE chicken frankfurters, which were inoculated with seven strains of *L. monocytogenes* (10^2-10^6 CFU/g) and stored for 14 days (at 5 or 15°C), was determined. They found that all the tested strains were able to survive and grow in the control sample at 5 and 15°C, while the addition of either 1 or 2% clove EO inhibited bacterial growth under both storage conditions. They indicated that clove EO (1% v/w) along with low-temperature storage could decrease possible contamination and growth of *L. monocytogenes* without having an adverse effect on flavor (76). Furthermore, Khaleque et al. studied the application of clove and cinnamon EO against *L. monocytogenes* in ground beef. They studied the effect of 5 and 10% of crude and commercial clove EO or 2.5 and 5.0% of crude and commercial cinnamon EO against *L. monocytogenes* in ground beef stored at 0 and 8°C for 7 days and at −18°C for 60 days. They realized that 10% of either crude or commercial clove EOs were able to entirely inhibit *L. monocytogenes* in ground beef 3 days post inoculation, regardless of storage temperature, while either crude or commercial clove EOs at 5% concentration was not able to effectively inactivate *L. monocytogenes* during storage. Furthermore, both 2.5 and 5.0% cinnamon EOs were not able to kill *L. monocytogenes* during storage. On the other hand, based on the storage time and temperature, a decrease of 3.5–4.0 log CFU/g in the *L. monocytogenes* population occurred with the addition of 5.0% commercial cinnamon EOs. This bacterial count reduction was achieved after 7 days of refrigeration and chilling temperatures and 60 days of freezing temperatures, indicating that anti-*Listeria* activity of cinnamon EO is affected by time and temperature. However anti-*Listeria* activity of clove EO was not affected by time and temperature, and therefore, clove EO can be more effective in the inactivation of *L. monocytogenes* in ground beef than cinnamon (4). Similarly, it has been reported by various investigations that clove and cinnamon EOs could be effective in the inhibition of *L. monocytogenes* and in expanding the shelf life of meat (77, 78). The antibacterial effect of clove is related to eugenol, a member of the phenylpropanoid class of compounds that cause the deterioration of the cell wall and lysis of bacterial cell (70). Furthermore, the antibacterial activity of cinnamon EOs is derived from compounds such as cinnamaldehyde, limonene, and eugenol (52). It was stated that eugenol could change the membrane and fatty acid profile, affect the transportation of ATP and ion, and inhibit ATPase, histidine decarboxylase, amylase, and protease enzymes (50, 79).

Additionally, in a study carried out by Raeisi et al., the effects of sodium alginate coating with nisin, cinnamon, and rosemary EOs individually and in combinations on the fate of *L. monocytogenes* in chicken meat during 15 days of refrigeration were studied. The control and the sample coated with alginate solution had the highest growth rate of *L. monocytogenes*, while other treated samples, especially those with the combined use of tested antimicrobial agents, resulted in the inhibition of *L. monocytogenes*, whereas the combination of cinnamon and rosemary EOs, rosemary EOs and nisin, and cinnamon EOs and nisin had the lowest final population, respectively, indicating the synergistic effect of these EOs and nisin in controlling *L. monocytogenes* (80). It seems that cinnamaldehyde and camphor as the main components of cinnamon and rosemary EOs contribute to their antibacterial activities by disrupting the function of the cytoplasmic membrane, electron flow, proton motive force, and coagulation of cell contents (53). It has been reported by Tajik et al. that *L. monocytogenes* was more inhibited by the simultaneous use of *Zataria multiflora* EO and grape seed extract in comparison with individual use of *Z. multiflora* EO in buffalo patties (81). Based on the type of antimicrobial agents and microorganisms, the combined effect of different antimicrobial compounds might be additive, antagonistic, or synergistic (82).

Firouzi et al. studied the effect of oregano and nutmeg EOs (1, 2, and 3 μl/g) on *L. monocytogenes* in ready-to-cook Iranian barbecued chicken that was inoculated with 6–7 log CFU/g of this pathogen and stored at 3, 8, and 20°C for 72 h. They reported that nutmeg with a MIC value of 0.20 μl/ml was more effective against *L. monocytogenes* than oregano EO with a MIC value of 0.26 μl/ml. Furthermore, MBC values of 0.50 and 0.54 μl/ml were obtained against *L. monocytogenes* by nutmeg and oregano EOs, respectively. They also found that there were no significant differences among all EO-treated and control Iranian barbecued chickens in *L. monocytogenes* growth during 72 h of storage at any of the three temperatures (3, 8, and 20°C) (83). Furthermore, the effect of *N,O*-carboxymethyl chitosan, oregano EO, and their combination on *L. monocytogenes* in raw chicken meat filets that were inoculated with low (10^3 CFU/g) and high (10^5 CFU/g) counts of bacteria and stored at 4°C for 14 days was studied by Khanjari et al. Their results showed that *N,O*-carboxymethyl chitosan exerted a significantly stronger antimicrobial activity against *L. monocytogenes* when compared to oregano EO. They found that *L. monocytogenes* was completely inhibited by the combination of *N,O*-carboxymethyl chitosan and oregano EO in the samples with low and high inoculation levels at days of 2 and 4 of storage, respectively (84). Similarly, Pavli et al. found that incorporation of oregano EO into sodium alginate edible films in ham slices led to a 1.5-log CFU/g decrease in population of *L. monocytogenes* at the end of the storage (40 days) at 8 and 12°C and an approximately 2.5-log CFU/g reduction at 4°C. They finally indicated that a significant reduction or absence of *L. monocytogenes* was achieved in ham slices by application of

high hydrostatic pressure and edible film containing oregano EO, together (85). Additionally, the combination effect of packaging atmosphere, oregano EO, and cold temperature on inhibition of *L. monocytogenes* Scott A in RTE smoked turkey meat was studied by Mahgoub et al. They found that inhibition of *L. monocytogenes* in RTE smoked turkey under MAP and MAP with oregano EO (MAPEO) was increased when compared to VP during the shelf life of the product. They stated that *L. monocytogenes* can be efficiently controlled by a combination of MAP and oregano EO, especially when it is difficult to keep constant temperature during transportation and retail display (86). The antimicrobial activity of oregano EO has been previously confirmed, and it has been utilized to control *L. monocytogenes* in meat and meat products (75, 87–89). The composition of oregano EO is a mixture of volatile terpenes, including α-terpinene, *p*-cymene, carvacrol, and thymol, that participate in antimicrobial properties of this EO, and the latter two compounds are the most important due to their activities on the membranes of bacteria (90). Furthermore, it has been reported that *Z. multiflora* Boiss EO, which mainly contains thymol and carvacrol, had antimicrobial activities against *L. monocytogenes* in meat and meat products, indicating the importance of these two components in the antimicrobial activities of the EOs (31, 91).

It has been stated that the outer membrane of microorganisms was disintegrated by thymol, and therefore the permeability of the cytoplasmic membrane increased and release of K+ and ATP was carried out (92, 93). Furthermore, its integration with the polar head-group placed in the lipid bilayer leads to cell membrane alteration (94). It has been also noted that the citrate metabolic pathway and the enzymes involved in ATP synthesis would be affected by thymol (95).

Carvacrol is another component that has antimicrobial activity. It was supposed that fatty acid profiles and the structure of the cell membrane are changed by carvacrol. Moreover, it was reported that carvacrol could influence proton motive force and the synthesis of flagellin and thus reduce bacterial motility (3, 93). Furthermore, it was indicated that membrane-bound ATPase activity of *L. monocytogenes* would be inhibited by components such as carvacrol, eugenol, and cinnamaldehyde (96).

Awaisheh et al. studied the anti-*Listeria* activity of fir or qysoom EOs alone (1% v/w) or in combination (0.5% each) in beef-luncheon meat products inoculated with *L. monocytogenes* and stored at 4°C for 14 days. They figured out that at the end of storage, for samples with low contamination (3 log CFU/g), fir EO, qysoom EO, and their mixture had ∼6.37, 6.04, and 5.53 log CFU/g of *L. monocytogenes*, respectively, compared to 6.90 log CFU/g of the control, while in the samples with a high contamination level (6 log CFU/g), bacterial counts reached 8.43, 8.88, and 6.75 log CFU/g for fir EO, and qysoom EO, and their mixture, respectively, compared to 9.90 log CFU/g of the control. They indicated that the combination of fir and qysoom exerts good anti-*Listeria* activity. It has been reported that α -and β-pinene, 1,8-cineol, and borneol that are present in fir and qysoom participate in the antimicrobial activities of these EOs (97).

In a study carried out by Carramiñana et al., the effect of savory (*Satureja montana)* EO [0.25, 0.5, 1, and 2.5 μl/g (v/w)] against inoculated *L. monocytogenes* serovar 4b (10^4 CFU/g) in minced pork meat stored at 4°C for up to 7 days was investigated. Furthermore, they utilized thyme (*T. vulgaris* F) and rosemary (*R. officinalis*) as reference ingredients. They found that just *S. montana* and *T. vulgaris* F EOs efficiently inhibited the growth of *L. monocytogenes* in pork, while *R. officinalis* had no remarkable antimicrobial effect. They indicated that the low antimicrobial effect of *R. officinalis* EO could be attributed to the absence of carvacrol and thymol in this EO (92). Similarly, Bukvički et al. (98) studied the effect of *Satureja horvatii* EO (0.16–20 mg/ml) on *L. monocytogenes* (10^7 CFU/ml) in pork meat medium stored at 4 and 25°C for 3 days. They also indicated that two concentrations of 10 and 20 mg/ml *S. horvatii* EO led to a 100 inhibition of *L. monocytogenes* regardless of the incubation temperature, while no inhibition was observed at the lowest concentrations of *S. horvatii* EO (0.16 and 0.32 mg/ml). GC-MS analysis of *S. horvatii* EO showed that the main components of this EO included *p*-cymene (33.14%), thymol (26.11%), thymol methyl ether (15.08%), γ-terpinene (4.05%), α-pinene (4.26%), and α-terpinene (4.02%) (98). It has been reported that the high antimicrobial potential of *Satureja* oil is related to the considerable amount of oxygenated monoterpenes thymol and thymol methyl ether compounds (99). Selected publications on the major compounds of various EOs and their anti-*Listeria* activity in meat and poultry products are summarized in **Table 1**.

LIMITATIONS AND FUTURE TRENDS OF EO APPLICATION IN MEAT AND POULTRY PRODUCTS

The use of EOs as additives and preservatives has a long history, and the use of these compounds along with their toxicity has been considered. One of the substantial aspects of EOs is that they are generally low-risk products. It has been reported by various studies that EOs and their chemical components possess a range of 1 to 20-g/kg body weight for LD50 values, but some exceptions are also observed (114).

Most EOs are nontoxic to mammals and fish, but they are good as pesticides (115). Generally, the chemical compounds that are present in EOs have no remarkable risk that is derived from oral intake. Therefore, the toxicity of EOs is principally related to the compounds that exist in the EO. It has been reported that most of the EO constituents, even at high levels, exhibited no carcinogenic effects (116).

EOs are widely utilized in various products including detergents, creams, lotions, perfumes, and soaps and different food products such as beverages, baked foods, puddings, and meat products. They are well known for their antimicrobial properties and can be applied as antiseptic agents (117, 118). Therefore, EOs can be considered as an alternative for chemical preservatives in food products such as meat and meat products (119).

The efficiency of various EOs has been reported in the control and inhibition of *L. monocytogenes* in meat and poultry products; however, it seems that the interaction of EOs with various

TABLE 1 | Major compounds of essential oils utilized in meat and poultry products against *Listeria monocytogenes*.

Meat product	Type of essential oil	Essential oil concentration	The mode of essential oils applications	Main components	Outcome	References
Minced beef meat	*Thymus capitata*	0.01, 0.05, 0.25, and 1.25% (v/w)	Addition of essential oil solution to minced beef meat	Carvacrol (88.98%), Linalol (1.57%), Terpinen-4-ol (1.41%), *p*-Cymene (1.14%), Caryophyllene epoxide (1.08%)	Due to high amount of carvacrol, *T. capitata* essential oil showed high antioxidant and antimicrobial activities. Application of 0.25 or 1% (v/w) of *T. capitata* essential oil along with low temperature storage can decrease potential contamination of *L. monocytogenes.*	(18)
Minced beef meat	*Ceratonia siliqua*	0.1, 0.2, and 0.4 mg/ml	Addition of essential oil solution to minced beef meat	Nonadecane (21.68%), Heneicosane (10.04%), Naphthalene (9.08%), 1,2-Benzenedicarboxylic acid dibutylester (8.88%), Heptadecane (6.56%), Hexadecanoic acid (5.83%), Octadecanoic acid (4.97%), 1,2-Benzenedicarboxylic acid (3.81%), Phenyl ethyl tiglate (2.76%), Eicosene (2.34%), Farnesol 3 (1.32%), Camphor (1.19%), Nerolidol (1.09%), and n-Eicosane (1.04%).	The concentration of 2.5 and 5 mg/ml (2 MIC) had bacteriostatic activity (MIC) while the 7.5 mg/mL exhibited a bactericidal activity (MBC). Antimicrobial activity of *C. siliqua* essential oil in contaminated minced beef meat (2×10^2 CFU/g of *L. monocytogenes*) that stored at 7°C for 10 days was evident and by increasing essential oil concentration, a gradual decrease in *L. monocytogenes* count was observed. 0.1, 0.2, and 0.4 mg/mL of *C. siliqua* essential oil resulted a 2-log decrease in bacterial population after 6,4, and 2 days of refrigerated storage.	(13)
Ground beef meat	*Zataria multiflora* Boiss	0.3, 0.5, 1, and 2%	Addition of essential oil solution to ground beef meat	Thymol (29.2%), carvacrol (19.64%), burneol (6.62%), thymol methyl ether (6.55%), and o-isopropyltoluene (5.34%)	Treatments with 0.5, 1, and 2% of Zataria multiflora essential oil resulted to a significant decrease in *L. monocytogenes* population during 9 days of storage at 7°C.	(91)
Ground beef	*Melaleuca alternifolia* (tea tree)	1.5% v/w	Addition of essential oil solution to ground beef	Terpinen-4-ol (43.1%), γ-Terpinene (22.8%), α-Terpinene (9.3%), α-Terpineol (5.2%), Terpinolene (3.5%), and α-Pinene (3.0%)	The values of 0.10 μL/g and 0.15 μL/mL were obtained for MIC and MBC, respectively. Based on the inoculation volum of *L. monocytogenes* (1.5 × 108 CFU/mL, 4.6 × 104 CFU/mL, 9.2 × 103 CFU/mL, and 1.2 × 102 CFU/mL), various result was obtained. High counts of *L. monocytogenes* was observed in the control samples at all concentration during storage (14 days at 4°C). No growth of *L. monocytogenes* was observed after the first 20 min of storage in the essential oil- treated samples with initial concentrations of 1.2×10^2 CFU/mL and 9.2×10^3 CFU/mL, while, a reduction of 2.41 log CFU/mL was occurred in the sample with initial counts of 4.6×10^4 CFU/mL. *M. alternifolia* essential oil was not significantly effective in the sample wit high initial inoculation level (1.5×10^8 CFU/mL).	(100)
Sous vide cook-chill beef	*Thymus vulgaris* L. and *Rosmarinus officinalis* L.	Thyme; 3.9 μl/mL Rosemary; 62.5 μl/mL	Essential oil was added directly on meat	*Thymus vulgaris* L.: Linalool (18.18%), thymol (7.48%), limonene (6.49%), endo-borneol (5.86%) and terpinen-4-ol (5.66%).	The sample with thyme essential oils and control sample had similar count of *L. monocytogenes*. On the other hand, a 2 log CFU/g reduction was occurred in the rosemary-treated samples stored at 2 and 8°C.	(74)

(Continued)

TABLE 1 | Continued

Meat product	Type of essential oil	Essential oil concentration	The mode of essential oils applications	Main components	Outcome	References
				Rosmarinus officinalis L.: Eucalyptol (13.05%), camphor (8.93%), verbenone (8.58%), endo-borneol (7.87%) and α-pinene (6.78%)	It is essential to provide adequate chilling storage to assure the safety of the sous vide cook-chill beef in terms of *L. monocytogenes*.	
Bovine ground meat	*Syzygium aromaticum* (clove) and *Cymbopogon citratus* (DC.) Stapf (lemongrass)	1.56, 3.125, and 6.25% (w/v)	Addition of essential oil solution to bovine ground meat	Clove: eugenol (89.80%), trans-caryophyllene (5.88%) and α-humulene (2.30%) Lemongrass: geranial (42.90%), neral (30.90%) and 2-undecanone (4.1296%)	The value of The MIC value of 56% was obtained for both essential oils. The population of bacteria significantly affected in the contaminated meat sample (106 CFU/g of *L. monocytogenes*) with clove and lemongrass essential oils during 3 days incubation at 5°C. The most remarkable reductions were occurred at higher concentrations of both tested essential oils, whereas at concentrations of 3.125 and 6.25% (w/v) no bacteria was detected after the second day of storage.	(101)
Wine marinated beef	*Juniperus communis* and *Satureja montana*	*J. communis* essential oil (0.25%) *S. montana* (0.125%) and their combination.	Marination	*J. communis*: α-pinene (47.8%), sabinene (11.0%), β-pinene (8.5%), and limonene (5.8%) *S. montana*: carvacrol (30.7%), thymol (18.0%), para-cymene (15.6%), borneol (5.9%), and γ-terpinene (5.5%).	Basic red wine marinades and the ones with essential oil or mixture of essential oils significantly reduced *L. monocytogenes* population in comparison with saline control sample during 15 days at 4°C. The marinade containing mixture of tested essential oil exhibited the most pronounced effect.	(7)
Minced beef meat	*Citrus limon* (lemon)	0.06 and 0.312 mg/g (2MIC and 3MIC, respectively)	Addition of essential oil solution to minced beef meat	β-Pinene (25.44%), Limonene (39.74%), Linalool (2.16%), α-Terpineol (7.30%), linalyl acetate(3.01%), Acétate geranyl (3.03%), Nerolidol (6.91%), Acetate neryl (1.74%), and Farnesol (4.28%).	Untreated samples had higher counts of bacteria over the storage period, while the addition of *C. limon* essential oil at 2 and 3 MIC had inhibitory effect on *L. monocytogenes* during 10 days storage at 4°C. The application of C. limon essential oil at a 0.06 and 0.312 mg/g may potentially considered as new strategies in controlling growth of pathogenic bacteria, especially *L. monocytogenes* during storage of minced beef meat at 4°C.	(102)
Turkey meat	*Zataria Multiflora* Boiss and *Bunium persicum* Boiss	0.5 and 1%	In chitosan coating nanoemulsion	*Zataria Multiflora* Boiss: carvacrol (51.55%), thymol (25.49%), ρ-cymene (5.23%), and γ-terpinene (4.44%) *Bunium persicum* Boiss: cumic aldehyde (38.39%), ρ-cymene (18.36%), and 2-Caren-10-al (13.26%).	Nanoemulsions of *Zataria Multiflora* Boiss had the MIC and MBC values of 0.25 and 0.5 mg/mL, respectively, while the MIC and MBC values of nanoemulsions *Bunium persicum* Boiss were 1 and 2 mg/mL.	(31)
Chicken breast filets	Ginger (*Zingiber officinale*)	Nanoemulsion-based edible sodium caseinate with ginger essential oils (3 and 6% wt)	In nanoemulsion-based edible sodium caseinate coating	a-zingiberene (24.96%) b-sesquiphellandrene (12.74%), sesquisabinene hydrate (6.19%), camphene (5.90%), zingiberenol (4.26%), (E)-citral (3.93%), sabinene (3.75%), (E)-farnesene (3.73%), and italicene (3.21%)	Nanoemulsion based edible coatings with 6% of ginger essential oils nanoemulsion led to remarkable reduction in *L. monocytogenes* in refrigerated chicken filets during 12 days.	(103)

(Continued)

TABLE 1 | Continued

Meat product	Type of essential oil	Essential oil concentration	The mode of essential oils applications	Main components	Outcome	References
Chicken meat filets	Rosemary and cinnamon essential oils	Sodium alginate active coating solutions containing, cinnamon and rosemary essential oils (5 mg/ml) and nisin (2000 IU/ml) individually or in combination.	In sodium alginate coating	Cinnamon: (E)-cinnamaldehyde (83.47%), α-copaene (2.57%), and α-muurolene (1.97%) Rosemary: Camphor (23.17%), α-Pinene (18.56%), and 1,8-Cineole (11.89%)	Application of tested essential oil and nisin was effective in *controlling L. monocytogenes* and the strongest effect was observed in samples coated with alginate solution containing both cinnamon and rosemary essential oil had highest effect, indicating synergist effect of cinnamon and rosemary essential oil.	(80)
Chicken meatballs	*Ziziphora clinopodioides*	0.1, 0.2, and 0.3% v/w	Addition of essential oil solution to chicken meatballs	Carvacrol (65.22%), thymol (19.51%), p-cymene (4.86%), and γ -terpinene (4.63%).	Contaminated samples (10^5 CFU/g of *L. monocytogenes*) with *Z. clinopodioides* essential oil had better microbial properties compared to the control during 12 days storage 4°C. In control sample although L. monocytogenes did not growth, however it survived in the refrigerated condition. While, the counts of *L. monocytogenes* reached to 2.66, 2.25, and 2.01 log CFU/g in the samples containing 0.1, 0.2, and 0.3% *Z. clinopodioides* essential oil, respectively. They concluded that *Z. clinopodioides* essential oil can be utilized as a natural substance to control L. monocytogenes in raw chicken meatball that stored at 4°C.	(19)
Sausages	Thyme essential oil	0.1%	In formula incorporation	Thymole(38.2%), p-cymene (25.4%) and terpineol with g terpirene (16.2%)	*L. monocytogenes* was inhibited by addition of thyme essential oil. The main antimicrobial component of thyme essential oil related to thymol that disturbs cell membrane and inhibits the ATPase activity of *L. monocytogenes*.	(104)
Italian mortadella	*Thymus vulgaris* L. and *Rosmarinus officinalis* L	0.025 and 0.05%	In formula incorporation	*Thymus vulgaris* L.: Thymol (45.9%), p-cymene (26.59%), linalool (4.96%) *Rosmarinus officinalis* L: α-Pinene (23.98%), camphor (22.62%), eucalyptol (18.76%), camphene (8.83%), β-pinene (5.61%)	Mixture of rosemary and thyme had anti-listeria activity in Italian mortadella. There was significant differences among control and treated samples in *L. monocytogenes,* at the end of storage (4°C, 30 d). In comparison to control samples, *L. monocytogenes* charges were almost lower of 2.29 and 2.79 log CFU/g in samples containing 0.025 and 0.05% essential oils, respectively.	(12)
Dry Fermented Sausages	*Juniperus communis* L.	0.01, 0.05, and 0.10 μL/g	In formula incorporation	β-myrcene (14.12%), sabinene (9.51%), d,l-limonene (8.36%), 4-terpineol (6.88%), α-amorphene (5.43%), β-pinene (5.39%), caryophyllene (3.94%), p-cymene (3.92%), germacrene D (3.81%),	No foodborne pathogens (*Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp. and sulfite-reducing clostridia) were observed in any sample throughout the storage period (225 days). The sample with 0.10 μL/g of *Juniperus communis* L. exhibited untypical flavor. *Juniperus communis* L. essential oil can be considered as partial replacement for sodium nitrite in dry fermented sausages.	(105)

(Continued)

TABLE 1 | Continued

Meat product	Type of essential oil	Essential oil concentration	The mode of essential oils applications	Main components	Outcome	References
Dry cured sausages (Portuguese chouriço de vinho)	Bay, garlic, nutmeg, oregano, rosemary, thyme	0.005 and 0.05%	In formula incorporation	Bay: Eucaliptol (58.20%), α-terpinenyl acetate (19.19), b-phellandrene (5.01) Garlic: Diallyl trisulfide (33.82%), diallyl disulfide (18.86%), diallyl tetrasulphide (10.97%), methyl allyl trisulfide (9.04%), diallyl sulfide (8.36%) Nutmeg: Myristicin (43.35%), sabinene (23.28%) Oregano: Thymol (93.34%), γ-Terpinene (1.29%) Rosemary: Camphor (22.4%), eucaliptol (13.24%), a-pinene (10.84%) Thyme: Thymol (93.94%) cis-Ocimene (1.29%)	Microbial counts of the tested pathogens was reduced by the addition of essential oils and by increasing concentration, higher antimicrobial effect was observed. However, due to sensory limitations, application of high concentrations was not practical. *L. monocytogenes* was not detected in the sample with 0.005% of oregano, rosemary and nutmeg, after 12 days of drying. Contribution of essential oil addition (0.005%) for remarkable reduction of pathogen's counts and for a shorter period to obtain the not detectable level, making the industry to decrease the drying period and increasing yield production.	(106)
Tuscan sausage	Bay leaf	0.05% and 0.1%	In formula incorporation	1.8-Cineole (35.50%), linalool (14.10%), α-terpinyl acetate (9.65%), sabinene (9.45%)	The count of psychrotrophic microorganisms such as *L. monocytogenes* were significantly lower ($P < 0.05$) in the treated samples than in the control during 14 days storage at 7°C. The count of psychrotrophic microorganisms reached to 7 log CFU/g on day 8 for the sample treated with 0.05% of essential oil, and on day 10 for the sample containing 0.1%, indicating a 2-day increase in shelf life with 0.1 g/100 g of bay leaf essential oil.	(107)
Sausage model	Chinese cinnamon and cinnamon bark	0.025 and 0.05 v/w mixed essential oil	Emulsified microbeads added into sausages formulation	Chinese cinnamon: Trans-Cinnamaldehyde (87.58%) and cinnamyl acetate (7.53%) Cinnamon bark: Transcinnamaldehyde (40.71%), cinnamyl acetate (14.25%), β-phellandrene (9.02%), and β -caryophyllene (7.41%)	Anti-listerial effects of essential oils, nisin, nitrite and organic acid salts in a sausage was studied during 7 days storage at 4°C. Application of 0.025 and 0.05% essential oil in combination with nitrite (100 ppm), organic acid salts (1.55%), and nisin (12.5 ppm), led to a 1.5 and 2.6 log CFU/g reduction in *L. monocytogenes* population, respectively in comparison with control at day 7 of storage.	(108)
Pork Sausages	Lemongrass	2%	Poly lactic acid films	Not detected	The population of inoculated *L. monocytogenes* in the sausage samples wrapped with the poly lactic acid films containing 2% lemongrass was 1.47 Log CFU/g lower than the control samples during 12 days storage at 4°C.	(109)
Turkey ham	Rosemary	1%	Addition of rosemary solution directly to the diced turkey ham	Not detected	The positive control and the sample treated with rosemary were similar in *L. monocytogenes* counts during 63 days at 4°C.	(110)

(Continued)

TABLE 1 | Continued

Meat product	Type of essential oil	Essential oil concentration	The mode of essential oils applications	Main components	Outcome	References
Bologna	Oregano	1 or 2%	Chitosan films with oregano essential oil	Not detected	Chitosan films alone decreased *L. monocytogenes* counts, by value of 2 logs CFU/g during 5 days storage at 4°C, while addition of 1 and 2% oregano essential oil resulted in a reduction of 3.6 and 4 logs CFU/g in population of *L. monocytogenes*, respectively, indicating antibacterial potentiality of oregano essential oil.	(111)
Ham Slices	Oregano	1% (v/v)	In Na-alginate edible films	Not detected	Control treatment had slightly higher counts of *L. monocytogenes*, compared to the sample treated with edible film containing oregano essential oil during 40 days at 4, 8 and 12°C. A 1.5 log CFU/g reduction in *L. monocytogenes* population was observed at the end of storage at 8 and 12°C, while at 4°C, a reduction 2.5 log CFU/g was occurred.	(85)
Mortadella-type sausages	*Zataria multiflora* Boiss	0.5 and 1%	Chitosan films containing essential oil	Not detected	The highest count of *L. monocytogenes* was observed in control sample during 6 days at 4°C. Essential oil addition inhibited *L. monocytogenes* growth and anti-listeria activity of chitosan film significantly changed by increasing essential oils concentration from 0.5 to 1%.	(112)
Chicken frankfurters	*Thymus daenensis* Celak, *Thymbra spicata* L. and *Satureja bachtiarica* Bunge	1% (v/w)	Spraying on the surface	Not detected	Control sample had significantly higher *L. monocytogenes* population compared to the samples treated with essential oils during 14 days of storage at 4°C.The highest decrease in bacterial population was observed in the sample treated with *Thymus daenensis* essential oil.	(113)

food constituents might decrease their antimicrobial properties (3, 53, 73, 120). The interaction of the phenolic compounds of EO with proteins and surrounding EO hydrophobic constituents with fat seem to limit their availability to microorganisms' target size, and therefore, their antimicrobial properties decrease (53). Furthermore, it has been indicated that antimicrobial activities of EOs increase by reduction of oxygen level and pH. The hydrophobicity of EOs would increase by a decrease in pH, making it easy for them to dissolve the cell membrane and interact with target sites of the microorganism (3, 120, 121). High concentration of EOs is required for acceptable antimicrobial activity. Consequently, due to the intense aroma of EOs, their application in higher amounts could result in sensory defects (60, 119).

In order to rectify this shortcoming, various approaches have been suggested. Incorporation of EOs to edible films and coatings, microencapsulation or nanoencapsulation of EOs, use of EO mixtures, and concomitant use of EOs with other preservation methods such as low temperature, new packaging methods, thermal and nonthermal processes can be named as examples of these strategies (3, 31, 119, 122, 123). It has been reported by Khaleque et al. that clove EO (10%) inactivated *L. monocytogenes* in ground beef meat; however, no consumer liked 10% clove EO-supplemented cutlets due to the strong flavor. Therefore, there is a limitation of using spices with a strong flavor like clove. They suggested that a combination of other preservatives such as acid or salt with clove EO and proper storage condition can be useful in reducing the unfavorable sensory effect of clove EO (4).

For example, Upadhyay et al. figured out that combinations of β-resorcylic acid (1.5%), carvacrol (0.75%), and *trans*-cinnamaldehyde (0.75%) with hydrogen peroxide (0.1%) inhibited the growth of *L. monocytogenes* more effectively than did individual tested antimicrobial compounds or hydrogen peroxide (71).

In addition, Noori et al. studied the antimicrobial properties of nanoemulsion-based edible sodium caseinate coating containing ginger EO (3 and 6% wt.) on chicken breast filets. They found that nanoemulsion-based edible coating containing 6% of ginger EO nanoemulsion significantly decreased total aerobic psychrophilic bacteria during 12 days of storage. It seems that nanoemulsion formation results in a reduction in EO droplet size and therefore there is faster penetration of the antimicrobial compounds into the bacterial cell. Hence, this increased antimicrobial activity of nanoemulsions, in comparison with conventional emulsions, allows the application of lower concentrations of EOs in food and active coating or packaging (103). Furthermore, it has been reported that chitosan-loaded nanoemulsion containing *Z. multiflora* EO increased reduction of *L. monocytogenes* in turkey meat in comparison to control during 18 days of storage at 4°C (31). Moreover, the effect of applying VP and MAP conditions with or without the bay EO on controlling *L. monocytogenes* in ground chicken breast was studied by Irkin et al. They found that VP and MAP efficiency against *L. monocytogenes* can be enhanced by the addition of bay EO in chicken meat (124). Furthermore, it has been reported by Pavli et al. that the combined effect of high-pressure processing and sodium alginate edible films containing oregano EO led to more reduction of *L. monocytogenes* in a shorter time and with the lowest final levels in ham slices in comparison to the ones where high-pressure processing and edible films with oregano EO were separately utilized (85). Additionally, it has been reported by Criado et al. that there was a synergistic effect among thyme EO and irradiation on the reduction of the *Listeria innocua* population and extension of the shelf life of ground meat (30). Similarly, the combined effect of gamma (γ)-irradiation and microencapsulated oregano and cinnamon EO and nisin against *L. monocytogenes* on RTE ham was investigated by Huq et al. They found that γ-irradiation treatment and microencapsulated antimicrobials showed a synergistic antimicrobial effect on RTE meat products during storage (125).

Additionally, Cui et al. studied the synergetic antimicrobial effects of cold nitrogen plasma and lemongrass oil against *L. monocytogenes* on pork loin. They found that antibacterial activities of lemongrass oil against *L. monocytogenes* was obtained at high doses, while with exertion of cold nitrogen plasma, a lower concentration of lemongrass oil is required to achieve acceptable antimicrobial effects. Therefore, the possible adverse sensory effect of high concentrations of EO can be mitigated after synergic treatment (126). Therefore, it could be stated that combined use of EOs as well as their application with other preservative and packaging methods has a synergistic antimicrobial effect on *L. monocytogenes* in meat and meat products, and further reduction of *L. monocytogenes* occurred when EOs are associated with these preservative methods (12, 31, 71, 80, 84, 86, 103).

CONCLUSION

This study has revealed that EOs could be potentially used as a replacement for chemical preservatives in meat and poultry products to mitigate or inhibit growth of *L. monocytogenes.* The antimicrobial properties of EOs are ascribed to the action of various compounds that are present in the EOs. Thymol, carvacrol, eugenol, carvone, cinnamaldehyde, limonene, α- and β-pinene, and *p*-cymene can be named as examples of major compounds of EOs that exert anti-*Listeria* activity through different mechanisms such as changing fatty acid profiles and the structure of cell membrane and increasing cell permeability as well as affecting membrane proteins and inhibition of functional properties of the cell wall. However, due to the possible negative effect of EOs, especially in high concentrations, on the organoleptic properties of meat and poultry products, the concentration of these substances utilized in meat and poultry products should be carefully taken into consideration. Furthermore, a combination of low amounts of EOs with other natural antimicrobial substances and technologies which exert synergistic antimicrobial effects could be applied to efficiently prevent growth of *L. monocytogenes* in meat and poultry products. Incorporation of EOs to edible films and coatings, microencapsulation or nanoencapsulation of EOs, use EO mixtures, and application of EOs with new

packaging methods and emerging technology such as high hydrostatic pressure, irradiation, high-intensity pulsed electric field, and cold plasma can be more efficient in inhibiting *L. monocytogenes* growth and enhancing the safety and quality of meat and poultry products, which should be studied in future investigations.

AUTHOR CONTRIBUTIONS

This study was design by HH. The manuscript was written by MY and NK. HH critically revised the manuscript, and finally, all authors listed have approved it for publication. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Mohamed SHS, Zaky WM, Kassem JM, Abbas HM, Salem MME, Said-Al Ahl HAH. Impact of antimicrobial properties of some essential oils on cheese yoghurt quality. *World Appl Sci J*. (2013) 27:497–507. doi: 10.5829/idosi.wasj.2013.27.04.13623
2. Aguilar-Veloz LM, Calderón-Santoyo M, Vázquez González Y, Ragazzo-Sánchez JA. Application of essential oils and polyphenols as natural antimicrobial agents in postharvest treatments: advances and challenges. *Food Sci Nutr*. (2020) 8:2555–68. doi: 10.1002/fsn3.1437
3. Khorshidian N, Yousefi M, Khanniri E, Mortazavian AM. Potential application of essential oils as antimicrobial preservatives in cheese. *Innov Food Sci Emerg Technol*. (2018) 45:62–72. doi: 10.1016/j.ifset.2017.09.020
4. Khaleque MA, Keya CA, Hasan KN, Hoque MM, Inatsu Y, Bari ML. Use of cloves and cinnamon essential oil to inactivate *Listeria monocytogenes* in ground beef at freezing and refrigeration temperatures. *LWT Food Sci Technol*. (2016) 74:219–23. doi: 10.1016/j.lwt.2016.07.042
5. Fratianni F, De Martino L, Melone A, De Feo V, Coppola R, Nazzaro F. Preservation of chicken breast meat treated with thyme and balm essential oils. *J Food Sci*. (2010) 75:M528–35. doi: 10.1111/j.1750-3841.2010.01791.x
6. Kurpas M, Wieczorek K, Osek J. Ready-to-eat meat products as a source of *Listeria monocytogenes*. *J Vet Res*. (2018) 62:49–55. doi: 10.2478/jvetres-2018-0007
7. Vasilijević B, Mitić-Culafić D, Djekic I, Marković T, KneŽević-Vukčević J, Tomasevic I, et al. Antibacterial effect of *Juniperus communis* and *Satureja montana* essential oils against *Listeria monocytogenes in vitro* and in wine marinated beef. *Food Control*. (2019) 100:247–56. doi: 10.1016/j.foodcont.2019.01.025
8. Aminzare M, Hashemi M, Hassanzad Azar H, Hejazi J. The use of herbal extracts and essential oils as a potential antimicrobial in meat and meat products; a review. *J Hum Environ Health Promot*. (2016) 1:63–74. doi: 10.29252/jhehp.1.2.63
9. Shamloo E, Hosseini H, Moghadam ZA, Larsen MH, Haslberger A, Alebouyeh M. Importance of *Listeria monocytogenes* in food safety: a review of its prevalence, detection, and antibiotic resistance. *Iran J Vet Res*. (2019) 20:241. doi: 10.26656/fr.2017.4(1).155
10. Cherifi T, Arsenault J, Pagotto F, Quessy S, Côté JC, Neira K, et al. Distribution, diversity and persistence of *Listeria monocytogenes* in swine slaughterhouses and their association with food and human listeriosis strains. *PLoS ONE*. (2020) 15:e0236807. doi: 10.1371/journal.pone.0236807
11. Bahrami A, Moaddabdoost Baboli Z, Schimmel K, Jafari SM, Williams L. Efficiency of novel processing technologies for the control of *Listeria monocytogenes* in food products. *Trends Food Sci Technol*. (2020) 96:61–78. doi: 10.1016/j.tifs.2019.12.009
12. Giarratana F, Muscolino D, Ragonese C, Beninati C, Sciarrone D, Ziino G, et al. Antimicrobial activity of combined thyme and rosemary essential oils against *Listeria monocytogens* in Italian mortadella packaged in modified atmosphere: thyme and rosemary EOs vs *L. monocytogenes*. *J Essen Oil Res*. (2016) 28:467–74. doi: 10.1080/10412905.2016.1165744
13. Hsouna AB, Trigui M, Mansour RB, Jarraya RM, Damak M, Jaoua S. Chemical composition, cytotoxicity effect and antimicrobial activity of *Ceratonia siliqua* essential oil with preservative effects against Listeria inoculated in minced beef meat. *Int J Food Microbiol*. (2011) 148:66–72. doi: 10.1016/j.ijfoodmicro.2011.04.028
14. Amit SK, Uddin MM, Rahman R, Islam SMR, Khan MS. A review on mechanisms and commercial aspects of food preservation and processing. *Agric Food Secur*. (2017) 6:51. doi: 10.1186/s40066-017-0130-8
15. Zhang ZH, Wang LH, Zeng XA, Han Z, Brennan CS. Non-thermal technologies and its current and future application in the food industry: a review. *Int J Food Sci Technol*. (2019) 54:1–13. doi: 10.1111/ijfs.13903
16. Picart-Palmade L, Cunault C, Chevalier-Lucia D, Belleville MP, Marchesseau S. Potentialities and limits of some non-thermal technologies to improve sustainability of food processing. *Front Nutr*. (2019) 5:130. doi: 10.3389/fnut.2018.00130
17. Mathys A. Perspective of micro process engineering for thermal food treatment. *Front Nutr*. (2018) 5:24. doi: 10.3389/fnut.2018.00024
18. El Abed N, Kaabi B, Smaali MI, Chabbouh M, Habibi K, Mejri M, et al. Chemical composition, antioxidant and antimicrobial activities of thymus capitata essential oil with its preservative effect against *Listeria monocytogenes* inoculated in minced beef meat. *Evid Based Complement Alternat Med*. (2014) 2014:152487. doi: 10.1155/2014/152487
19. Shahbazi Y, Karami N, Shavisi N. Effect of *Ziziphora clinopodioides* essential oil on shelf life and fate of *Listeria monocytogenes* and *Staphylococcus aureus* in refrigerated chicken meatballs. *J Food Saf*. (2018) 38:e12394. doi: 10.1111/jfs.12394
20. Mehdizadeh T, Tajik H, Langroodi AM, Molaei R, Mahmoudian A. Chitosan-starch film containing pomegranate peel extract and *Thymus kotschyanus* essential oil can prolong the shelf life of beef. *Meat Sci*. (2020) 163:108073. doi: 10.1016/j.meatsci.2020.108073
21. Carocho M, Morales P, Ferreira ICFR. Natural food additives: Quo vadis? *Trends Food Sci Technol*. (2015) 45:284–95. doi: 10.1016/j.tifs.2015.06.007
22. Ribeiro JS, Santos MJMC, Silva LKR, Pereira LCL, Santos IA, da Silva Lannes SC, et al. Natural antioxidants used in meat products: a brief review. *Meat Sci*. (2019) 148:181–8. doi: 10.1016/j.meatsci.2018.10.016
23. Arab M, Hosseini SM, Nayebzadeh K, Khorshidian N, Yousefi M, Razavi SH, et al. Microencapsulation of microbial canthaxanthin with alginate and high methoxyl pectin and evaluation the release properties in neutral and acidic condition. *Int J Biol Macromol*. (2019) 121:691–8. doi: 10.1016/j.ijbiomac.2018.10.114
24. Asli MY, Khorshidian N, Mortazavian AM, Hosseini H. A review on the impact of herbal extracts and essential oils on viability of probiotics in fermented milks. *Curr Nutr Food Sci*. (2017) 13:6–15. doi: 10.2174/1573401312666161017143415
25. Valdivieso-Ugarte M, Gomez-Llorente C, Plaza-Díaz J, Gil Á. Antimicrobial, antioxidant, and immunomodulatory properties of essential oils: a systematic review. *Nutrients*. (2019) 11:2786. doi: 10.3390/nu11112786
26. Pilevar Z, Hosseini H, Abdollahzadeh E, Shojaee-Aliabadi S, Tajedin E, Yousefi M, et al. Effect of *Zataria multiflora* Boiss. Essential oil, time, and temperature on the expression of *Listeria monocytogenes* virulence genes in broth and minced rainbow trout. *Food Control*. (2020) 109:106863. doi: 10.1016/j.foodcont.2019.106863
27. Abdollahzadeh E, Ojagh SM, Hosseini H, Irajian G, Ghaemi EA. Predictive modeling of survival/death of *Listeria monocytogenes* in liquid media: bacterial responses to cinnamon essential oil, ZnO nanoparticles, and strain. *Food Control*. (2017) 73:954–65. doi: 10.1016/j.foodcont.2016.10.014
28. Abdollahzadeh E, Rezaei M, Hosseini H. Antibacterial activity of plant essential oils and extracts: the role of thyme essential oil, nisin, and their combination to control *Listeria monocytogenes* inoculated in minced fish meat. *Food Control*. (2014) 35:177–83. doi: 10.1016/j.foodcont.2013.07.004
29. Aziz M, Karboune S. Natural antimicrobial/antioxidant agents in meat and poultry products as well as fruits and vegetables: a review. *Crit Rev Food Sci Nutr*. (2018) 58:486–511. doi: 10.1080/10408398.2016.1194256
30. Criado P, Fraschini C, Jamshidian M, Salmieri S, Desjardins N, Sahraoui A, et al. Effect of cellulose nanocrystals on thyme essential oil release from alginate beads: study of antimicrobial activity against *Listeria innocua* and

ground meat shelf life in combination with gamma irradiation. *Cellulose*. (2019) 26:5247–65. doi: 10.1007/s10570-019-02481-2
31. Keykhosravy K, Khanzadi S, Hashemi M, Azizzadeh M. Chitosan-loaded nanoemulsion containing *Zataria Multiflora* Boiss and *Bunium persicum* Boiss essential oils as edible coatings: Its impact on microbial quality of turkey meat and fate of inoculated pathogens. *Int J Biol Macromol*. (2020) 150:904–13. doi: 10.1016/j.ijbiomac.2020.02.092
32. Liu Y, Sun W, Sun T, Gorris LGM, Wang X, Liu B, et al. The prevalence of *Listeria monocytogenes* in meat products in China: a systematic literature review and novel meta-analysis approach. *Int J Food Microbiol*. (2020) 312:108358. doi: 10.1016/j.ijfoodmicro.2019.108358
33. Olaimat AN, Al-Holy MA, Shahbaz HM, Al-Nabulsi AA, Abu Ghoush MH, Osaili TM, et al. Emergence of antibiotic resistance in *Listeria monocytogenes* isolated from food products: a comprehensive review. *Compr Rev Food Sci Food Saf*. (2018) 17:1277–92. doi: 10.1111/1541-4337.12387
34. Gómez D, Iguácel LP, Rota M, Carramiñana JJ, Ariño A, Yangüela J. Occurrence of *Listeria monocytogenes* in ready-to-eat meat products and meat processing plants in Spain. *Foods*. (2015) 4:271–82. doi: 10.3390/foods4030271
35. Borović B, Baltić T, Lakićević B, Janković V, Mitrović R, Jovanović J, et al. Prevalence of *Listeria monocytogenes* in ready-to-eat food of animal origin. *Tehnologija mesa*. (2014) 55:117–22. doi: 10.5937/tehmesa1402117B
36. Lakicevic B, Nastasijevic I. *Listeria monocytogenes* in retail establishments: Contamination routes and control strategies. *Food Rev Int*. (2017) 33:247–69. doi: 10.1080/87559129.2016.1175017
37. Aghakhani ES, Jalali M, Mirlohi M, Moghadam ZA, Aghakhani ES, Maracy MR, et al. Prevalence of listeria species in raw milk in Isfahan, Iran. *J Isfahan Med School*. (2012) 30:204–8. doi: 10.4103/2277-9183.150384
38. Donovan S. Listeriosis: A rare but deadly disease. *Clin Microbiol Newslett*. (2015) 37:135–40. doi: 10.1016/j.clinmicnews.2015.08.001
39. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, et al. Foodborne illness acquired in the United States-major pathogens. *Emerg Infect Dis*. (2011) 17:7–15. doi: 10.3201/eid1701.P11101
40. Martín B, Perich A, Gómez D, Yangüela J, Rodríguez A, Garriga M, et al. Diversity and distribution of *Listeria monocytogenes* in meat processing plants. *Food Microbiol*. (2014) 44:119–27. doi: 10.1016/j.fm.2014.05.014
41. Wang W, Zhou X, Suo Y, Deng X, Cheng M, Shi C, et al. Prevalence, serotype diversity, biofilm-forming ability and eradication of *Listeria monocytogenes* isolated from diverse foods in Shanghai, China. *Food Control*. (2017) 73:1068–73. doi: 10.1016/j.foodcont.2016.10.025
42. Ingham SC, Buege DR, Dropp BK, Losinski JA. Survival of *Listeria monocytogenes* during storage of ready-to-eat meat products processed by drying, fermentation, and/or smoking. *J Food Protect*. (2004) 67:2698–702. doi: 10.4315/0362-028X-67.12.2698
43. Carpentier B, Cerf O. Review - Persistence of *Listeria monocytogenes* in food industry equipment and premises. *Int J Food Microbiol*. (2011) 145:1–8 doi: 10.1016/j.ijfoodmicro.2011.01.005
44. Pietrysiak E, Smith S, Ganjyal GM. Food safety interventions to control *Listeria monocytogenes* in the fresh apple packing industry: a review. *Comprehen Rev Food Sci Food Saf*. (2019) 18:1705–26. doi: 10.1111/1541-4337.12496
45. Oloketuyi SF, Khan F. Inhibition strategies of *Listeria monocytogenes* biofilms—current knowledge and future outlooks. *J Basic Microbiol*. (2017) 57:728–43. doi: 10.1002/jobm.201700071
46. Bridier A, Dubois-Brissonnet F, Greub G, Thomas V, Briandet R. Dynamics of the action of biocides in *Pseudomonas aeruginosa* biofilms. *Antimicrob Agents Chemother*. (2011) 55:2648–54. doi: 10.1128/AAC.01760-10
47. Khan I, Khan J, Miskeen S, Tango CN, Park YS, Oh DH. Prevalence and control of *Listeria monocytogenes* in the food industry - a review. *Czech J Food Sci*. (2016) 34:469–87. doi: 10.17221/21/2016-CJFS
48. Zhu M, Du M, Cordray J, Ahn DU. Control of *Listeria monocytogenes* contamination in ready-to-eat meat products. *Comprehen Rev Food Sci Food Saf*. (2005) 4:34–42. doi: 10.1111/j.1541-4337.2005.tb00071.x
49. Bajpai VK, Park I, Lee J, Shukla S, Nile SH, Chun HS, et al. Antioxidant and antimicrobial efficacy of a biflavonoid, amentoflavone from *Nandina domestica in vitro* and in minced chicken meat and apple juice food models. *Food Chem*. (2019) 271:239–47. doi: 10.1016/j.foodchem.2018.07.159
50. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*. (2013) 6:1451–74. doi: 10.3390/ph6121451
51. Solórzano-Santos F, Miranda-Novales MG. Essential oils from aromatic herbs as antimicrobial agents. *Curr Opin Biotechnol*. (2012) 23:136–41. doi: 10.1016/j.copbio.2011.08.005
52. Calo JR, Crandall PG, O'Bryan CA, Ricke SC. Essential oils as antimicrobials in food systems - a review. *Food Control*. (2015) 54:111–9. doi: 10.1016/j.foodcont.2014.12.040
53. Burt S. Essential oils: Their antibacterial properties and potential applications in foods - a review. *Int J Food Microbiol*. (2004) 94:223–53. doi: 10.1016/j.ijfoodmicro.2004.03.022
54. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils - a review. *Food Chem Toxicol*. (2008) 46:446–75. doi: 10.1016/j.fct.2007.09.106
55. Skubij N, Dzida K. Essential oil composition of summer savory (*Satureja hortensis* L.) cv. Saturn depending on nitrogen nutrition and plant development phases in raw material cultivated for industrial use. *Indust Crops Prod*. (2019) 135:260–70. doi: 10.1016/j.indcrop.2019.04.057
56. Tohidi B, Rahimmalek M, Arzani A. Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of Thymus species collected from different regions of Iran. *Food Chem*. (2017) 220:153–61. doi: 10.1016/j.foodchem.2016.09.203
57. Angioni A, Barra A, Coroneo V, Dessi S, Cabras P. Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas* L. ssp. stoechas essential oils from stem/leaves and flowers. *J Agric Food Chem*. (2006) 54:4364–70. doi: 10.1021/jf0603329
58. Tajkarimi MM, Ibrahim SA, Cliver DO. Antimicrobial herb and spice compounds in food. *Food Control*. (2010) 21:1199–218. doi: 10.1016/j.foodcont.2010.02.003
59. Luque De Castro MD, Jiménez-Carmona MM, Fernández-Pérez V. Towards more rational techniques for the isolation of valuable essential oils from plants. *TrAC Trends Anal Chem*. (1999) 18:708–16. doi: 10.1016/S0165-9936(99)00177-6
60. Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Front Microbiol*. (2012) 3:12. doi: 10.3389/fmicb.2012.00012
61. Bajpai VK, Baek KH, Kang SC. Control of Salmonella in foods by using essential oils: a review. *Food Res Int*. (2012) 45:722–34. doi: 10.1016/j.foodres.2011.04.052
62. Fisher K, Phillips C. The mechanism of action of a citrus oil blend against *Enterococcus faecium* and *Enterococcus faecalis*. *J Appl Microbiol*. (2009) 106:1343–9. doi: 10.1111/j.1365-2672.2008.04102.x
63. Lambert RJW, Skandamis PN, Coote PJ, Nychas GJE. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J Appl Microbiol*. (2001) 91:453–62. doi: 10.1046/j.1365-2672.2001.01428.x
64. Friedly EC, Crandall PG, Ricke SC, Roman M, O'Bryan C, Chalova VI. *In vitro* antilisterial effects of citrus oil fractions in combination with organic acids. *J Food Sci*. (2009) 74:M67–72. doi: 10.1111/j.1750-3841.2009.01056.x
65. Li M, Muthaiyan A, O'Bryan CA, Gustafson JE, Li Y, Crandall PG, et al. Use of natural antimicrobials from a food safety perspective for control of *Staphylococcus aureus*. *Curr Pharm Biotechnol*. (2011) 12:1240–54. doi: 10.2174/138920111796117283
66. Smith-Palmer A, Stewart J, Fyfe L. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Lett Appl Microbiol*. (1998) 26:118–22. doi: 10.1046/j.1472-765X.1998.00303.x
67. Shelef LA. Antimicrobial effects of spices. *J Food Saf*. (1984) 6:29–44. doi: 10.1111/j.1745-4565.1984.tb00477.x
68. Sokovicx M, Glamočlija J, Marin PD, Brkić D, Van Griensven LJLD. Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an *in vitro* model. *Molecules*. (2010) 15:7532–46. doi: 10.3390/molecules15117532
69. Yousefi M, Shariatifar N, Tajabadi Ebrahimi M, Mortazavian AM, Mohammadi A, Khorshidian N, et al. *In vitro* removal of polycyclic aromatic hydrocarbons by lactic acid bacteria. *J Appl Microbiol*. (2019) 126:954–64. doi: 10.1111/jam.14163

70. Vergis J, Gokulakrishnan P, Agarwal RK, Kumar A. Essential oils as natural food antimicrobial agents: a review. *Crit Rev Food Sci Nutr*. (2015) 55:1320–3. doi: 10.1080/10408398.2012.692127
71. Upadhyay A, Upadhyaya I, Kollanoor-Johny A, Ananda Baskaran S, Mooyottu S, Karumathil D, et al. Inactivation of *Listeria monocytogenes* on frankfurters by plant-derived antimicrobials alone or in combination with hydrogen peroxide. *Int J Food Microbiol*. (2013) 163:114–8. doi: 10.1016/j.ijfoodmicro.2013.01.023
72. Moon H, Kim NH, Kim SH, Kim Y, Ryu JH, Rhee MS. Teriyaki sauce with carvacrol or thymol effectively controls *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and indigenous flora in marinated beef and marinade. *Meat Sci*. (2017) 129:147–52. doi: 10.1016/j.meatsci.2017.03.001
73. Gutierrez J, Barry-Ryan C, Bourke P. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *Int J Food Microbiol*. (2008) 124:91–7. doi: 10.1016/j.ijfoodmicro.2008.02.028
74. Gouveia AR, Alves M, de Almeida JMMM, Monteiro-Silva F, González-Aguilar G, Silva JA, et al. The antimicrobial effect of essential oils against *Listeria monocytogenes* in sous vide cook-chill beef during storage. *J Food Process Preserv*. (2017) 41:e13066. doi: 10.1111/jfpp.13066
75. Pesavento G, Calonico C, Bilia AR, Barnabei M, Calesini F, Addona R, et al. Antibacterial activity of Oregano, Rosmarinus and Thymus essential oils against *Staphylococcus aureus* and *Listeria monocytogenes* in beef meatballs. *Food Control*. (2015) 54:188–99. doi: 10.1016/j.foodcont.2015.01.045
76. Mytle N, Anderson GL, Doyle MP, Smith MA. Antimicrobial activity of clove (*Syzgium aromaticum*) oil in inhibiting *Listeria monocytogenes* on chicken frankfurters. *Food Control*. (2006) 17:102–7. doi: 10.1016/j.foodcont.2004.09.008
77. Friedman M, Henika PR, Mandrell RE. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *J Food Protect*. (2002) 65:1545–60. doi: 10.4315/0362-028X-65.10.1545
78. Jamilah MB, Abbas KA, Rahman RA. A review on some organic acids additives as shelf life extenders of fresh beef cuts. *Am J Agric Biol Sci*. (2008) 3:566–74. doi: 10.3844/ajabssp.2008.566.574
79. Wendakoon CN, Morihiko S. Inhibition of amino acid decarboxylase activity of enterobacter aerogenes by active components in spices. *J Food Protect*. (1995) 58:280–3. doi: 10.4315/0362-028X-58.3.280
80. Raeisi M, Tabaraei A, Hashemi M, Behnampour N. Effect of sodium alginate coating incorporated with nisin, *Cinnamomum zeylanicum*, and rosemary essential oils on microbial quality of chicken meat and fate of *Listeria monocytogenes* during refrigeration. *Int J Food Microbiol*. (2016) 238:139–45. doi: 10.1016/j.ijfoodmicro.2016.08.042
81. Tajik H, Aminzare M, Mounesi Raad T, Hashemi M, Hassanzad Azar H, Raeisi M, et al. Effect of *Zataria multiflora* Boiss essential oil and grape seed extract on the shelf life of raw buffalo patty and fate of inoculated *Listeria monocytogenes*. *J Food Process Preserv*. (2015) 39:3005–13. doi: 10.1111/jfpp.12553
82. Fu Y, Zu Y, Chen L, Shi X, Wang Z, Sun S, et al. Antimicrobial activity of clove and rosemary essential oils alone and in combination. *Phytother Res*. (2007) 21:989–94. doi: 10.1002/ptr.2179
83. Firouzi R, Shekarforoush SS, Nazer AHK, Borumand Z, Jooyandeh AR. Effects of essential oils of oregano and nutmeg on growth and survival of *Yersinia enterocolitica* and *Listeria monocytogenes* in barbecued chicken. *J Food Protect*. (2007) 70:2626–30. doi: 10.4315/0362-028X-70.11.2626
84. Khanjari A, Karabagias IK, Kontominas MG. Combined effect of N,O-carboxymethyl chitosan and oregano essential oil to extend shelf life and control *Listeria monocytogenes* in raw chicken meat fillets. *LWT Food Sci Technol*. (2013) 53:94–9. doi: 10.1016/j.lwt.2013.02.012
85. Pavli F, Argyri AA, Skandamis P, Nychas GJ, Tassou C, Chorianopoulos N. Antimicrobial activity of oregano essential oil incorporated in sodium alginate edible films: control of *Listeria monocytogenes* and spoilage in ham slices treated with high pressure processing. *Materials*. (2019) 12:3726. doi: 10.3390/ma12223726
86. Mahgoub SA, El-Mekkawy RM, Abd El-Hack ME, El-Ghareeb WR, Suliman GM, Alowaimer AN, et al. Inactivation of *Listeria monocytogenes* in ready-to-eat smoked turkey meat by combination with packaging atmosphere, oregano essential oil and cold temperature. *AMB Express*. (2019) 9:54. doi: 10.1186/s13568-019-0775-8
87. Menezes NMC, Martins WF, Longhi DA, de Aragão GMF. Modeling the effect of oregano essential oil on shelf-life extension of vacuum-packed cooked sliced ham. *Meat Sci*. (2018) 139:113–9. doi: 10.1016/j.meatsci.2018.01.017
88. Dussault D, Vu KD, Lacroix M. *In vitro* evaluation of antimicrobial activities of various commercial essential oils, oleoresin and pure compounds against food pathogens and application in ham. *Meat Sci*. (2014) 96:514–20. doi: 10.1016/j.meatsci.2013.08.015
89. Ghalfi H, Benkerroum N, Doguiet DDK, Bensaid M, Thonart P. Effectiveness of cell-adsorbed bacteriocin produced by *Lactobacillus curvatus* CWBI-B28 and selected essential oils to control *Listeria monocytogenes* in pork meat during cold storage. *Lett Appl Microbiol*. (2007) 44:268–73. doi: 10.1111/j.1472-765X.2006.02077.x
90. Muriel-Galet V, Cran MJ, Bigger SW, Hernández-Muñoz P, Gavara R. Antioxidant and antimicrobial properties of ethylene vinyl alcohol copolymer films based on the release of oregano essential oil and green tea extract components. *J Food Eng*. (2015) 149:9–16. doi: 10.1016/j.jfoodeng.2014.10.007
91. Raeisi M, Hashemi M, Sadeghi AR, Aminzare M, Khodadadi M, Ahmadzadeh AM, et al. *Salmonella typhimurium* and *Listeria monocytogenes* growth inhibition by *Zataria multiflora* essential oil in ground meat. *J Human Environ Health Promot*. (2017) 2:261–9. doi: 10.29252/jhehp.2.4.261
92. Carramiñana JJ, Rota C, Burillo J, Herrera A. Antibacterial efficiency of Spanish *Satureja montana* essential oil against Listeria monocytogenes among natural flora in minced pork. *J Food Protect*. (2008) 71:502–8. doi: 10.4315/0362-028X-71.3.502
93. Xu J, Zhou F, Ji BP, Pei RS, Xu N. The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. *Lett Appl Microbiol*. (2008) 47:174–9. doi: 10.1111/j.1472-765X.2008.02407.x
94. Di Pasqua R, Betts G, Hoskins N, Edwards M, Ercolini D, Mauriello G. Membrane toxicity of antimicrobial compounds from essential oils. *J Agric Food Chem*. (2007) 55:4863–70. doi: 10.1021/jf0636465
95. Di Pasqua R, Mamone G, Ferranti P, Ercolini D, Mauriello G. Changes in the proteome of *Salmonella enterica* serovar Thompson as stress adaptation to sublethal concentrations of thymol. *Proteomics*. (2010) 10:1040–9. doi: 10.1002/pmic.200900568
96. Sikkema J, De Bont JAM, Poolman B. Mechanisms of membrane toxicity of hydrocarbons. *Microbiol Rev*. (1995) 59:201–22. doi: 10.1128/MMBR.59.2.201-222.1995
97. Awaisheh SS. Efficacy of Fir and Qysoom essential oils, alone and in combination, in controlling *Listeria monocytogenes in vitro* and in RTE meat products model. *Food Control*. (2013) 34:657–61. doi: 10.1016/j.foodcont.2013.06.017
98. Bukvički D, Stojković D, Soković M, Vannini L, Montanari C, Pejin B, et al. Satureja horvatii essential oil: *in vitro* antimicrobial and antiradical properties and in situ control of *Listeria monocytogenes* in pork meat. *Meat Sci*. (2014) 96:1355–60. doi: 10.1016/j.meatsci.2013.11.024
99. Lakušić B, Ristić M, Slavkovska V, Stanković JA, Milenković M. Chemical composition and antimicrobial activity of the essential oil from *Satureja horvatii* Šilić (Lamiaceae). *J Serb Chem Soc*. (2008) 73:703–11. doi: 10.2298/JSC0807703L
100. Silva CDS, Figueiredo HMD, Stamford TLM, Silva LHMD. Inhibition of *Listeria monocytogenes* by *Melaleuca alternifolia* (tea tree) essential oil in ground beef. *Int J Food Microbiol*. (2019) 293:79–86. doi: 10.1016/j.ijfoodmicro.2019.01.004
101. De Oliveira TLC, Cardoso MG, Soares RA, Ramos EM, Piccoli RH, Tebaldi VMR. Inhibitory activity of *Syzygium aromaticum* and *Cymbopogon citratus* (DC.) Stapf. essential oils against *Listeria monocytogenes* inoculated in bovine ground meat. *Braz J Microbiol*. (2013) 44:357–65. doi: 10.1590/S1517-83822013005000040
102. Ben Hsouna A, Ben Halima N, Smaoui S, Hamdi N. Citrus lemon essential oil: chemical composition, antioxidant and antimicrobial activities with its preservative effect against *Listeria monocytogenes* inoculated in minced beef meat. *Lipids Health Dis*. (2017) 16:146. doi: 10.1186/s12944-017-0487-5
103. Noori S, Zeynali F, Almasi H. Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (*Zingiber officinale*)

essential oil and its effect on safety and quality attributes of chicken breast fillets. *Food Control*. (2018) 84:312–20. doi: 10.1016/j.foodcont.2017.08.015
104. Blanco-Lizarazo CM, Betancourt-Cortés R, Lombana A, Carrillo-Castro K, Sotelo-Díaz I. *Listeria monocytogenes* behaviour and quality attributes during sausage storage affected by sodium nitrite, sodium lactate and thyme essential oil. *Food Sci Technol Int*. (2017) 23:277–88. doi: 10.1177/1082013216686464
105. Tomović V, Šojić B, Savanović J, Kocić-Tanackov S, Pavlić B, Jokanović M, et al. New formulation towards healthier meat products: *Juniperus communis* L. essential oil as alternative for sodium nitrite in dry fermented sausages. *Foods*. (2020) 9:1066. doi: 10.3390/foods9081066
106. García-Díez J, Alheiro J, Pinto A, Soares L, Falco V, Fraqueza M, et al. Behaviour of food-borne pathogens on dry cured sausage manufactured with herbs and spices essential oils and their sensorial acceptability. *Food Control*. (2016) 59:262–70. doi: 10.1016/j.foodcont.2015.05.027
107. da Silveira SM, Luciano FB, Fronza N, Cunha A Jr, Scheuermann GN, Vieira CRW. Chemical composition and antibacterial activity of *Laurus nobilis* essential oil towards foodborne pathogens and its application in fresh Tuscan sausage stored at 7 C. *LWT Food Sci Technol*. (2014) 59:86–93. doi: 10.1016/j.lwt.2014.05.032
108. Ghabraie M, Vu KD, Huq T, Khan A, Lacroix M. Antilisterial effects of antibacterial formulations containing essential oils, nisin, nitrite and organic acid salts in a sausage model. *J Food Sci Technol*. (2016) 53:2625–33. doi: 10.1007/s13197-016-2232-x
109. Yang H-J, Song KB. Application of lemongrass oil-containing polylactic acid films to the packaging of pork sausages. *Korean J Food Sci Anim Resour*. (2016) 36:421. doi: 10.5851/kosfa.2016.36.3.421
110. Ruiz A, Williams S, Djeri N, Hinton A Jr, Rodrick G. Nisin, rosemary, and ethylenediaminetetraacetic acid affect the growth of *Listeria monocytogenes* on ready-to-eat turkey ham stored at four degrees Celsius for sixty-three days. *Poult Sci*. (2009) 88:1765–72. doi: 10.3382/ps.2008-00521
111. Zivanovic S, Chi S, Draughon AF. Antimicrobial activity of chitosan films enriched with essential oils. *J Food Sci*. (2005) 70:M45–51. doi: 10.1111/j.1365-2621.2005.tb09045.x
112. Moradi M, Tajik H, Razavi Rohani SM, Oromiehie AR. Effectiveness of *Zataria multiflora* Boiss essential oil and grape seed extract impregnated chitosan film on ready-to-eat mortadella-type sausages during refrigerated storage. *J Sci Food Agric Food Secur*. (2011) 91:2850–7. doi: 10.1002/jsfa.4531
113. Pirbalouti A, Rahimi E, Moosavi S. Antimicrobial activity of essential oils of three herbs against *Listeria monocytogenes* on chicken frankfurters. *Acta Agric Slovenica*. (2010) 95:219. doi: 10.2478/v10014-010-0013-1
114. Kuttan R, Liju VB. *Safety Evaluation of Essential Oils. Essential Oils in Food Processing: Chemistry, Safety and Applications*. (2017). p. 339–58.
115. Regnault-Roger C, Vincent C, Arnason JT. Essential oils in insect control: low-risk products in a high-stakes world. *Annu Rev Entomol*. (2012) 57:405–24. doi: 10.1146/annurev-ento-120710-100554
116. Smith RL, Cohen SM, Doull J, Feron VJ, Goodman JI, Marnett LJ, et al. A procedure for the safety evaluation of natural flavor complexes used as ingredients in food: essential oils. *Food Chem Toxicol*. (2005) 43:345–63. doi: 10.1016/j.fct.2004.11.007
117. Lee K, Lee JH, Kim SI, Cho MH, Lee J. Anti-biofilm, anti-hemolysis, and anti-virulence activities of black pepper, cananga, myrrh oils, and nerolidol against *Staphylococcus aureus*. *Appl Microbiol Biotechnol*. (2014) 98:9447–57. doi: 10.1007/s00253-014-5903-4
118. Irkin R, Korukluoglu M. Growth inhibition of pathogenic bacteria and some yeasts by selected essential oils and survival of l. monocytogenes and *C. albicans* in apple-carrot juice. *Foodborne Pathog Dis*. (2009) 6:387–94. doi: 10.1089/fpd.2008.0195
119. Gray JA, Chandry PS, Kaur M, Kocharunchitt C, Bowman JP, Fox EM. Novel biocontrol methods for *Listeria monocytogenes* biofilms in food production facilities. *Front Microbiol*. (2018) 9:605. doi: 10.3389/fmicb.2018.00605
120. Skandamis P, Tsigarida E, Nychas G-J. Ecophysiological attributes of *Salmonella typhimurium* in liquid culture and within a gelatin gel with or without the addition of oregano essential oil. *World J Microbiol Biotechnol*. (2000) 16:31–5. doi: 10.1023/A:1008934020409
121. Holley RA, Patel D. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiol*. (2005) 22:273–92. doi: 10.1016/j.fm.2004.08.006
122. Sánchez-González L, Vargas M, González-Martínez C, Chiralt A, Cháfer M. Use of essential oils in bioactive edible coatings: a review. *Food Eng Rev*. (2011) 3:1–16. doi: 10.1007/s12393-010-9031-3
123. Nguefack J, Tamgue O, Dongmo JBL, Dakole CD, Leth V, Vismer HF, et al. Synergistic action between fractions of essential oils from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* against *Penicillium expansum*. *Food Control*. (2012) 23:377–83. doi: 10.1016/j.foodcont.2011.08.002
124. Irkin R, Esmer OK. Control of *Listeria monocytogenes* in ground chicken breast meat under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of bay essential oil at 4°C. *Food Sci Technol Res*. (2010) 16:285–90. doi: 10.3136/fstr.16.285
125. Huq T, Vu KD, Riedl B, Bouchard J, Lacroix M. Synergistic effect of gamma (γ)-irradiation and microencapsulated antimicrobials against *Listeria monocytogenes* on ready-to-eat (RTE) meat. *Food Microbiol*. (2015) 46:507–14. doi: 10.1016/j.fm.2014.09.013
126. Cui H, Wu J, Li C, Lin L. Promoting anti-listeria activity of lemongrass oil on pork loin by cold nitrogen plasma assist. *J Food Saf*. (2017) 37:12316–25. doi: 10.1111/jfs.12316

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.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 11 February 2021
doi: 10.3389/fnut.2020.619023

Thyme Oil Enhances the Inactivation of *Salmonella enterica* on Raw Chicken Breast Meat During Marination in Lemon Juice With Added *Yucca schidigera* Extract

Samuel Kiprotich[1], Aubrey Mendonça[1,2], James Dickson[2,3], Angela Shaw[1], Emalie Thomas-Popo[2], Shecoya White[4], Rkia Moutiq[1] and Salam A. Ibrahim[5]*

[1] Department of Food Science and Human Nutrition, Iowa State University, Ames, IA, United States, [2] Interdepartment Microbiology Graduate Program, Iowa State University, Ames, IA, United States, [3] Department of Animal Science, Iowa State University, Ames, IA, United States, [4] Department of Food Science, Nutrition and Health Promotion, Mississippi State University, Starkville, MS, United States, [5] Food Microbiology and Biotechnology Laboratory, Food and Nutritional Sciences Program, College of Agriculture and Environmental Sciences, North Carolina A & T State University, Greensboro, NC, United States

OPEN ACCESS

Edited by:
Maria João Fraqueza, University of Lisbon, Portugal

Reviewed by:
Dejan S. Stojkovic, University of Belgrade, Serbia
Luis Patarata, University of Trás-os-Montes and Alto Douro, Portugal
Marta Laranjo, University of Evora, Portugal

***Correspondence:**
Aubrey Mendonça
amendon@iastate.edu

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

Received: 19 October 2020
Accepted: 30 December 2020
Published: 11 February 2021

Citation:
Kiprotich S, Mendonça A, Dickson J, Shaw A, Thomas-Popo E, White S, Moutiq R and Ibrahim SA (2021) Thyme Oil Enhances the Inactivation of Salmonella enterica on Raw Chicken Breast Meat During Marination in Lemon Juice With Added Yucca schidigera Extract. Front. Nutr. 7:619023. doi: 10.3389/fnut.2020.619023

Enteric pathogens such as *Salmonella enterica* can survive in low pH conditions and pose a food safety threat during marinating of raw poultry meat. A study was conducted to investigate the effectiveness of thyme oil for killing *S. enterica* on raw chicken during marination in lemon juice containing yucca extract. Samples of raw chicken breast were inoculated with a five-serovar mixture of *S. enterica* ($\sim 10^8$ CFU/mL) and immersed for 2, 4, 6, and 8 h in four lemon-based marinades at 22°C: lemon juice alone (L), L with added 0.5% yucca extract (L + Y), L + Y and 0.5% thyme oil (L + Y + 0.5% TO) and L + Y + 1.0% TO. The L and L + Y served as controls. Survivors were determined by surface plating chicken homogenates on xylose-lysine tergitol-4 (XLT4) agar and XLT4 agar overlaid with non-selective agar (TAL) and counting bacterial colonies after 48 h of incubation (35°C). Marinades containing Y and TO significantly reduced initial viable populations of *S. enterica* compared to control (L and L + Y) solutions ($P < 0.05$). Based on *S. enterica* survivors on TAL medium, the L and L + Y reduced initial populations by 1.12 and 1.42 Log CFU/sample, respectively, after 8 h whereas, Log reductions caused by L + Y + 0.5% TO and L + Y + 1.0% TO, respectively, were 2.62 and 3.91 ($P < 0.05$). Numbers of survivors were higher on TAL compared to XLT4 agar ($P < 0.05$); however, the extent of sub-lethal injury caused by the marinades was not statistically significant ($P > 0.05$). The death rate of *S. enterica* increased significantly ($P < 0.05$) in the marinades containing TO (0.5 or 1.0%) compared to control (L + Y). Based on these results, thyme oil has good potential to increase the antimicrobial efficacy of lemon juice marinade against *Salmonella* on raw chicken breast and enhance the microbial safety of this popular poultry product.

Keywords: *Salmonella*, thyme oil, yucca extract, lemon juice, marinade

INTRODUCTION

Non-typhoidal *Salmonella enterica* are commonly implicated in foodborne disease outbreaks and are a leading cause of bacterial foodborne illnesses worldwide (1, 2). From 1998 to 2017 there were 298 salmonellosis outbreaks involving contaminated chicken meat in the United States. Those outbreaks were linked to 7,881 reported cases, 905 hospitalizations, and 4 deaths (3). *Salmonella* frequently inhabits the intestinal tract of poultry (4); therefore, a major cause of *Salmonella* contamination during poultry processing is spillage of intestinal contents during evisceration. Eradication of *Salmonella* is difficult because of numerous animal reservoirs for this pathogen and its ubiquity in the natural environment (5). Moreover, the ability of *Salmonella* to survive in poultry processing facilities increases the incidence of cross contamination to previously non-infected carcasses (6). The relatively high prevalence of *Salmonella* in retail poultry (7–9) suggests that, to date, poultry processors have been unable to completely prevent dispersion of this pathogen during production and marketing of raw poultry meat (10). Thus, due to numerous opportunities for microbial contamination in poultry processing, multiple pathogen control strategies and intervention kill steps are necessary to ensure microbial safety of poultry meat from farm to consumer (11). In this regard, antimicrobial marinade formulations may have enhanced potential as an intervention strategy to reduce enteric pathogens on raw poultry meat in the farm-to-consumer continuum.

Marination typically involves the soaking or pre-incubation of raw meats in an emulsion or water-based solution that might contain a wide variety of ingredients such as vinegar, wine, fruit juices, organic acids, spices and different aroma additives (12–15). The main purpose of marination is to improve meat tenderness, juiciness, yield, flavor, texture (14, 16, 17), and microbial quality (18). Smith and Acton (19) estimated that more than 50% of raw poultry may be marinated prior to consumption; therefore, marination presents an ideal opportunity to exploit the antimicrobial activity of certain aromatic components of herbs and spices against meat-borne pathogens. Low pH marinades containing lemon juice or vinegar have exhibited antimicrobial properties (20, 21); however, the ineffectiveness of acidic marinades with regard to completely inactivating pathogens in raw meat continues to be problematic (22). The antimicrobial activity of acidic marinades can be improved by compounds from different spices and herbs that are utilized for flavoring purposes (17, 23) as well as the addition of certain plant essential oils (EOs) (24) or EO components (25).

The EOs are extracts of aromatic plants that exhibit potent antimicrobial activity (26–29). The oily (hydrophobic) characteristic of EOs is a major impediment to their application in water-based (hydrophilic) marinades that may consist of water, salt and phosphate or mainly citrus juices such as lime or lemon juice. The EOs are not miscible in water and therefore require the addition of a surfactant for their solubilization (29–31). Considering the increasing consumer demand for more natural alternatives to synthetic food additives, natural surfactants such as yucca extract are gaining much attention from food processors. For example, yucca extract from the *Yucca schidigera* plant has FDA GRAS status and is approved for use as an ingredient in foods and beverages (Code of Federal Regulations 21CFR 172-510, FEMA number 2973).

While there is a growing body of knowledge on the application of EOs as antimicrobials in various food products, published reports on the addition of thyme oil to low pH marinades for pathogen control in poultry meat are scarce. In addition, there are no published reports on the use of yucca extract to disperse EOs in marinade solutions. Accordingly, the main objective of the present study was to evaluate the effectiveness of thyme oil for killing *S. enterica* on artificially inoculated raw chicken breast meat during marination in lemon juice with added yucca extract. A secondary objective was to determine the extent of sub-lethal injury to *S. enterica* survivors on marinated chicken breast meat.

MATERIALS AND METHODS

Bacterial Strain and Culture Conditions

Five serotypes of *Salmonella enterica* (Enteritidis ATCC13076, Heidelberg ATCC 8326, Typhimurium ATCC 14802, Gaminara ATCC 8324, and Oranienburg ATCC 9239) were obtained from the culture collection of the Microbial Food Safety Laboratory at Iowa State University. The cultures were maintained frozen (−80°C) in brain heart infusion (BHI) broth (Difco; Becton, Dickinson and Company, Sparks, MD) with 10% (v/v) added glycerol. Each frozen stock culture was thawed under cold running water and activated in tryptic soy broth (Difco; Becton, Dickinson and Company, Sparks, MD) supplemented with 0.6% (w/v) yeast extract (TSBYE) at 35°C. Prior to each experiment, two consecutive 24-h transfers of each activated stock culture were performed in TSBYE (35°C) to prepare working cultures.

Preparation of Inoculum

Equal volumes (6-mL) of each of the five working cultures of *S. enterica* were combined in a sterile centrifuge tube. The cells were harvested by centrifugation (10,000 × g, 10 min, 4°C) using a Sorvall Super T21 centrifuge (American Laboratory Trading, Inc., East Lyme, CT). The pelleted cells were suspended in 3.0 mL of 0.85% (w/v) NaCl (saline) to obtain a final viable cell concentration of 10 $\log_{10}$ colony-forming units (CFU)/mL for use in the *in-vitro* experiments. For inoculation of chicken breast meat samples, pelleted cells re-suspended in 30 mL of saline to give 9.0 Log_{10} CFU/mL were used. Colony counts of the cell suspensions were evaluated by serially diluting (10-fold) and surface plating samples on tryptic soy agar (Difco; Becton Dickinson) supplemented with 0.6% yeast extract (TSAYE) followed by the counting of bacterial colonies on TSAYE after incubation (35°C) for 24 h.

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of thyme oil for *S. enterica* were evaluated using a broth dilution assay (32). One milliter of filter-sterilized thyme oil was added to 99 mL of sterile BHI broth (pH 7.4) containing 0.5% (v/v) yucca extract to give an initial concentration of 1.0% (v/v). Two-fold dilutions of thyme

oil (1.0%) were prepared in BHI broth with added yucca extract to obtain the following thyme oil concentrations: 0.5, 0.25, 0.125, 0.062, 0.031, and 0.015% (v/v). The *S. enterica* cell suspension (9.0 Log_{10} CFU/mL) in saline was diluted in fresh saline to obtain 7.0 Log CFU/mL. Aliquots (0.1-mL) of the diluted cell suspension were used to inoculate tubes of sterile BHI broth (10 mL/tube) containing various concentrations of thyme oil to obtain an initial *S. enterica* concentration of ∼5.0 Log_{10} CFU/mL. Inoculated and non-inoculated BHI broth with yucca extract served as positive and negative control, respectively. All tubes of BHI broth were incubated at 35°C and checked for turbidity after 24 h. The MIC of thyme oil was determined as the lowest concentration at which no turbidity was observed after 24 h. To determine the MBC, 0.1 mL aliquots from the tubes showing no turbidity were surface plated on TSAYE. The TSAYE plates were then incubated (35°C) and bacterial colonies were counted after 48 h. The lowest concentration of thyme oil that demonstrated ≥99.9% (3-log) kill of the pathogen was deemed as the MBC (33).

Preparation of Lemon Juice Marinade

Whole Sunkist® lemons (Citrus *limon* L) from the same production lot were purchased from a local grocery store in Ames, Iowa. The lemons were washed with potable water and cut into halves using a sanitized knife. The lemon juice for the marinade solutions was prepared by juicing the lemons using a manual citrus juicer. The fresh juice was titrated with NaOH solution and its citric acid content was expressed as gram citric acid per 100 mL based on a citric acid standard curve. Prior to each experiment four treatment solutions including control lemon juice alone (control) were prepared by aseptically transferring 100 mL of lemon juice into each of four sterile screw-capped glass bottles. Appropriate amounts of filter-sterilized stock solutions of yucca extract (Garuda International, Inc, Exeter, CA) (34) alone or in emulsion with certified food grade thyme oil (Sigma-Aldrich, Milwaukee, WI) in lemon juice were added to the three remaining bottles of lemon juice to obtain thyme oil concentrations of 0, 0.1, and 0.2%. Except for lemon juice alone, all other marinade solutions contained 0.5% (w/v) yucca extract. The capped bottles containing the marinade solutions were vigorously shaken and held at 4°C until used.

Inoculation of Lemon Juice Marinade Solutions

For *in vitro* time-kill studies, 20-mL aliquots of each of the four marinade solutions were aseptically transferred to separate sterile 50-mL plastic tubes and inoculated with 0.2 mL of a five-serovar concentrated cell suspension (10 Log_{10} CFU/mL) of *S. enterica* to obtain a final viable cell concentration of 8.0 Log_{10} CFU)/mL. Each inoculated treatment solution was thoroughly mixed by vortexing and samples were removed for microbial analysis at 0, 3, 6, 9, 12, and 15 min. Time 0 min represented about 15 s of exposure of the pathogen to the marinade before the samples were transferred to buffered peptone water (BPW; Difco) for further dilution and plating.

Preparation and Inoculation of Chicken Breast Meat

Fresh, skinless chicken breast filets were obtained from a local grocery store and transported on ice in a cooler to the Microbial Food Safety Laboratory at Iowa State University. The filets were refrigerated at 4°C and used within 24 h of purchase. Five chicken breast filets were randomly selected, and samples (∼50 g each) of chicken breast meat were aseptically excised using a sanitized cylindrical plastic corer 30 mm in diameter. Each sample was inoculated with 0.1 mL of *Salmonella* (∼9.0 log CFU/mL) in order to obtain a final concentration of ∼8.0 Log_{10}CFU/sample. The inoculum was spread over the surface of the meat sample using a sterile bent glass rod. The inoculated chicken breast slices were then held for 30 min at ambient temperature (22 ± 1°C) in a laminar flow bio-hazard chamber (with the blower on) and for an additional 1.5 h without the blower to allow for cell attachment to the meat surface and drying of the inoculum.

Marination of Inoculated Chicken Breast Meat

For marination, four 50-g samples of inoculated chicken breast slices were each transferred to a separate sterile beaker containing a marinade treatment solution at 22 ± 1°C. The four marinade solutions consisted of lemon juice only, and lemon juice with 0, 0.5, and 1.0% (v/v) thyme oil. Each of the three latter marinades contained 0.5% (w/v) yucca extract. Chicken breast meat samples were immersed (inoculated side down) in the marinade solutions with a marinade to meat ratio of 2:1 (100 mL/50 g). Meat samples were removed from the marinade and drained for 30 s on a sanitized stainless-steel grill before being analyzed for *Salmonella* survivors.

Microbiological Analysis

Samples of non-inoculated raw juice were analyzed for aerobic plate count (APC) and naturally occurring salmonellae. In this respect, one-mL samples of juice were each added to 2-mL of double-strength (2X) buffered peptone water (BPW; pH 7.2). To determine the APC, aliquots (1.0- and 0.1-mL) of the diluted juice were surface plated on TSAYE followed by incubation (35°C) and counting bacterial colonies after 48 h. For enumerating naturally occurring salmonellae, aliquots of the diluted juice were surface plated on XLT agar overlaid with TSAYE (TAL) followed by incubation (35°C) for 48 h. For the *in-vitro* study, microbiological analysis of the inoculated lemon juice was performed to determine *Salmonella* survivors after 3, 6, 9, 12, and 15 min of inoculation. Ten-fold serial dilutions of each treatment solution were prepared in BPW. Aliquots (0.1-mL) of appropriate dilutions were surface plated (in duplicate) on XLT-4 agar and XLT-4 agar overlaid with TSAYE (TAL). The inoculated agar plates were incubated at 35°C, and bacterial colonies were counted after 48 h. *Salmonella* survivors on chicken breast meat were determined after 2, 4, 6, and 8 h of marination. At each sampling time, the meat samples were drained for 30 s on a sanitized stainless steel grill then transferred to Seward Stomacher sterile strainer/filter bags (Fisher Scientific, Fair Lawn, NJ) each containing 50 mL of 2X BPW. The bagged samples

were each pummeled for 1.0 min in a laboratory stomacher blender operating at medium speed. Aliquots (1-mL) of the sample homogenate were serially diluted in BPW and 0.1-mL portions were spread-plated on both XLT-4 agar and TAL media. The inoculated agar plates were incubated (35°C) and bacterial colonies were counted after 48 h.

Calculation of *D*-Values

The *D*-values (time of exposure to marinade that results in 90% reduction in viable salmonellae) were determined by plotting the log number of survivors per ml of marinade or per sample (marinated chicken) vs. exposure time using Microsoft Excel 2000 Software (Microsoft Inc., Redmond, WA). Using linear regression analysis the line of best fit for each set of data was determined. The D-value was evaluated by calculating the negative reciprocal of the slope of the regression line.

Determination of Sub-lethal Injury

The TAL medium was used to recover both non-injured and sub-lethally injured *Salmonella* and was prepared by aseptically layering 14 mL of sterile TSAYE (49°C) onto 20 mL of solidified XLT-4 agar in petri dishes (35). Plates of solidified TAL media were used within 2 h after preparation for surface-plating samples. Sub-lethal injury in the surviving *Salmonella* population was determined as described by Wuytack et al. (36). Briefly, the numbers bacterial colonies recovered on XLT4 agar and TAL media were used to calculate the reduction factor (RF) after each exposure of the pathogen to the marinade. The RF is the ratio of *Salmonella* colony counts (CFU/ml) of the control to that of the treated sample. For each sampling time, the log of the RF was calculated for *Salmonella* recovered on each of the two plating media using the following equation:

$$Log\ RF = Log\left(\frac{CFU\ before\ treatment}{CFU\ after\ treatment}\right)$$

For each sampling time, the log RF values for XLT4 agar and the TAL medium were plotted on the Y-axis and X-axis, respectively. Linear regression lines were fitted through the data points and sub-lethal injury was determined when the values of the slope and intercept deviated significantly ($P < 0.05$) from 1.0 and 0, respectively (36, 37).

Measurement of pH of Marinades

The pH value of each marinade was measured initially (0 h) and after 2, 4, 6, and 8 h of immersion of non-inoculated raw chicken breast meat in the marinades. The pH measurements were performed using an Orion Model 525 pH meter (Orion Research, Inc., Boston, MA) fitted with a glass electrode.

GC-MS Analysis of Thyme Oil

The thyme oil was analyzed with Agilent Technologies Model 6890A Gas Chromatography system coupled to a Model 5973N inert XLMSD with Triple–Axis Detector. An Agilent Rxi-5SilMS (30 m × 0.25 mm × 0.25 mm) Capillary column was used, and each injected sample consisted of 1 μL of essential oil diluted in 1.0 ml Hexane (HPLC grade) using split-less injection. The inlet temperature and the helium flow rate were 250°C and 1.0 mL/min, respectively. Ionization voltage was 70 eV with interface temperature of 280°C. The MS source temperature was 230°C and the MS Quad temperature was 150°C with the temperature sequence was as follows: initial temperature 50°C, ramp 5°C per min to 180°C, then 10°C per min to 280°C with a total run time of 36 min per sample. A mixture of homologous series of normal alkanes from C10 to C26 was analyzed under the same conditions as listed above. The compounds present in the essential oil were identified by comparing the mass spectra of each component with those from National Institute of Standards Technology (NIST) by Automated Mass Spectral Deconvolution and Identification System (AMDIS). The identification was also based on a comparison between the literature and estimated Kovat's retention indices using the formula:

$RI_x = 100\ [n + (t_x - t_n)/(t_{n+1} - t_n)]$ ((38)). The t_n and t_{n+1} represent retention times of the reference normal alkane hydrocarbons eluting closely before and after the chemical compound to identify "*x*." The t_x is the retention time of that compound "*x*" whereas "*n*" represents the number of carbons.

Statistical Analysis

All experiments were performed in triplicate and the results were reported as averages. The SAS software (SAS version 9.3, SAS Institute, Cary, NC) was used for two-way Analysis of Variance (ANOVA) to evaluate treatment means with significant differences. The Welch test was used to determine significant differences between paired treatments. Mean pH values were analyzed by using JMP Pro statistical software version 15 (SAS Institute, Inc., Cary, NC). The pH means were evaluated for significant differences at a 5% significance level using the Student's *t*-test.

RESULTS

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

Of the thyme oil concentrations ranging from 0.015 to 1.0% (v/v) in BHI broth (pH 7.4), 0.03 to 1.0% thyme oil inhibited growth of *S. enterica*. No turbidity was observed in the respective tubes of broth. The MIC of thyme oil for the pathogen in BHI broth (35°C) for 24 h was 0.03%. The MBC of thyme oil was 0.06%, which resulted in 3.2 Log CFU/mL reduction in initial viable count of *S. enterica*.

Viability of Pathogens in Marinade Solutions

The APC and numbers of viable salmonellae in non-inoculated raw lemon juice were each <3.0 CFU/mL. **Figures 1A,B** show numbers of *S. enterica* survivors in artificially inoculated lemon juice marinade solutions based on bacterial colony counts on TAL medium (**Figure 1A**) and XLT-4 agar (**Figure 1B**). The average initial viable count of *S. enterica* in marinade solutions was 8.0 ± 0.4 Log_{10} CFU/mL. Lemon juice alone decreased numbers of the pathogen from ∼8.0 to 4.3 Log CFU/mL after 15 min (**Figure 1A**). Survivors in lemon juice with only yucca extract were consistently lower than those in

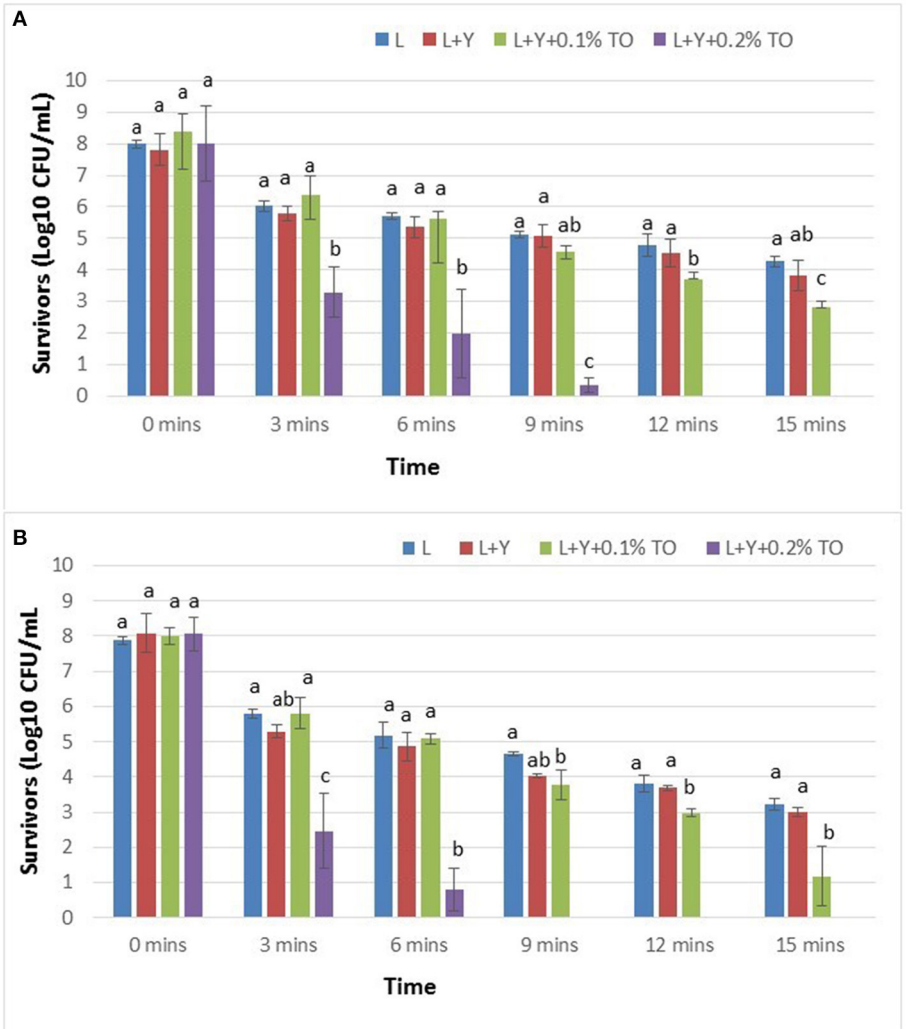

FIGURE 1 | Survivors of *Salmonella enterica* planktonic cells in lemon juice marinades based on bacterial colony counts on TAL medium **(A)** and on XLT4 agar **(B)**.

lemon juice alone; however, differences were not statistically significant ($p > 0.05$) (**Figure 1A**). In lemon juice containing yucca extract and thyme oil (0.1%), initial numbers of *S. enterica* decreased from ∼8.0 log CFU/mL (0 min) to 6.38, 5.62, 4.58, 3.70, and 2.81 Log CFU/mL after 3, 6, 9, 12, and 15 min, respectively (**Figure 1A**). Lemon juice with thyme oil (0.2%) exhibited the highest antibacterial effect whereby initial numbers of the pathogen decreased from ∼8.0 (0 min) to 3.29, 1.96 and <1.0 Log CFU/mL, respectively, after only 3, 6, and 9 min. No *S. enterica* survivors were detected in that same juice after 12 and 15 min (**Figure 1A**). Irrespective of plating medium, lemon juice with added yucca extract and thyme oil (0.2%) exhibited the strongest bactericidal effect against the pathogen (**Figures 1A,B**). For those two juices that contained thyme oil (0.1 and 0.2%), higher numbers of *S. enterica* survivors were observed on TAL medium compared to XLT-4 agar ($P < 0.05$).

Survival of *Salmonella* on Raw Chicken Meat

No salmonellae was detected on non-inoculated samples of chicken breast meat. The numbers of *S. enterica* survivors on marinated raw chicken breast based on colony counts on TAL medium and XLT4 agar, respectively, are presented in **Figures 2A,B**. The initial viable count of *S. enterica* on artificially inoculated chicken breast filets was ∼8.0 Log_{10} CFU/sample based on microbial analysis of the cell suspension used to inoculate the chicken samples. The numbers of survivors on the non-treated inoculated chicken breast after 2 h at ambient temperature (22 ± 1°C) were ∼7.08 log CFU/sample representing a 0.92 Log CFU decrease in cell viability. Compared to lemon juice alone or juice with added yucca extract, all treatments containing thyme oil significantly reduced initial numbers of viable *S. enterica* on raw chicken irrespective of the type of agar medium used for counting bacterial colonies

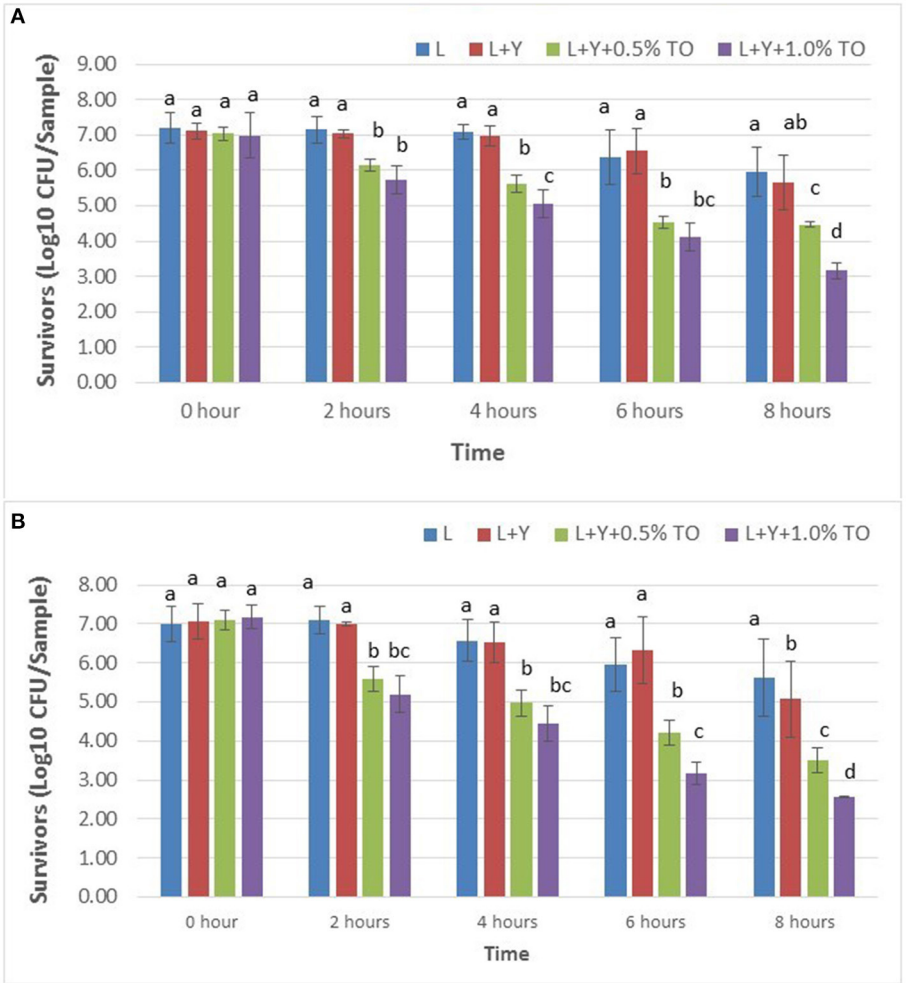

FIGURE 2 | Survivors of *Salmonella enterica* attached to raw chicken breast meat in lemon juice marinades based on bacterial colony counts on TAL medium **(A)** and on XLT4 agar **(B)**.

($P < 0.05$). After 8 h, initial numbers of viable *S. enterica* on chicken in lemon juice alone and with yucca extract decreased from 7.08 to 5.96 and 5.66 Log CFU/sample, respectively, based on numbers of survivors on TAL medium (**Figure 2A**). In contrast, after 8 h, initial numbers of the pathogen (Log CFU/sample) on chicken in marinades with added thyme oil decreased to 4.46 (0.5% TO) and 3.17 (1.0% TO) (**Figure 2A**). Higher numbers of *Salmonella* survivors were consistently recovered on TAL medium compared to XLT agar.

Decimal Reduction Times (D-Values)

Table 1 shows the effect of marination on D-values for *S. enterica* planktonic cells (A) and cells attached to raw chicken breast meat (B). No significant differences in D-values were observed for cells treated with lemon juice alone compared to lemon juice containing only yucca extract irrespective the state of the cells (planktonic or attached) or plating medium ($P > 0.05$). For planktonic cells in marinade solutions the addition of thyme oil (0.1 or 0.2%) significantly decreased the D-value of the pathogen compared to control (lemon juice and lemon juice + yucca extract) irrespective of the plating medium ($P < 0.05$). Based on numbers of pathogen survivors on TAL medium, a similar observation was made for cells attached to chicken whereby significant reductions in D-values ($P < 0.05$) occurred with the addition of thyme oil to marinade solutions (**Table 1B**). Based on numbers of survivors on XLT-4 agar, no significant differences in D-values ($P > 0.05$) were observed for *S. enterica* cells attached to marinated raw chicken (**Table 1B**).

Sub-lethal Injury in *Salmonella* Survivors

Table 2 shows sub-lethal injury (expressed by linear regression parameters) in *S. enterica* survivors resulting from exposure of inoculated chicken breast meat to the marinade solutions. That table displays the slopes and intercepts from linear regression plots, which showed reduction in culturability of the pathogen. Based on those slope and intercept parameters, the extent of sub-lethal injury in pathogen survivors caused by each of the marinade treatments was not statistically significant ($P > 0.05$).

TABLE 1 | Decimal reduction times (D values) for *Salmonella enterica* as planktonic cells **(A)** and cells attached to raw chicken breast meat **(B)** during exposure to lemon juice marinade solutions at 22 ± 1°C.

	D value (minutes)[x]	
Treatment	**TAL**	**XLT4**
(A) PLANKTONIC CELLS		
Lemon juice	4.83 ± 0.18[a]	3.55 ± 0.26[a]
Lemon juice + yucca*	5.21 ± 0.44[a]	3.50 ± 0.08[a]
Lemon juice + yucca + 0.1% thyme oil	2.99 ± 0.21[b]	2.41 ± 0.40[b]
Lemon juice + yucca + 0.2% thyme oil	1.02 ± 0.46[c]	0.74 ± 0.28[c]
	D value (hours)[x]	
Treatment	**TAL**	**XLT4**
(B) ATTACHED CELLS		
Lemon juice	4.59 ± 1.39[a,b]	4.04 ± 1.50[a]
Lemon juice + yucca	6.44 ± 2.09[a]	3.68 ± 1.54[a]
Lemon juice + yucca + 0.5% thyme oil	2.46 ± 0.19[b]	2.04 ± 0.15[a]
Lemon juice + yucca + 1.0% thyme oil	2.25 ± 0.33[b]	1.84 ± 0.22[a]

**yucca extract (0.5%); TAL, thin agar layer medium; XLT4, xylose lysine tergitol agar.*
[x]For each group of cells (planktonic or attached) average D values with different superscripts (a, b) within a column are significantly different (P < 0.05).

TABLE 2 | Slopes and intercepts from regression plots for reduction in viability of *Salmonella enterica* on chicken breast meat in lemon juice marinade.

Treatment	[a]Slope	[b]Intercept	R-Squared
Lemon juice alone	1.283 ± 0.227	0.349 ± 0.227	0.918
Lemon+ Yucca	1.223 ± 0.066	0.104 ± 0.047	0.857
Lemon+ Yucca+ 0.5% Thyme oil	1.235 ± 0.245	−0.222 ± 0.736	0.907
Lemon+ Yucca+ 1.0% Thyme oil	1.204 ± 0.108	0.431 ± 0.291	0.889

[a]Values for slope are not significantly different from 1.0 (P > 0.05).
[b]Values for intercept are not significantly different from 0 (P > 0.05).
Each value represents the mean ± standard deviation from 3 replicate experiments.

The pH of Marinade Solutions

The pH value of the lemon juice was 2.44 and its citric acid content was 7.0 g/100 mL. The pH values for marinade solutions (22 ± 1°C) with or without raw chicken are shown in **Table 3**. The initial pH of the marinades ranged from 2.44 to 2.46. The pH of control marinade solution (without raw chicken) remained largely unchanged (pH ∼ 2.44) through 8 h. In contrast, significant increases ($P < 0.05$) in the pH values were observed for all marinades that contained raw chicken. Increases in pH values at 4 and 8 h averaged 0.16 and 0.44, respectively. Compared to control, all other marinades exhibited a higher pH value after 2, 4, 6, and 8 h in contact with raw chicken samples ($P < 0.05$) with pH values ranging from 2.88 to 2.90 after 8 h.

GC-MS Analysis of Thyme Oil

Results of gas chromatography-mass spectrometry (GC-MS) analysis of thyme oil used in the present study are presented in **Table 4**. Fifteen different components representing major and minor components of that essential oil were identified at concentrations ranging from 0.14 to 51.07%. Thymol and O-cymene were the top two major components at concentrations of 51.07 and 24.1%, respectively.

DISCUSSION

In vitro microbial susceptibility tests such as MIC and MBC, are usually performed to evaluate the sensitivity of an organism to an antimicrobial agent such as an antibiotic or chemical preservative. Since the MIC and MBC of thyme oil depend on several variables including composition and concentration of this EO's bioactive components and the cultural conditions for the test organisms, comparison of results with those of other studies involving thyme oil is not simple (39). Considering these limitations, Lu and Wu (40) reported 0.1 and 0.2% as MIC and MBC, respectively, for each of four *S. enterica* serotypes namely, Typhimurium, Enteritidis, Sefentenberg, and Kentucky. Those concentrations are higher than those (MIC = 0.03% and MBC = 0.06%) for the five serovar mixture (Enteritidis ATCC13076, Heidelberg ATCC 8326, Typhimurium ATCC 14802, Gaminara ATCC 8324, and Oranienburg ATCC 9239) reported in the present study. While the MIC and MBC concentrations differ between the two studies, one common finding is that the MBC is twice that of the MIC. In this respect, when the ratio of MBC to MIC is ≤4, the antimicrobial is bactericidal (41). Since the MBC:MIC ratio of thyme oil for *S. enterica* is 2, that EO is bactericidal and should be effective for augmenting the antibacterial activity of citrus juice marinades against *S. enterica*.

Citrus juices from lime and lemon fruit are major sources of citric acid and are widely used by consumers to marinate raw poultry meat in preparation for cooking. Several published reports have highlighted consumers' belief that diluted lemon juice, lime juice, or vinegar may destroy pathogens on raw poultry meat to improve microbial safety of this product (42, 43). In the present study, exposure of planktonic *S. enterica* cells to undiluted lemon juice (pH 2.44) at 22 ± 1°C for 15 min decreased initial numbers of the pathogen by 3.7 Log CFU/mL based on numbers of survivors on TAL medium (**Figure 1A**). This result indicates that lemon juice alone exerts a bactericidal effect on *S. enterica*. The low pH (2.44) of the lemon juice plus the citric acid in that juice are likely responsible for the observed antimicrobial action as similar findings were reported by others (22, 44). The 0.92 Log CFU decrease in initial numbers of *S. enterica* after 2 h of inoculating the chicken suggests a loss of viability of some cells during drying of the inoculum at ambient temperature (22 ± 1°C). In this regard, 7.08 Log CFU/sample was used as the actual initial viable count for calculating reductions in populations of the pathogen on chicken breast meat.

When samples of inoculated raw chicken breast meat were marinated for 8 h in lemon juice alone, reduction in the initial viable count was only 1.12 Log CFU/sample (**Figure 2A**) which was lower than that observed for planktonic cells exposed to lemon juice for 15 min (**Figure 1A**). These results are not surprising considering the fact that bacteria attached to surfaces are more tolerant to antimicrobial agents compared to planktonic bacteria (45). For example, *Salmonella* cells attached

TABLE 3 | Changes in pH of marinade solutions during marination of non-inoculated raw chicken breast meat at 22 ± 1°C.

Treatment	[a]pH of marinade solutions				
	0 h	2 h	4 h	6 h	8 h
L (NC)*	2.44 ± 0.01Ax	2.44 ± 0.02Ay	2.43 ± 0.05Ay	2.45 ± 0.02Ay	2.44 ± 0.02Ay
L	2.44 ± 0.02Ex	2.51 ± 0.03Dx	2.59 ± 0.04Cx	2.66 ± 0.02Bx	2.89 ± 0.02Ax
L + Y	2.46 ± 0.02Ex	2.54 ± 0.04Dx	2.62 ± 0.03Cx	2.68 ± 0.01Bx	2.88 ± 0.02Ax
L + Y + 0.5% TO	2.45 ± 0.03Ex	2.55 ± 0.02Dx	2.60 ± 0.02Cx	2.68 ± 0.02Bx	2.90 ± 0.02Ax
L + Y + 1.0% TO	2.46 ± 0.02Dx	2.54 ± 0.04Cx	2.64 ± 0.02Bx	2.67 ± 0.03Bx	2.88 ± 0.03Ax

[a]*Each pH value represents the mean ± standard deviations of three replicate experiments.*

**NC, no chicken in marinade.*

L, lemon juice; Y, yucca extract (0.5%); TO, thyme oil.

Means that do not share the same letter (A, B, C, D, E) within the same row or within each column (x, y) are significantly different ($P < 0.05$).

TABLE 4 | Compounds identified in thyme oil based on analysis using GC-MS.

Component	RT	RI_s	RI_{Nist}	Percent (%)
α-pinene	5.98	942.5	937	1.78
Camphene	6.37	956.8	952	0.42
β-Myrcene	7.29	990	991	0.55
o-Cymene	8.24	1,024.3	1,022	**24.1**
Eucalyptol	8.45	1,032	1,032	1.67
γ-Terpinene	9.14	1,057.2	1,060	5.42
4-Carene/Linalool	10.27	1,097.9	1,099	4.4
Camphor	11.63	1,147	1,145	1.49
Isoborneol	12.09	1,164	1,157	0.14
Endo-borneol	12.32	1,172.6	1,167	0.73
Terpinen-4-ol	12.56	1,181.2	1,177	0.72
Thymol	15.57	1,293.2	1,291	**51.07**
Carvacrol	15.82	1,301.5	1,299	3.33
Caryophyllene	18.99	1,422.6	1,419	0.2
Caryophyllene oxide	22.95	1,585.4	1,581	0.18

RT, Retention time; RI_s, Kovat's Retention index of thyme oil compounds found in the sample used in this study; RI_{Nist}, Kovat's Retention index in NIST14 library. Values in bold type indicate the amounts (%) of two major components of thyme oil.

to surfaces exhibited far more resistance than planktonic cells to disinfectants used during poultry processing (46). Moreover, the increased resistance of the attached cells may be partly attributed to the protective effect that the chicken breast offers *S. enterica*. It is likely that cell attachment to the chicken meat surface precludes full contact of the pathogen with the marinade treatment solutions. Additionally, production of biofilm on the meat surface could offer some protection to the attached cells. Dimakopoulou-Papazoglou et al. (47) reported an increased tendency of *S. enterica* to produce biofilm rapidly especially when they were exposed to conditions of very low pH. However, for planktonic *S. enterica* cells, such protection via attachment and biofilms is not possible because the cells are directly exposed to marinade solutions.

The survival of *S.enterica* on chicken breast meat in lemon juice suggests that, depending on the initial level of *Salmonella* contamination, survivors of that pathogen may persist on raw chicken in lemon juice even after several hours and pose a health risk to consumers. Our results are supported by those of Yang et al. (22) who reported that lemon juice marinade (pH 2.6–2.8) was not effective for completely inactivating *Salmonella* Enteritidis, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on raw beef. In the present study, the addition of thyme oil (0.1 or 0.2% wt/vol) to the lemon juice significantly ($P < 0.05$) increased in the bactericidal effect of lemon juice marinades against both planktonic (**Figure 1A**) and attached cells (**Figure 2A**) of *S. enterica*.

There are several published reports on the potential of essential oils (EOs) including thyme oil to control foodborne pathogens due to their bacteriostatic and bactericidal properties (24, 26, 27, 29, 48–50). Although the precise antibacterial mode of action of thyme oil is not fully elucidated, the antimicrobial effects of this EO might be attributed to additive or synergistic effects of several of its major and/or minor components (26, 51). The results of GC-MS analysis of the composition of the thyme oil used in the present study (**Table 4**) revealed that thymol (51.1%) and O-cymene (24.1%) were two major components. Recently, compositional analysis of thyme oil extracted from thyme leaves in Cordoba, Argentina, revealed that the two major components of that EO were also thymol and O-cymene at concentrations of 34.8 and 37.1%, respectively (52). (53) reported that the antibacterial mode of action of thymol involves increased cell permeability, dissipation of the pH gradient across the cytoplasmic membrane, and cellular leakage of inorganic ions. The other components of thyme oil may further contribute to the bactericidal properties as they may act synergistically in contribution to thyme oil's antibacterial effect. For example, p-cymene, another main component of essential oils, has relatively weak antimicrobial activity; however, it may enhance the antimicrobial action of other EO components via synergism and additive effects ((54))

Other plausible factors that likely contributed to the antibacterial effect of lemon juice marinade containing thyme oil are increased hydrophobicity and miscibility of the thyme oil. Hydrophobicity of EOs increases at low pH levels (55) and increased hydrophobicity enhances partitioning of EOs in bacterial membrane lipids to disrupt cell functioning (26, 56, 57). In this regard, lemon juice (pH 2.44) used in the present study, served as a low pH medium for enhancing the bactericidal effect of thyme oil against *Salmonella* on raw chicken

meat. The hydrophobic characteristic of thyme oil precludes its miscibility in the lemon juice, which is hydrophilic. Poor miscibility EOs in water-based solutions is one of the challenges to their widespread application in foods systems (29, 30). When an antimicrobial agent is poorly miscible in foods, it does not readily contact foodborne microorganisms to exert its bacteriostatic or bactericidal effect. Improved miscibility of EOs via use of emulsifiers can enhance their antimicrobial activity (58). To improve the miscibility of EOs, researchers have used synthetic emulsifiers such as Tween 20 or Tween 80 (24, 59, 60). However, considering growing consumer demand for more natural alternatives to synthetic food additives, we used yucca extract in the present study to emulsify the thyme oil in the lemon juice. Thomas-Popo et al. (31) were the first researchers to report the application of yucca extract for solubilizing an EO component (isoeugenol) in raw pineapple juice to kill enteric pathogens. Yucca extract from the Mohave Yucca plant (*Yucca schidigera)* is a natural surfactant, which is FDA-approved for use in the food, cosmetic, and feed industries (61). That plant extract contains saponins, which have both lipophilic and hydrophilic characteristics (62). Also, saponins are known to have antimicrobial properties (63). Based on the previously stated information, we speculate that both the low pH of the lemon juice (pH 2.44) and the emulsifying property of yucca extract improved the antibacterial activity of thyme oil against *S. enterica* as planktonic cells and as cells attached to chicken breast meat.

The D-values (**Table 1**) for *S. enterica* exposed to marinade solutions represent the times required for a 10-fold (1.0 Log) destruction of the initial viable population of the pathogen. The significant ($P < 0.05$) decreases in D-value for planktonic cells of *S. enterica* in marinades containing thyme oil at 0.1 and 0.2% (v/v) suggest a faster death rate of the pathogen in those marinades compared to lemon juice alone or with added yucca extract (**Table 1A**). Based on numbers of survivors on TAL medium, the death rate for *S. enterica* cells attached to chicken breast meat was significantly ($P < 0.05$) faster in lemon juice marinades with added thyme oil at 0.5 and 1.0% (v/v) compared to lemon juice with added yucca extract (**Table 1B**). Most of the antimicrobial activity of thyme oil seem to be associated with its phenolic components such as carvacrol and thymol (64–66). The antimicrobial action of phenolic compounds is mainly associated with membrane disruption in Gram-negative and Gram-positive bacteria (67). While carvacrol constitutes only 3.33% of the components of thyme oil used in the present study, thymol (51%) is a major phenolic component (**Table 4**). The increased death rate of *S. enterica* cells in lemon juice with added thyme oil further suggests that the antimicrobial components of thyme oil inflicted additional damage to the pathogen beyond that caused by the low pH of the lemon juice.

Our observed increase in pH of the marinades (22 ± 1°C) during 8 h of marination of chicken breast meat (**Table 3**) was likely due to dilution of the marinade by juices from the raw chicken. Similar increases in pH of meat marinades have been reported (12, 68). Tan et al. (69) demonstrated that raw chicken meat immersed in a buffered saline solution (pH 2.0) at 4°C increased the pH of that medium to 4.74 after 24 h. In those previously mentioned reports, the increases in pH were likely attributed to the high buffering capacity of meat proteins (12). The buffering effect of chicken meat protected *S. enterica* from the effects of acidic pH (69). Our findings and those of others regarding the increase in pH of acidic solutions containing poultry meat suggest that raw poultry meat can decrease the inhibitory properties of acidic marinades over time. This in turn can reduce the lethal effect of the marinade and allow survival of human enteric pathogens to pose a food safety risk to consumers. This problem is further exacerbated considering that some *Salmonella* serovars possess acid-adaptation systems that enhance their survival at pH levels as low as 2.5 (70, 71). After 8 h, in spite of the increase in pH of the marinades containing raw chicken breast meat, the added thyme oil (1.0% v/v) inactivated the initial population of *S. enterica* by 3.91 and 4.52 Log CFU/sample, based on survivors on TAL medium and XLT4 agar, respectively (**Table 2**) ($P < 0.05$). Based on this result thyme oil at 1.0% (v/v) exhibits good potential for killing *S. enterica* on chicken meat in acidic marinades that undergo increases in pH during several hours of marination at ambient temperature (22 ± 1°C).

Despite recommendations by government agencies globally (72–74) that meats should be marinated under refrigeration, some consumers marinate meats at room temperature. This is especially true in some areas of the world where refrigeration is unavailable. In the present study, raw chicken breast meat immersed in lemon juice was held at 22 ± 1°C for 8 h to simulate temperature abuse during marination. The recommendation for marinating meats under refrigeration (<5°C) for no more than 2 days (74) is to prevent growth of pathogenic microorganisms. In the present study, addition of thyme oil to lemon juice marinade significantly increased the death rate of *S. enterica* on the chicken compared to marinade without thyme oil ($P < 0.05$). These findings suggest that thyme oil can decrease the survival of *S. enterica* in lemon juice marinade under temperature abuse conditions for 8 h. Therefore, addition of thyme oil to acidic marinades may serve as an alternative to refrigeration to ensure microbial safety of chicken breast meat for 8 h at room temperature.

In determining the numbers of *S. enterica* survivors in lemon juice marinades, we plated diluted samples of marinade on two agar media, namely, TAL medium and XLT4 agar. Two agar media were used to evaluate the extent of sub-lethal injury in *S. enterica* survivors. Hurst (75) described sub-lethal injury as a result of exposure of microbes to a chemical or physical process that damages but does not kill them. The TAL medium allowed resuscitation and growth of sub-lethally injured survivors and growth of non-injured survivors of the pathogen without interference (growth) from background organisms (35, 76). The XLT4 agar is a selective medium, which allowed growth of non-injured survivors while preventing growth of sub-lethally injured organisms and background microflora. Therefore, the occurrence of larger populations of *S. enterica* on TAL medium compared to XLT4 agar indicated sub-lethal injury. For both planktonic cells and cells attached to raw chicken, numbers of *S. enterica* survivors were significantly higher on TAL medium compared to XLT4 agar ($P < 0.05$). On this basis, these results indicate that the acidic marinade treatments caused sub-lethal

injury in the part of the surviving population of *S. enterica*. However, based on the slope intercept method described by Wuytack et al. (36) the extent of sub-lethal injury caused by each of the marinades was not statistically significant ($P > 0.05$; **Table 3**).

The consistently lower numbers of *S. enterica* survivors observed on XLT4 agar suggests that the use of selective media for evaluating pathogen survivors in acidic marinades can erroneously overestimate the extent of inactivation of the target pathogen. This is because selective media such as XLT4 are unable to support growth of injured pathogens (77). More importantly sub-lethally injured organisms might be able to resuscitate and develop increased resistance to antimicrobial treatments (78). From a food safety perspective, our results are important considering that some *S. enterica* serovars have a low infectious dose (79). Therefore, failure to detect even small amounts of that pathogen due to sub-lethal injury causes critical limitations in food diagnostics because of overestimating pathogen inactivation and the possibility of false negative results (80).

CONCLUSIONS

Salmonella enterica can persist for several hours on raw chicken breast meat during marination in lemon juice at room temperature (22°C). The addition of thyme oil at 0.5 or 1.0 % (v/v) to lemon juice marinade containing yucca extract significantly increases the death rate of *S. enterica* on raw chicken during marination at 22°C. Thyme oil combined with yucca extract can improve the antimicrobial effectiveness of lemon juice against *Salmonella* on raw chicken breast and enhance the microbial safety of this popular poultry product.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

AM conceived the research idea, designed the study, and guided the research performed by SK. SK performed the experiments and drafted the manuscript. RM performed GC-MS analysis of thyme oil. ET-P and SK performed statistical analysis of the data. JD, AS, ET-P, SW, and SI provided revisions of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Iowa Agriculture and Home Economics Experiment Station Project No. IOW04202, sponsored by Hatch Act and State of Iowa funds.

ACKNOWLEDGMENTS

The authors thank Allison Little for laboratory assistance, and Melanie Mendonca-Stephens, Garuda International, Inc., for providing the *Yucca schidigera* extract.

REFERENCES

1. Bajpai VK, Baek K-H, Kang SC. Control of *Salmonella* in foods by using essential oils: a review. *Food Res Int*. (2012) 45:722–34. doi: 10.1016/j.foodres.2011.04.052
2. Boore AL, Hoekstra RM, Iwamoto M, Fields PI, Bishop RD, Swerdlow DL. *Salmonella enterica* infections in the United States and assessment of coefficients of variation: a novel approach to identify epidemiologic characteristics of individual serotypes, 1996-2011. *PLoS ONE*. (2015) 10:e0145416. doi: 10.1371/journal.pone.0145416
3. Centers for Disease Control and Prevention. *National Outbreak Reporting System (NORS), Outbreaks per State, Salmonella, Chicken*. (2020). Available online at: https://wwwn.cdc.gov/norsdashboard/ (accessed March 15, 2020).
4. Smith DP, Northcutt JK, Cason JA, Hinton A, Buhr RJ, Ingram KD. Effect of external or internal fecal contamination on numbers of bacteria on prechilled broiler carcasses. *Poult Sci*. (2007) 86:1241–4. doi: 10.1093/ps/86.6.1241
5. Wigley P. Genetic resistance to *Salmonella*. infection in domestic animals. *Res Vet Sci*. (2004) 76:165–9. doi: 10.1016/S0034-5288(03)00117-6
6. Carramiñana JJ, Yangüela J, Blanco D, Rota C, Agustin AI, Ariño A, et al. *Salmonella* incidence and distribution of serotypes throughout processing in a Spanish poultry slaughterhouse. *J Food Prot*. (1997) 60:1312–7. doi: 10.4315/0362-028X-60.11.1312
7. Golden CE, Mishra A. Prevalence of *Salmonella* and *Campylobacter* spp. in alternative and conventionally produced chicken in the United States: a systematic review and meta-analysis. *J Food Prot*. (2020) 83:1181–97. doi: 10.4315/JFP-19-538
8. Khan AS, Georges K, Rahaman S, Abdela W, Adesiyun AA. Prevalence and serotypes of *Salmonella* spp. on chickens sold at retail outlets in Trinidad. *PLoS ONE*. (2018) 13:e0202108. doi: 10.1371/journal.pone.0202108
9. Regalado-Pineda ID, Rodarte-Medina R, Resendiz-Nava CN, Saenz-Garcia CE, Castañeda-Serrano P, Nava GM. Three-year longitudinal study: prevalence of *Salmonella enterica*. in chicken meat is higher in supermarkets than wet markets from Mexico. *Foods*. (2020) 9:264. doi: 10.3390/foods9030264
10. D'Aoust J, Maurer JJ. *Salmonella* species. In: P Doyle M, Beuchat LR, Montville TJ, editors. *Food Microbiology: Fundamentals and Frontiers*. Washington, DC: ASM Press (2007). p. 141–78.
11. White PL, Baker AR, James WO. Strategies to control *Salmonella* and *Campylobacter* in raw poultry products. *Rev Sci Tech*. (1997) 16:525–41. doi: 10.20506/rst.16.2.1046
12. Björkroth J. Microbiological ecology of marinated meat products. *Meat Sci*. (2005) 70:477–80. doi: 10.1016/j.meatsci.2004.07.018
13. Quelhas I, Petisca C, Viegas O, Melo A, Pinho O, Ferreira IMPLVO. Effect of green tea marinades on the formation of heterocyclic aromatic amines and sensory quality of pan-fried beef. *Food Chem*. (2010) 122:98–104. doi: 10.1016/j.foodchem.2010.02.022
14. Nisiotou A, Chorianopoulos NG, Gounadaki A, Panagou EZ, Nychas G-JE. Effect of wine-based marinades on the behavior of *Salmonella* Typhimurium and background flora in beef fillets. *Int J Food Microbiol*. (2013) 164:119–27. doi: 10.1016/j.ijfoodmicro.2013.04.008
15. Lytou AE, Nychas G-JE, Panagou EZ. Effect of pomegranate based marinades on the microbiological, chemical and sensory quality of chicken meat: a metabolomics approach. *Int J Food Microbiol*. (2018) 267:42–53. doi: 10.1016/j.ijfoodmicro.2017.12.023
16. Alvarado C, McKee S. Marination to improve functional properties and safety of poultry meat. *J Appl Poult Res*. (2007) 16:113–20. doi: 10.1093/japr/16.1.113

17. Pathania A, McKee SR, Bilgili SF, Singh M. Antimicrobial activity of commercial marinades against multiple strains of *Salmonella* spp. *Int J Food Microb*. (2010) 139:214–7. doi: 10.1016/j.ijfoodmicro.2010.01.039
18. Lytou AE, Renieri CT, Doulgeraki AI, Nychas GJE, Panagou EZ. Assessment of the microbiological quality and safety of marinated chicken products from Greek retail outlets. *Int J Food Microbiol*. (2020) 320:108506. doi: 10.1016/j.ijfoodmicro.2019.108506
19. Smith DP, Acton JC. Marination, cooking, and curing of poultry products. In: Owens CM, Alvarado CZ, Sams AR, editors. *Poultry Meat Processing*. Boca Raton, FL: CRC Press (2001). p. 257–79.
20. Henley SC, Launchi N, Quinlan JJ. Survival of *Salmonella* on raw poultry exposed to 10% lemon juice and vinegar washes. *Food Control*. (2018) 94:229–32. doi: 10.1016/j.foodcont.2018.06.034
21. Lytou AE, Tzortzinisa K, Skandamis PN, Nychas G-JE, Panagoua EZ. Investigating the influence of organic acid marinades, storage temperature and time on the survival/inactivation interface of *Salmonella* on chicken breast fillets. *Int J Food Microbiol*. (2019) 299:47–57. doi: 10.1016/j.ijfoodmicro.2019.03.019
22. Yang J, Lee D, Afaisen S, Gadi R. Inactivation by lemon juice of *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes* in beef marinating for the ethnic food kelaguen. *Int J Food Microbiol*. (2013) 160:353–9. doi: 10.1016/j.ijfoodmicro.2012.11.009
23. Bremer PJ, Osborne CM. Efficacy of marinades against Listeria monocytogenes cells in suspension or associated with green shell mussels (*Perna canaliculus*). *Appl. Environ. Microbiol*. (1995) 61:1514–9. doi: 10.1128/AEM.61.4.1514-1519.1995
24. Van Haute S, Raes K, Van der Meeren P, Sampers I. The effect of cinnamon, oregano and thyme essential oils in marinade on the microbial shelf life of fish and meat products. *Food Control*. (2016) 68:30–9. doi: 10.1016/j.foodcont.2016.03.025
25. Karam L, Roustom R, Abiad MG, El-Obeidd T, Savvaidis IN. Combined effects of thymol, carvacrol and packaging on the shelf-life of marinated chicken. *Int J Food Microbiol*. (2019) 291:42–7. doi: 10.1016/j.ijfoodmicro.2018.11.008
26. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. *Int J Food Microbiol*. (2004) 94:223–53. doi: 10.1016/j.ijfoodmicro.2004.03.022
27. Tajkarimi MM, Ibrahim SA, Cliver DO. Antimicrobial herb and spice compounds in food. *Food Control*. (2010) 21:1199–218. doi: 10.1016/j.foodcont.2010.02.003
28. Gyawali R, Ibrahim SA. Natural products as antimicrobial agents. *Food Control*. (2014) 46:412–29. doi: 10.1016/j.foodcont.2014.05.047
29. Mendonca A, Jackson-Davis A, Moutiq R, Thomas-Popo E. Use of natural antimicrobials of plant origin to improve the microbiological safety of foods. In: Ricke SC, Atungulu GG, Rainwater CE, Park SH, editors. *Food and Feed Safety Systems and Analysis*. Amsterdam: Academic Press (2018). p. 249–72. doi: 10.1016/B978-0-12-811835-1.00014-2
30. Samperio C, Boyer R, Eigel WN, Holland KW, McKinney JS, O'Keefe SF, et al. Enhancement of plant essential oils' aqueous solubility and stability using alpha and beta cyclodextrin. *J Agric Food Chem*. (2010) 58:12950–6. doi: 10.1021/jf103275a
31. Thomas-Popo E, Mendonca A, Dickson J, Shaw A, Coleman S, Daraba A, et al. Isoeugenol significantly inactivates *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* in refrigerated tyndallized pineapple juice with added *Yucca schidigera* extract. *Food Control*. (2019) 106:106727. doi: 10.1016/j.foodcont.2019.106727
32. López-Malo A, Barreto-Valdivieso J, Palou E, Martín FS. *Aspergillus flavus* growth response to cinnamon extract and sodium benzoate mixtures. *Food Control*. (2007) 18:1358–62. doi: 10.1016/j.foodcont.2006.04.010
33. CLSI. *Methods for Determining Bactericidal Activity of Antimicrobial Agents*. Wayne, PA: Approved Guideline, Clinical and Laboratory Standards Institute document (CLSI) (1999), M26–A.
34. Garuda International, Inc. *Yucca Schidigera Extract NP Raw Material (Food Grade)*. (2013). Available online at: http://www.garudaint.com/prodspec.php?prod_code=YUCEXTNP (accessed October 12, 2020).
35. Kang D-H, Fung DYC. Thin agar layer method for recovery of heat-injured *Listeria monocytogenes*. *J Food Prot*. (1999) 62:1346–9. doi: 10.4315/0362-028X-62.11.1346
36. Wuytack EY, Phuong LD, Aertsen A, Reyns KM, Marquenie D, De Ketelaere B, et al. Comparison of sublethal injury induced in *Salmonella enterica* serovar Typhimurium by heat and by different nonthermal treatments. *J Food Prot*. (2003) 66:31–7. doi: 10.4315/0362-028X-66.1.31
37. Wang F, Mendonça A, Brehm-Stecher BF, Dickson J, DiSpirito A, Shaw A, et al. Long-term survival phase cells of *Salmonella* Typhimurium ATCC 14028 have significantly greater resistance to ultraviolet radiation in 0.85% saline and apple Juice. *Foodborne Pathog Dis*. (2018) 15:538–43. doi: 10.1089/fpd.2018.2423
38. Van den Dool H, Kratz PD. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J Chromat*. (1963) A11:463–71. doi: 10.1016/S0021-9673(01)80947-X
39. Verheyen D, Baka M, Van Impe JFM. Sublethal injury caused to *Listeria monocytogenes* by natural plant extracts: case study on grape seed extract and garlic extract. *Foods*. (2019) 9:264. doi: 10.3390/app9132731
40. Lu Y, Wu C. Reduction of *Salmonella enterica* contamination on grape tomatoes by washing with thyme oil, thymol, and carvacrol as compared with chlorine treatment. *J Food Prot*. (2010) 73:2270–5 doi: 10.4315/0362-028X-73.12.2270
41. Pankey GA, Sabath LD. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. *Clin Infect Dis*. (2004) 38:864–70. doi: 10.1086/381972
42. Drexel University. *Don't Wash Your Chicken!*. (2013). Available online at: https://drexel.edu/dontwashyourchicken/ (accessed October 17, 2020).
43. Henley SC, Stein SE, Quinlan JJ. Identification of unique food handling practices that could represent food safety risks for minority consumers. *J Food Prot*. (2012) 75:2050–4. doi: 10.4315/0362-028X.JFP-12-146
44. Bjornsdottir K, Breidt F, McFeeters RF. Protective effects of organic acids on survival of *Escherichia coli* O157:H7 in acidic environments. *Appl Environ Microbiol*. (2006) 72:660–4. doi: 10.1128/AEM.72.1.660-664.2006
45. Sereno M, Ziech R, Druziani J, Pereira J, Bersot L. Antimicrobial susceptibility and biofilm production by *Salmonella* spp. strains isolated from frozen poultry carcasses. *Braz J Poult Sci*. (2017) 19:103–8. doi: 10.1590/1806-9061-2016-0268
46. Cadena M, Kelman T, Marco ML, Pitesky M. Understanding antimicrobial resistance (AMR) profiles of *Salmonella* biofilm and planktonic bacteria challenged with disinfectants commonly used during poultry processing. *Foods*. (2019) 8:275. doi: 10.3390/foods8070275
47. Dimakopoulou-Papazoglou D, Lianou A, Koutsoumanis KP. Modelling biofilm formation of *Salmonella enterica* ser. Newport as a function of pH and water activity. *Food Microbiol*. (2016) 53:76–81. doi: 10.1016/j.fm.2015.09.002
48. Thanissery R, Smith DP. Marinade with thyme and orange oils reduces *Salmonella* Enteritidis and *Campylobacter coli* on inoculated broiler breast fillets and whole wings. *Poult Sci*. (2014) 93:1258–62. doi: 10.3382/ps.2013-03697
49. Manu D, Mendonca AF, Daraba A, Dickson JS, Sebranek J, Shaw A, et al. Antimicrobial Efficacy of cinnamaldehyde against *Escherichia coli* O157:H7 and *Salmonella enterica* in carrot juice and mixed berry juice held at 4°C and 12°C. *Foodborne Pathog Dis*. (2017) 14:5. doi: 10.1089/fpd.2016.2214
50. Possas A, Posada-Izquierdo GD, Pérez-Rodríguez F, Valero A, García-Gimeno RM, Duarte MCT. Application of predictive models to assess the influence of thyme essential oil on *Salmonella* Enteritidis behaviour during shelf life of ready-to-eat turkey products. *Int J Food Microbiol*. (2017) 240:40–6. doi: 10.1016/j.ijfoodmicro.2016.08.003
51. Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Front Microbiol*. (2012) 3:12. doi: 10.3389/fmicb.2012.00012
52. Prieto MC, Lapaz MI, Lucini EI, Pianzzola MJ, Grosso NR, Asensio CM. Thyme and suico essential oils: promising natural tools for potato common scab control. *Plant Biol*. (2020) 22:81–9. doi: 10.1111/plb.13048
53. Lambert RJW, Skandamis PN, Coote PJ, Nychas GJE. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J Appl Microbiol*. (2001) 91:453–62. doi: 10.1046/j.1365-2672.2001.01428.x

54. Marchese A, Arciola CR, Barbieri R, Silva AS, Nabavi SF, Tsetegho Sokeng AJ, et al. Update on monoterpenes as antimicrobial agents: a particular focus on p-Cymene. *Materials.* (2017) 10:E947. doi: 10.3390/ma10080947
55. Negi PS. Plant extracts for the control of bacterial growth: efficacy, stability and safety issues for food application. *Int J Food Microbiol.* (2012) 156:7–17. doi: 10.1016/j.ijfoodmicro.2012.03.006
56. Juven BJ, Kanner J, Schved F, Weisslowicz H. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. *J Appl Bacteriol.* (1994) 76:626–31. doi: 10.1111/j.1365-2672.1994.tb01661.x
57. Hyldgaard M, Mygind T, Piotrowska R, Foss M, Meyer RL. Isoeugenol has a non-disruptive detergent-like mechanism of action. *Front Microbiol.* (2015) 6:754. doi: 10.3389/fmicb.2015.00754
58. Hammer KA, Carson CF, Riley TV. Influence of organic matter, cations and surfactants on the antimicrobial activity of *Melaleuca alternifolia* (tea tree) oil *in vitro*. *J Appl Microbiol.* (1999) 86:446–52. doi: 10.1046/j.1365-2672.1999.00684.x
59. Budzynska A, Wieckowska-Szakiel M, Kalemba D, Sadowska B, Rozalska B. The optimization of methods utilized for testing the antibacterial activity of essential oils. *Med Dosw Mikrobiol.* (2009) 61:281–7.
60. Remmal A, Bouchikhi T, Tantaoui-Elaraki A, Ettayebi M. Inhibition of antibacterial activity of essential oils by tween 80 and ethanol in liquid medium. *J Pharm Belg.* (1993) 48:352–6.
61. USFDA 21 CFR.172.510. *Natural Flavoring Substances and Natural Substances Used in Conjunction With Flavors. Code of Federal Regulations (CFR). Title21 Food and Drugs Chapter 1 Subpart F, Section 172.510*. Washington, D.C.: Government Printing Office. (2020).
62. Cheeke PR. Actual and potential applications of *Yucca schidigera* and *Quillaja saponaria* saponins in human and animal nutrition. *J Animal Sci.* (2000) 77 (Suppl. E):1–10. doi: 10.1007/978-94-015-9339-7_25
63. Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z, et al. Antimicrobial activity of saponins from Medicago sp.: structure-activity relationship. *Phytother Res.* (2006) 20:454–7. doi: 10.1002/ptr.1876
64. Davidson PM, Naidu AS. Phytophenol. In: Naidu AS, editor. *Natural Food Antimicrobial Systems*. Boca Raton, FL: CRC Press (2000). p. 265–94.
65. Skocibusic M, Bezic N, Dunkic V. Phytochemical composition and antimicrobial activities of essential oils from *Satureja subspicata* Vis. growing in Croatia. *Food Chem.* (2006) 96:20–8. doi: 10.1016/j.foodchem.2005.01.051
66. Rota MC, Herrera A, Martinez RM, Sotomayor JA, Jordan MJ. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. *Food Control.* (2007) 19:681–7. doi: 10.1016/j.foodcont.2007.07.007
67. Lima MC, Paiva de Sousa C, Fernandez-Pradad C, Harеld J, Dubreuild JD, De Souza EL. A review of the current evidence of fruit phenolic compounds as potential antimicrobials against pathogenic bacteria. *Microb Pathog.* (2019) 130:259–70. doi: 10.1016/j.micpath.2019.03.025
68. Baltic T, Baltic ZM, Misic D, Ivanovic J, Janjic J, Boskovic M, et al. Influence of marination on *Salmonella* spp. growth in broiler breast fillets. *Acta Vet Beograd.* (2015) 65:417–28. doi: 10.1515/acve-2015-0034
69. Tan SM, Lee SM, Dykes GA. Buffering effect of chicken skin and meat protects *Salmonella enterica* strains against hydrochloric acid but not organic acid treatment. *Food Control.* (2014) 42:329–34. doi: 10.1016/j.foodcont.2014.02.031
70. Baik HS, Bearson S, Dunbar S, Foster JW. The acid tolerance response of *Salmonella* Typhimurium provides protection against organic acids. *Microbiology.* (1996) 142:3195–200. doi: 10.1099/13500872-142-11-3195
71. Waterman SR, Small PLC. Acid-sensitive enteric pathogens are protected from killing under extremely acidic conditions of pH 2.5 when they are inoculated onto certain solid food sources. *Appl Environ Microbiol.* (1998) 64:3882–6. doi: 10.1128/AEM.64.10.3882-3886.1998
72. Government of Canada. *Food Safety Tips for Barbequing.* (2018). Available online at: https://www.canada.ca/en/health-canada/services/general-food-safety-tips/food-safety-tips-barbecuing.html (accessed October 9, 2020).
73. National Environment Agency, Singapore. *Importance of Cold Chain Management and Guidelines on Storing Food Safely, Food Safety Bulletin, Issue 4:5. July – December*. (2016). Available online at: https://www.sfa.gov.sg/docs/default-source/our-services/nea-food-safety-bulletin-issue-4.pdf (accessed October 9, 2020).
74. USDA Food Safety and Inspection Service. *Chicken From Farm to Table*. (2014). Retrieved online at: https://www.fsis.usda.gov/wps/wcm/connect/ad74bb8d-1dab-49c1-b05e-390a74ba7471/Chicken_from_Farm_to_Table.pdf?MOD=AJPERES (accessed October 9, 2020).
75. Hurst A. Bacterial injury: a review. *Can J Microbiol.* (1977) 23:935–44. doi: 10.1139/m77-139
76. Lavieri NA, Sebranek JG, Cordray JC, Dickson JS, Jung S, Manu DK, et al. Evaluation of the thin agar layer method for the recovery of pressure-injured and heat-injured *Listeria monocytogenes*. *J Food Prot.* (2014) 77:828–31. doi: 10.4315/0362-028X.JFP-13-374
77. Wesche AM, Gurtler JB, Marks BP, Ryser ET. Stress, sub-lethal injury, resuscitation, and virulence of bacterial foodborne pathogens. *J Food Prot.* (2009) 72:1121–38. doi: 10.4315/0362-028X-72.5.1121
78. Brashears MM, Amezquita A, Stratton J. Validation of methods used to recover *Escherichia coli* O157: H7 and *Salmonella* spp. subjected to stress conditions. *J Food Prot*. (2001) 64:1466–71. doi: 10.4315/0362-028X-64.10.1466
79. Blaser MJ, Newman LS. A review of human salmonellosis: I. Infective dose. *Rev Infect Dis*. (1982) 4:1096–106. doi: 10.1093/clinids/4.6.1096
80. Noriega E, Velliou EG, Vanderlinden E, Mertens L, Van Impe J. Effect of cell immobilization on heat-induced sublethal injury of *Escherichia coli*, *Salmonella typhimurium* and *Listeria innocua*. *Food Microbiol.* (2013) 36:355–64. doi: 10.1016/j.fm.2013.06.015

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.

frontiers in Nutrition

REVIEW
published: 08 June 2021
doi: 10.3389/fnut.2021.628723

Combination of Natural Compounds With Novel Non-thermal Technologies for Poultry Products: A Review

Soukaina Barroug[1†], *Sonal Chaple*[1†] *and Paula Bourke*[1,2*]

[1] School of Biosystems and Food Engineering, University College Dublin, Dublin, Ireland, [2] School of Biological Sciences, Institute Global Food Security, The Queens University Belfast, Belfast, United Kingdom

Ensuring safe, fresh, and healthy food across the shelf life of a commodity is an ongoing challenge, with the driver to minimize chemical additives and their residues in the food processing chain. High-value fresh protein products such as poultry meat are very susceptible to spoilage due to oxidation and bacterial contamination. The combination of non-thermal processing interventions with nature-based alternatives is emerging as a useful tool for potential adoption for safe poultry meat products. Natural compounds are produced by living organisms that are extracted from nature and can be used as antioxidant, antimicrobial, and bioactive agents and are often employed for other existing purposes in food systems. Non-thermal technology interventions such as high-pressure processing, pulsed electric field, ultrasound, irradiation, and cold plasma technology are gaining increasing importance due to the advantages of retaining low temperatures, nutrition profiles, and short treatment times. The non-thermal unit process can act as an initial obstacle promoting the reduction of microflora, while natural compounds can provide an active obstacle either in addition to processing or during storage time to maintain quality and inhibit and control growth of residual contaminants. This review presents the application of natural compounds along with emerging non-thermal technologies to address risks in fresh poultry meat.

Keywords: poultry, non-thermal processing, natural compounds, *Campylobacter*, essential oils

OPEN ACCESS

Edited by:
Maria João Fraqueza, University of Lisbon, Portugal

Reviewed by:
Rosario Ramirez Bernabé, Center for Scientific and Technological Research of Extremadura (CICYTEX), Spain
Isabel Odriozola-Serrano, Universitat de Lleida, Spain

***Correspondence:**
Paula Bourke
paula.bourke@ucd.ie

[†] 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: *12 November 2020*
Accepted: *18 March 2021*
Published: *08 June 2021*

Citation:
Barroug S, Chaple S and Bourke P (2021) Combination of Natural Compounds With Novel Non-thermal Technologies for Poultry Products: A Review. Front. Nutr. 8:628723. doi: 10.3389/fnut.2021.628723

INTRODUCTION

Fresh poultry meat and poultry products are highly perishable products but also have high potential as sources of human infection due to the presence and persistence of key pathogens in the poultry process chain. Outbreaks of foodborne illnesses in association with poultry products are one of the primary causes of outbreaks in the US and the EU. Among the reported numbers and notification rates of confirmed zoonoses in the EU in 2018, the top 5 are *campylobacteriosis* (246,158), salmonellosis (91,662), yersiniosis (6,823), and Shiga toxin-producing *Escherichia coli* (STEC) infections (6,073) (1, 2). Also, according to the Centers for Disease Control and Prevention (3), around 11, 2, and 1% of foodborne outbreaks are associated with chicken, turkey, and other poultry products, respectively, while turkey (609 illnesses) had the most outbreak-associated illnesses followed by chicken (487 illnesses). The pathogen *Campylobacter* caused up to 1.5 million illnesses each year in the US (4); thus, a focus on comprehensive and emerging methods for safety control in poultry processing is warranted.

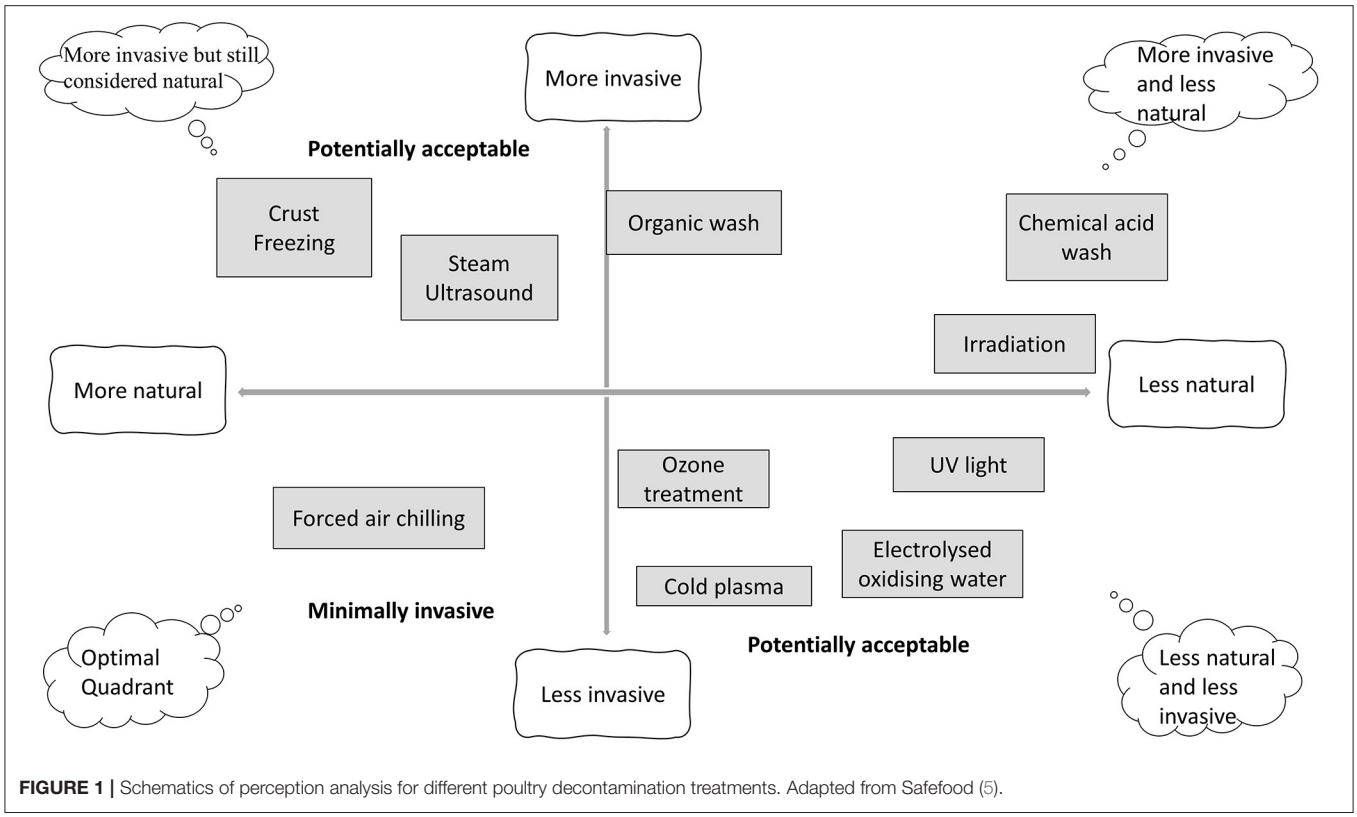

FIGURE 1 | Schematics of perception analysis for different poultry decontamination treatments. Adapted from Safefood (5).

In order to provide safer poultry products, the food industry has developed and implemented preventive measures based on the Hazard Analysis and Critical Control Points (HACCP) and food safety management systems in combination with technological interventions, such as sanitization processes, refrigeration, and modified atmosphere packaging that can control identified potential microbial hazards during food processing and storage. However, according to the policy and consumer demand requiring sustainable, safe, and high-quality minimally processed foods, the food industry seeks alternative approaches to extend safe shelf life; such as irradiation, high-pressure processing (HPP), and natural green chemicals including bio-preservation or intelligent packaging. **Figure 1** (6) illustrates the consumers' perception of 10 different meat decontamination processes based on how natural and invasive the process is considered.

The intensity of non-thermal processing treatment is vital for product safety and inactivation of pathogenic microbes. Han et al. (7) reported reactivation of sublethally injured microorganisms in a favorable environment during storage in meat and meat products. Spore-forming organisms such as *Clostridium botulinum* are resistant to HPP (8). The increase in a non-thermal process intensity to deal with recalcitrant microbiological issues may adversely affect foods' sensory properties (9). HPP can also alter the structure of polysaccharides and proteins, leading to textural changes in terms of hardness (10). Meat tenderness is the essential attribute that drives its consumer acceptability (11). Post slaughter, meat tenderization results from protease proteolysis of myofibrillar and cytoskeletal proteins as well as from the degradation of connective tissue substances, in particular collagen (12). Applying power ultrasound can produce free radicals, which imparts effective microbiological control, but which can also impact the product quality of high-fat foods due to oxidation (13). Similarly, irradiation can also cause undesirable organoleptic changes to high-fat foods and can induce color, odor, and taste effect on fresh meat products (14).

Thus, to overcome these shortcomings, there is potential to optimize non-thermal technology effects in combination with incorporation of natural compounds. Careful consideration of the mechanisms of action of individual non-thermal approaches may reveal what combinations can be successful across a range of food systems. For example, pulsed electric field (PEF) can cause cell membrane damage of microorganisms, enhancing sensitivity to antimicrobial agents like nisin (15). Similarly, application of naturally occurring antioxidant compounds such as rosemary extract, blueberry, or ascorbic acid can be used as an alternative for synthetic preservatives like butylated hydroxyanisole, butylated hydroxytoluene, and tertiary butylhydroquinone (16). Synergistic inactivation of microorganisms using non-thermal technology with natural compounds can be a promising way to increase the safety of poultry products while diminishing undesirable effects on some food characteristics. This review summarizes the key risks and published findings where non-thermal technologies have been combined with natural compounds and comments on the effects on the microbiological and physicochemical characteristics.

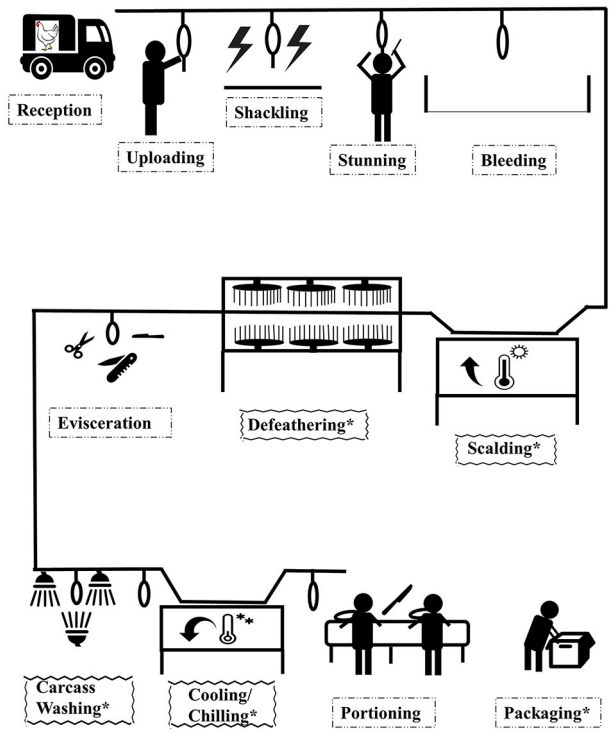

FIGURE 2 | Overview of poultry processing commercial chain. "*" represents a post slaughter stage point where antimicrobial interventions can take place.

CURRENT INTERVENTION PROCESS TECHNOLOGIES ENSURING POULTRY MICROBIOLOGICAL SAFETY

There are many approaches applied in poultry processing to maintain fresh poultry meat safety, which are generally classed as biological, physical, or chemical interventions (6). Electrolyzed water, hot water combined with rapid cooling, chilling and freezing (cold air and ice water), activated oxygen, and organic acids (lactic acid, oxidizing acids, and peroxyacetic acid) are examples of these decontamination processes currently used in poultry processing plants (17). The overview of the poultry processing plant is given in **Figure 2**. The proposed application of antimicrobial interventions in poultry processing can be implemented at carcass washing, scalding, defeathering, chilling/cooling, or packaging. The US Department of Agriculture (USDA) recommends the use of hot water above 74°C for sanitizing effect on carcasses.

High pressure, steam, and steam vacuum, as well as hot and cold water are some physical treatments commonly applied to meat carcass surface decontamination. Physical decontamination approaches currently used for chicken are steam and immersion in hot water (18). Due to the high temperature (100°C), microorganisms including natural microflora should be inactivated on the surface of the product within limited exposure times. Some limitations with stream usage are the deterioration of sensory characteristics attributed to changes of the color, and samples that look partially cooked, with shrunken skin (19). In steam vacuum processes, steam or hot water is sprayed on carcasses followed by vacuum treatment. This process is an effective method for spot decontamination at the slaughtering unit before the final chilling. The solutions of organic acids are frequently used in the chemical rinse to decontaminate the entire surface of carcasses. The most commonly used organic acids are acetic and lactic acids (20).

Peracetic acid, disodium phosphate, hexadecylpyridinium chloride, and sodium hypochlorite are the most utilized sanitizers during poultry processing (scalding and pre/post chilling) in poultry plants. Treatments with these antimicrobials can be online or off-line for reprocessing at different stages and temperatures and for different treatment times (21). Peracetic acid is an artificial disinfectant retaining good efficacy against poultry meat-related pathogens: *Salmonella* and *Campylobacter* (22). Sodium hypochlorite is commonly applied in water used for chilling/cleaning by spaying and/or immersion to reduce microbial load; however, the efficiency drops down significantly because of the interaction between organic matter in the meat and chlorine (23). Chlorine usage is prohibited in some countries including Germany, Denmark, and Belgium because of the potential interactions with organic matter within poultry carcasses, which can generate harmful chlorinated compounds (halo acetic acids, trihalomethanes, and chloramines) reported to be mutagenic and carcinogenic (23).

However, chlorine dioxide has also been used for sterilization, sanitization, and as disinfectant depending on its form (liquid or gas). Acidified sodium chlorite is an oxidative antimicrobial agent with a large activity spectrum (yeast, fungi, protozoa, viruses, pathogens, and molds). It is authorized by Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) to be used in poultry (24). One of the best alternatives to classical sodium hypochlorite is electrolyzed water due to it low cost, high sterilization effect, and non-harmful effect (23). Specific organic acids can also be effective in terms of microbial inactivation and stability in the presence of organic mateial, namely, citric acid, lactic acid, succinic acid, and acetic acid. Nevertheless, some limitations are associated with the usage of such organic acids such as off-colors, odors, and flavors in addition to material corrosion (24).

MICROBIOLOGICAL CONTAMINANTS OF CONCERN IN THE POULTRY SECTOR

Microbial Spoilage

Owing to microbial spoilage, millions of pounds per annum of fresh poultry meat products are lost (25, 26). Poultry meat spoilage has dramatic effects by limiting shelf life (27) and negatively affecting the economy (28). The deterioration may result from quality and sensory damage to change in texture, odor, color, taste (29), and slime formation (27, 30). These changes are induced by enzymatic reactions, lipid oxidation (30), and action of the natural microflora within the poultry meat (28). Odor quality is affected by the production of volatile catabolites, while the deterioration in color happens throughout storage that

is frequently related to biochemical reactions (between meat pigments, oxygen, and volatile microbial catabolites) and higher meat pH (30).

Several bacteria may be involved in poultry meat spoilage including coliforms, Enteriobacteriaceae, *Brochothrix thermosphacta*, *Pseudomonas*, *Aeromonas* sp., *Serratia*, lactic acid bacteria as *Lactobacillus oligofermentans*, *Leuconostoc gelidum* subsp. *gasicomitatum* (29), *Lactococcus*, *Vagococcus*, and *Carnobacterium* (27). The dominant spoilage bacteria is *Pseudomonas* spp. (26) due to its ability to assimilate, penetrate, and metabolize many meat compounds that other bacteria cannot use (30). When the total viable count achieves or exceeds 7 Log CFU/g, spoilage is deemed to ensue (29, 30).

Pathogens

Campylobacter, *Salmonella*, *Listeria monocytogenes*, and *Staphylococcus aureus* are some of the primary pathogens naturally present or contaminants of poultry meat products (31, 32). *Clostridium perfringens*, *Listeria innocua*, and *Aeromonas* spp. were also identified in poultry meat (29). Recently, *Helicobacter pullorum* and *Acrobacter* gained considerable attention as relevant agents of poultry meat infections (33). *Staphylococcus saprophyticus* is another relevant agent of poultry product contamination, which varies from *S. aureus* because of its virulence factors and genetic profile (34). *C. perfringens* is widespread in the tract of birds (35), is responsible for enteric diseases in poultry (36), and is known for potential to generate extracellular enzymes and large numbers of toxins (37). At a certain microbial load, toxin production is upregulated (38). The enterotoxins delivered are associated with human gastrointestinal illnesses—enterotoxemia where toxins induce organ damage upon entering the circulation (36, 38).

Campylobacter spp.

Poultry is the natural host/reservoir of *Campylobacter* spp. (39), which is present in the intestinal tract of birds, skin, and feathers (40). *Campylobacter jejuni* and *Campylobacter coli* are the main zoonotic enteropathogen causative serovars of human campylobacteriosis (41) and with high prevalence (42). *C. jejuni* is dominant by comparison with *C. coli* (39). At each point of the production chain, the proportions of these two isolates may be reversed by passing from one stage to the other. This could be attributable to the resistance or the susceptibility to a particular isolation technique introduced during the test of the collected samples and/or the feed withdrawal (41). They are microaerophilic and thermophilic (43) and require specific environmental conditions to develop (44).

It was reported that for 25.7% of chicken broiler carcasses tested, both liver (surface and internal tissue) and ceca were *Campylobacter* positive. However, for 83% (58/70) of carcasses tested, *Campylobacter* was isolated at least once in one of these compartments (42), and it is of note that the study pointed out that dissimilar subtypes of *Campylobacter* could simultaneously contaminate the same broiler carcasses.

Salmonella spp.

Salmonella spp. is a facultative anaerobic Gram-negative genus (44), belonging to Enterobacteriaceae family. It is motile (except for *Salmonella enterica* Gallinarum and Pullorum) (45). It grows at optimum environmental conditions of pH 6.5–7 and temperature around 37°C (45). It is ubiquitous and able to survive in water for several months and in a dry environment for up to 2 weeks (46).

Salmonella is one of the main causative agents of foodborne disease globally (47). Animal-based foods such as beef, poultry, and pork are the major sources of salmonellosis (48), where human salmonellosis is mostly due to consumption of poultry products (44). *Salmonella* can persist throughout the processing chain from the farm to the fork (47). Enteritidis, Newport, and Typhimurium are serotypes commonly identified (49). The main genes encoding for the virulence are in both virulence-associated plasmid and pathogenicity islands. These genes are involved in internalization, epithelial cell invasion, survival, and replication, have a significant role in systemic infection (50), and may present a target for intervention technologies design.

NATURAL COMPOUND-BASED INTERVENTIONS SIGNIFICANT TO POULTRY SECTOR

Spices and Herbs

These natural compounds are used in foods for flavoring and preservation and as additives but also for medicinal and therapeutic goals (anti-oxidative, immune modulators, anti-inflammatory, and antimutagenic). Their utilization in foods can have beneficial effects for shelf life extension as well as improvement of the organoleptic characteristics (51). The antioxidant potential of herbs or spices can prevent or decrease lipid oxidation (52) attributed to the action of phenolic compounds (53).

Clove and rosemary are two aromatic spices known for their antimicrobial and antioxidant potential. Antimicrobial activities of rosemary and clove extracts were tested separately or in combination against pathogenic and spoilage bacteria related to meat, namely, *L. monocytogenes, E. coli*, *Pseudomonas fluorescens*, and *Lactobacillus sake*, as well as against native microflora in poultry meat samples, where the combination of both provided enhanced microbial inactivation potential. The combination of the herb and spice also maintained or improved the sensory characteristics of fresh meat, reduced lipid oxidation, and extended shelf life up to 15 days (53).

Curcumin is a US FDA-approved safe plant pigment used for cooking, reputed for its health benefits. It acts as antioxidant, anti-inflammatory, and antiproliferative, but drawbacks for use in foods are related to the color, taste, and quality alterations (54). Corrêa et al. (54) analyzed the antimicrobial effect of two photonic approaches, ultraviolet (UV)-C and curcumin-mediated photodynamic inactivation (PDI), for control of *E. coli* and *S. aureus* inoculated on chicken breast cubes. Limitations were associated with these treatments, as the UV-C light or the curcumin-mediated PDI treatment with emission at 450 nm were

not absorbed in all areas and did not significantly penetrate the chicken meat surface, with 1–2 $\log_{10}$ CFU/ml reductions observed (54).

Essential Oils

Essential oils (EOs) are aromatic secondary metabolites and concentrated plant extracts. They can be obtained by steam distillation, expression or supercritical extraction with carbon dioxide from different parts of the plant, for example, bark, flower, fruits, seeds, leaves, or roots. Many EOs have found application in poultry feed as an alternative to antibiotics due to antioxidant, antiseptic, and insect repellent properties as well as immune-modulatory effects (55). EOs are chemically diverse compounds; hence, their antimicrobial activity varies from compound to compound. However, due to their hydrophobic nature, they are likely to enter cell membranes of microbes or eukaryotes (56). The main limitations of using EOs in food products are the strong flavor imparted on foods (56), the heat-labile nature, and volatile characteristics (57).

Rosemary extract is well-known for its antimicrobial activity, which is related to its phenolic composition (e.g., rosmarinic and carnisic acids). Inactivation of cellular enzymes was seen to result from the effect of phenolic compounds (16). Treatment of *Salmonella typhimurium* with thyme EO caused a rise in the electrical conductivity, which appears to result from the destruction of the cell membrane as well as electrolyte leakage. Additionally, quantitative analysis reported a significant drop in the protein contents, DNA, and ATP by 55.42, 54.03, and 52.64%, respectively, when compared to the control (57). Likewise, EOs (lemon oil, lemon grass oil, lime oil, garlic oil, onion oil, pimento berry oil, oregano oil, thyme oil, and rosemary oil) had higher antimicrobial potential against four *Campylobacter* strains when compared to organic acid (ascorbic acid, citric acid, and lactic acid). Oregano EOs specifically displayed the higher inactivation potential against *C. jejuni*, where the minimal inhibitory concentration was equivalent to 62.5 ppm (18).

The application of 0.1% oregano EO was effective for extending the shelf life up to 5–6 days for fresh chicken breast meat before packaging (58). The authors pipetted 0.1% of oregano EO in the low-density polyethylene (LDPE)/polyamide (PA)/LDPE barrier pouches, which was later subjected to either air or modified atmosphere packaging (MAP). The lipid peroxidation and deterioration of sarcoplasmic proteins were controlled to extend the shelf life of chicken breast up to 2 weeks at 4°C with the application of 0.5% of both thyme and *Melissa officinalis* balm EOs. These EOs were applied on the chicken breast slices by dipping method for 15 min. The results highlighted that thyme (0.5%) was more effective in inhibiting the growth of *E. coli*, whereas balm (0.5%) was more effective on the *Salmonella* spp. (59). The combined effect of ethylenediaminetetraacetic acid (EDTA) (1.5% w/w) lysozyme (1.5% w/w), rosemary oil (0.2% v/w), and oregano oil (0.2% v/w) was effective on extending the shelf life of vacuum-packed semi-cooked coated chicken filets stored at 4°C (60). EDTA and lysozyme were applied by spraying technique on the surface of the chicken surface, while rosemary and oregano oil were pipetted in the LDPE/PA/LDPE pouch barriers containing chicken samples.

Organic Acids

Organic acids are naturally occurring compounds present in many foods and can be produced during the fermentation process. They are added in foods as acidulants, preservatives, or flavorants. The commonly used organic acids are lactic, acetic, malic, and ascorbic acid, etc. The mechanism of inactivation of these acids is through lowering of pH, pKa value along with penetration of undissociated compounds through the cell membrane and its dissociation inside the cell, thus affecting the bacterial membrane (61, 62). In poultry products, the salts of organic acids such as potassium or sodium lactate and sodium diacetate are used to inactivate *L. monocytogenes*, and buffered citrate is used to enhance flavor (63). The maximum level for potassium and sodium lactate is 4.8% by weight of total formulation in various meat and poultry products. For sodium diacetate, the maximum permitted level is 0.25% by weight of total formulation when used as either antimicrobial agent or flavoring agent (64).

NON-THERMAL TECHNOLOGIES AND THEIR COMBINATIONS WITH NATURAL COMPOUNDS IN POULTRY PROCESSING

Non-thermal technologies, such as HPP, PEF, ultrasound, UV, irradiation, and cold atmospheric plasma (CAP) can retain nutritional as well as sensory properties of food in shorter treatment times and low operational temperatures (65). Extensive research on the application of various non-thermal technologies to poultry meat has been conducted in recent years. The effect of non-thermal technologies on microbial and physicochemical properties will be discussed in this section. The incorporation of natural compounds with non-thermal technologies can give an additional hurdle enhancing antimicrobial efficacy. Additionally, this will help adjust the processing conditions at lower intensity, giving improved physicochemical properties (66). Few studies have focused on combining natural compounds with the non-thermal treatment as summarized in **Table 1** illustrating its impact on both physicochemical and microbial properties. A pictorial representation of governing processing parameters, mechanism for microbial decontamination, and key physicochemical parameters to take into consideration while application of the non-thermal technology in the poultry products is depicted in **Figure 3**, and it will be further discussed in coming sections.

High-Pressure Processing

HPP is a non-thermal technology for the sterilization and preservation of food products in which the product is subjected to high pressure (300–600 MPa) with or without the combination of heat. With the application of HPP, covalent bonds in food matrices are not broken and the effect on the food characteristics is minimal. HPP is based on Le Chatelier's principle and the

TABLE 1 | Summary of combination trials of different non-thermal technologies with natural compounds on poultry products.

Non-thermal technology	Natural compounds	Poultry products	Chemical observations	Microbiological observations	References
CAP	Rosemary extract	Poultry ground meats	NA	- Reduction of the bacterial functional diversity - The lowest Maximum Population Size (54.65, 95% confidence interval [CI95%] ranges, 54.03–55.16) and slowest growth rate (hour) (0.03258, CI95% ranges, 0.0179–0.04726) in day 0 - At day 5 of storage at 4°C, the maximum population sizes of treated samples were statistically not significant comparing to day 0	(67)
	Rosemary extract	Ground chicken patties	- Lower pH values for rosemary samples - a* value was significantly affected by rosemary addition - Addition of rosemary extract prevented lipid oxidation for CAP	Rosemary extract significantly reduced the total plate counts with and without cold plasma treatment	(16)
	Thyme oil (TO)/ Silk fibroin (SF) nanofiber	Chicken and duck meat	- Thyme oil release was enhanced due to surface modification of SF by plasma treatment - Higher overall acceptability of chicken meat treated with plasma treatment and combination of TO/SF nanofiber	- The population of *Salmonella* Typhimurium on treated chicken meat reached 1.15 and 1.96 log CFU/g when stored at respectively 4 and 25°C for 7 days after been wrapped with plasma-Thymol oil-Silk fibroin nanofibers. Identic effects seen with the dusk meat treated with the same process	(57)
	Essential oils: *Crocus sativus* L., *Allium sativum* L., and *Zataria multiflora Boiss*	Breast chicken fillets	Overall acceptability and no undesirable impacts on both flavour and odour	- Associating CP and essential oils treatments of breast chicken fillet infected by *S. aureus* and *E. coli* lead to significant microbial reductions by at least 3–4 logCFU/g. - A synergetic effect due to the combination of three different EOs (*Crocus sativus L., Allium sativum L., and Zataria multiflora Boiss*.) and CP treatment reaching microbial reductions to great extent. - After 14 days storage, 2–2.7 logCFU/g microbial inactivation reported comparing to 4.9 logCFU/g of samples treated with only EOs	(68)
HPP	Articoat-DLP (lactic acid, acetic acid and sodium diacetate-active compounds)	Chicken breast fillets	- Significant increase in *L*-value - TBARS value remained same during storage - Increase in pH due to HPP	- *Pseudomonas* spp., *B. thermosphacta, coliforms, E coli* inactivated below detection limit - LAB reformed after 7 days storage time	(69)
	Carvacrol	Turkey breast ham	- Higher TBARS value for pressurised samples - Carvacrol addition decreased TBARS value of samples	- Carvacrol+HPP extend the lag phase for *Listeria* - Reduced the growth rate of LAB spoilage groups	(70)
	Thymol	Ground chicken	NA	- addition of thymol impacted the HPP sensitivity for iPEC O157:H7 and UPEC	(71)
PEF	Oregano essential oils	Raw chicken	NA	- No significant inhibition of C, jejuni if only treatment with PEF (0.25–1 kV/cm) applied. - Sequential treatment of PEF with immersion for 20 min in oregano essential oil (15.625 ppm) were effective against *C. jejuni* 1146 DF with maximum reduction of 1.5 log CFU/g	(18)
Ultrasound	Lactic acid	Broiler drumstick skin	NA	Ultra-sonication alone and with 1% lactic acid did not significantly affect aerobic plate count	(72)
	Lactic acid	Poultry skin	NA	- *Pseudomonas* was most sensitive to lactic acid than other gram-negative bacteria - Degree of reduction of gram-negative bacteria was dependent on treatment time and liquid medium (water or lactic acid)	(73)
	Oregano essential oil	Chicken breast	NA	0.3% oregano oil and ultrasound showed better inactivation of lactic acid bacteria, mesophiles and anaerobic bacteria at day 0 and during 21 days of storage	(74)

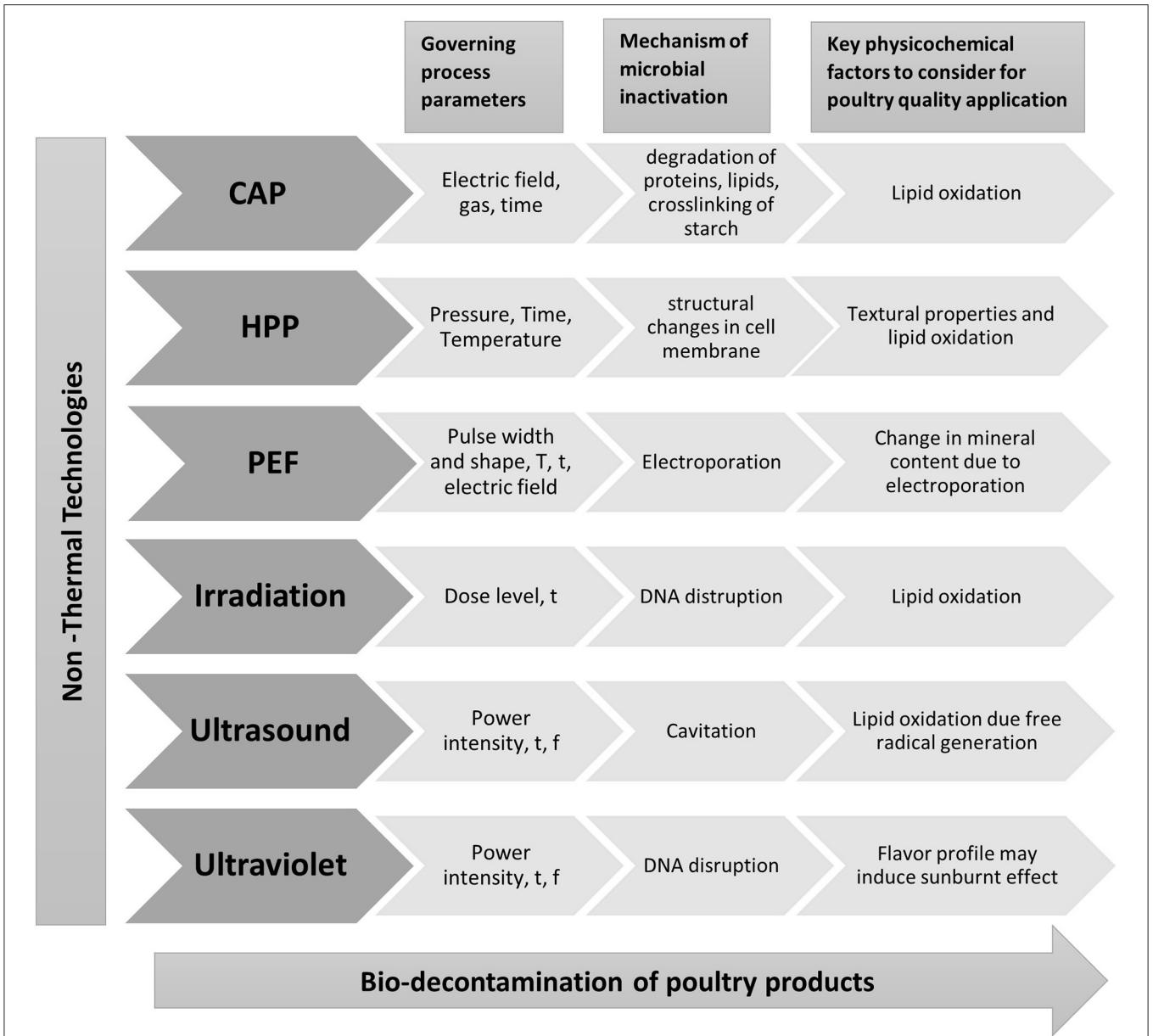

FIGURE 3 | Illustration of key parameters of non-thermal technologies, their mechanism of action, and critical factors to consider for poultry application, where T represents temperature, t is time, and f is frequency. CAP, cold atmospheric plasma; HPP, high-pressure processing; PEF, pulsed electric field.

isostatic principle. Le Chatelier's principle states that "if a change in conditions is applied on a system in equilibrium, then the system will try to counteract that change and restore the equilibrium." The isostatic principle states that food products are compressed by uniform pressure from every direction and then returned to their original shape when pressure is released. HPP is currently used for liquid and high-moisture solid products (75). The first commercialized HPP meat products available are sliced cooked ham and precooked meals containing poultry, pork, chorizo, and different sausages in Spanish market (76, 77). Further details about commercialized meat product of HPP are detailed elsewhere (78).

Effect of High-Pressure Processing on Microbial Decontamination of Poultry Products

Tracz et al. (2015) investigated the potential of HPP to destroy a mixed culture of three stains of *C. jejuni* inoculated in chicken breast under different pressure conditions (200, 300, and 400 MPa) and treatment times (5, 10, and 15 min), where D values were lowest at the highest pressure applied (79). According to the pressure applied, the temperature varies from 0 to 10°C. When the lowest pressure (200 MPa) was applied, *C. jejuni* exposed resistance and no significant reduction was achieved regardless of the duration of HPP treatment. Gram-negative bacteria are generally more susceptible to pressure compared

to Gram-positive bacteria (79). Sheen et al. (2015) reported also that the inactivation of *Salmonella* spp. in ground chicken was dependent on both treatment time and pressure level applied (80). It was highlighted that even at high pressure (550 MPa), while the highest temperature reached was 28°C, *Salmonella* recovered and resuscitated over storage at 10°C to achieve ∼6 Log CFU/g at day 9 of storage. Therefore, despite the mechanisms noted of surface structure damage or disintegration, internal cell compound disappearance, and appearance of internal voids in the cells, some cells survived the HPP 15-min treatment (80), pointing to a need for combination approaches. Argyri et al. (2018) HPP treatment at 500 MPa for 10 min at 18–20°C resulted in a significant reduction, below detection limit, of both the native microbiota of chicken and a cocktail culture of three different strains of *Salmonella* (31). Furthermore, *Salmonella enteritidis* inoculated on chicken at different initial concentration levels stayed below or just at detection limits during the storage at 4°C over 18 days (30). Working with a cocktail of *Listeria monocytogenes*, also Argyri et al. (2019) perceived the capability of HPP in maintaining safety and extending the shelf life of chicken (30, 32). Xu et al. (2020) reported D_{10} values for multi-isolated cocktails of extraintestinal pathogenic *E. coli* (ExPEC) to HPP (400 MPa, 0–25 min) on ground chicken, where 3.26 min was the average and the highest temperature value reached during the treatment was 25°C (81). Increasing the pressure to 600 MPa provided more than 6 log reduction within 3 min with no bacterial recovery after 4 min (81). The inactivation effect of HPP on two different strains of *E. coli* on ground chicken was assessed while the temperature remained under 40°C, where a significant resistance of uropathogenic *E. coli* (UPEC) by comparison with intestinal pathogenic *E. coli* (iPEC) O157:H7 at 450 and 500 MPa was reported (71). Liu et al. (2012) stated that *C. jejuni* HCJ2316 exhibited high resistance to pressure (2.8 Log CFU/g reduction), whereas the others were more susceptible to treatment and achieved 5 Log CFU/g reduction (82). However, the microbial recovery upon pressure treatment of *C. jejuni* is iron-dependent (82).

Effect of High-Pressure Processing on Physicochemical Properties of Poultry Products

Lipid oxidation is one of the major causes of deterioration of meat during storage. The chicken meat contains a higher amount of unsaturated fatty acids compared to other animal meats, which makes it more susceptible to lipid oxidation. The most common method to determine the lipid oxidation is Thiobarbituric acid reactive substances (TBARS) analysis (24). The lipid oxidation was particularly affected by working pressure; for low pressure (400 MPa and less), significantly less change in TBARS value was reported, while high pressure (500 MPa) have a higher impact on TBARS value (83). Similar effect was observed when chicken breast filets was treated with 450 and 600 MPa, while no significant change in TBARS value was observed at 300 MPa (84). The pressure of 800 MPa has the most detrimental on TABRS value (85).

Combined Effect of High-Pressure Processing and Natural Compounds on Poultry Products

A synergistic effect was recorded using nisin (200 ppm) and HPP (450 MPa) at 20°C, enhancing the microbial reduction of mechanically recovered poultry meat, specifically, the inactivation of both aerobic mesophile and psychrotroph populations was greater by comparison with HPP treatment on its own (86). Other researchers outlined the strong synergistic effect of combining hydrostatic pressure treatment (250 MPa for 30 min at 25°C) and 1% food additive (citric acid, nisin, and wasabi extract) in completely reducing the microbial concentration of *S. enteritidis* to undetectable levels (87). Combining HPP (300–400 MPa) with thymol (100–200 ppm) provided a large inactivation effect on separate cocktails of iPEC O157:H7 (0.94–5.16 Log CFU/g) and UPEC (0.41–4.66 Log CFU/g) in ground chicken samples (71).

Pulsed Electric Field

PEF uses short pulses of high voltage (5–80 kV) for microbial inactivation. The food is placed between two electrodes, and an external electric field is applied, which induces the movement of ions along the direction of lines of force of the applied electric field inside as well as outside the cells. This causes the accumulation of ions on the membranes, causing polarization of the cell, which results in thickness reduction of the membranes due to the forces of attraction between oppositely charged ions on either side of the membrane (88). Because of the potential for cell membrane permeabilization, PEF is a promising technology to modify several qualities of meat, such as color, texture, and water-holding capacity, and enhance mass transfer during curing and brining. However, applications to date can be limited in solid products due to conductivity requirements (89).

Effect of Pulsed Electric Field on Microbial Decontamination of Poultry Products

The cell membrane is commonly referred to as the only target of PEF contributing to bacterial cell death (90). Treatments with PEF display reversible or irreversible damages on the cell membrane by disorganizing the structure, which yields the breakdown of the semipermeable barrier due to formation of pores in the membrane (18), leading to irreversible electro-permeabilization of the cell membrane; however, recovery can occur in optimal conditions (91). Process parameters of pulse frequency and strength of the electric field of PEF have been demonstrated to affect the microbial inactivation (92).

Reduction in population densities of *S. enteritidis* and *S. typhimurium* strains suspended in citrate-phosphate buffer was greater with increasing both treatment time and electric field above 9 kV/cm (93). It was demonstrated by Clemente et al. (18) that the treatment of chicken thighs with PEF did not result in any significant reduction of *C. jejuni.* PEF was not sufficient to reduce cell concentration of *S. enteritidis*, *E. coli*, and *C. jejuni* on raw chicken (92). However, this non-thermal technology is suggested to be suitable for treating process waters used in poultry processing as well as for poultry scald (92).

Effect of Pulsed Electric Field on Physicochemical Properties of Poultry Products

Several studies have reported the ability of PEF treatment to modify sensory characteristics, texture, and water-holding property of the meat products, further improving the mass transfer properties (94–96). Meat is considered an excellent source of minerals, such as zinc, iron, phosphorus, and calcium (97). PEF induces irreversible electroporation in meat and affects cellular permeability and mass transfer. Studies suggest that PEF-applied products have changes in mineral content when compared to control. Khan et al. (98) examined the effect of low (2.5 kV, 200 Hz) and high PEF (10 kV, 200 Hz) on four nutritionally important minerals (P, K, Fe, and Zn) of raw and cooked chicken breast. For raw chicken, non-significant changes in mineral content were noted; however, with cooking, a decrease in P, K, and Zn was observed and the concentration of Fe was not affected by treatment or cooking. In another study conducted by Khan et al. (99), the authors found higher concentrations of Ni and Cu for both low (2.5 kV, 200 Hz) and high PEF (10 kV, 200 Hz) than control. Thus, it is vital to study the migration of minerals into meat products due to PEF treatment and should be checked under regulatory limits.

Combined Effect of Pulsed Electric Field and Natural Compounds on Poultry Products

Recent work revealed that chicken oyster thigh artificially contaminated by *C. jejuni* 1,146 DF (final concentration 4.41 ± 0.20 $\log_{10}$ CFU/g) and treated with only PEF (0.25–1 kV/cm) did not demonstrate any significant inhibition potential. However, sequential treatment of PEF (1 kV/cm) and immersion in buffer with oregano EO (15.625 ppm) for 20 min resulted in a significant reduction close to 1.5 $\log_{10}$ CFU/g (18).

Ultraviolet

UV light is electromagnetic radiation with wavelength from 10 to 400 nm. UV lights fall in the range between visible light and X-rays. To control surface contaminations on food products, UV-C light has received US FDA approval (Approval-2010). High-intensity pulsed UV light has been approved by FDA up to 12 J/cm^2 (100). UV-C light can be used in Europe; however, in Germany, the use is limited to water, fruit, vegetables, and stored hard cheese (101). UV-C has a wavelength range of 220–300 nm (102) and is known for its antimicrobial effect (103), where the specific mechanisms of action include targeting of the nucleic acids (DNA, RNA) within the bacterial cell and generation of pyrimidine dimers (104). This latter results in the bonding of two adjacent pyrimidine bases, provoking obstruction of transcription and translation, respectively, and suspending vital cellular functions (102, 105, 106).

Effect of UV on Microbial Decontamination of Poultry Products

There are some limitations of using UV in poultry processing: UV-C light is not absorbed and cannot penetrate the chicken meat surface, which may affect microbial reduction. The antimicrobial efficacy of pulsed UV and UV-C has limitations also in terms of product density and treatment time (104, 107). The bactericidal effect of UV-C irradiation against *C. jejuni*, *L. monocytogenes*, and *S. typhimurium* on chicken breast was dose-dependent, where treatment at 5 kJ/m^2 reduced *L. monocytogenes*, *C. jejuni*, and *S. typhimurium*, respectively by 1.29, 1.26, and 1.19 log cycles (102). Haughton et al. (105) examined UV effects against *S. enteritidis*, *E. coli*, and *Campylobacter* (*C. jejuni* and *C. coli*) when inoculated in liquid matrix, chicken skin and skinless chicken breast, food contact surfaces, as well as packaging materials. Treatment at a high dose equivalent to 0.192 J/cm^2 provided complete microbial inactivation of *Campylobacter* strains suspended in a liquid matrix. By contrast, *Salmonella* and *E. coli* were more resistant to the similar UV dose (105). Food surface topography can shield microorganisms and limit UV treatment efficacy (104, 107). Isohanni and Lyhs (103) highlighted that although UV treatment was effective in reducing *C. jejuni* on surface medium by 6.3 log cycles per square centimeter. However, only 0.8 and 0.7 log cycles reduction were achieved on broiler skin and on broiler meat, respectively, with a dose of 32.9 mW/s per square centimeter. UV light seems to works well on smooth surfaces (108). Bacterial multilayer overloading as well as overlapping, and in the presence of cell, organic compounds protect to target bacteria from UV irradiation (104, 107).

Effect of UV on Physicochemical Properties of Poultry Products

UV light can form off-flavors due to the photochemical effect on the lipid fractions of product or due to absorption of ozone and oxides of nitrogen (101). This leads to the development of lipid peroxidation causing off-flavor. The hexanal aldehyde is a volatile secondary lipid oxidation product, and it is indicative of fatty aldehydes by headspace/gas chromatography–mass spectrometry (GC-MS). McLeod et al. (104) detected an increase in hexanal content of the raw chicken filets treated with 10.8 J/cm^2 in air, which was noted by the sensory panel as a "sunburnt flavor" giving a low sensory score. Interestingly, when the same chicken samples were cooked, the sensory panel was unable to identify the difference, and it scored fairly with the untreated sample (104).

Combined Effect of UV and Natural Compounds on Poultry Products

The combination treatment of UV-C light and clove EO was assessed against poultry-related pathogen *S. typhimurium* biofilms, generated on stainless steel coupon surfaces. Treatment with 1.2 mg/ml of clove EO followed with UV-C (76.41 mJ/cm^2) induced a synergistic effect and resulted in no surviving cells (6.8 log CFU/cm^2) embedded within the biofilms. It was demonstrated that the contact with clove EO made the cell easily accessible by UV-C due to the morphological damage occurring: flatter structure (109).

Ultrasound

Ultrasound as a non-thermal approach applies sound waves with higher frequency (above 20 kHz) than the normal human hearing. The ultrasound frequencies used in the food industry are classified into three categories based on the frequency-power ultrasound: low frequency, high power range (20–100 kHz) and

large-amplitude waves where typical applications are within altering physicochemical properties or structure of foods. For low-intensity ultrasound, in the range of 100 kHz to 1 MKz, chemical reactions are activated, and free radicals can form like hydroxyl ions that can have antimicrobial properties. High-frequency ultrasound is usually used in the food processing and food safety industry. When the cavitation bubble breaks, it forms hydroxyl ions, which can have antimicrobial properties (110).

Effect of Ultrasound on Microbial Decontamination of Poultry Products

The mechanisms of action are connected to cavitation generation, which eventually disturbs the cell permeability as well as causing thinning (111) and damage on the bacterial membrane (112) and "localized heating" (73) that yields cell inactivation. The cell metabolism is disturbed due to ion penetration of the cell cytoplasm upon permeability disruption caused by pressure gradients of ultrasound. These mechanisms are the result of the collapse of cavitation bubbles during the acoustic cavitation (73). A further utility of ultrasound treatments is the "de-agglomeration of bacterial clusters" (73).

Apparent characteristics of target microorganism type, physiological state, and morphology determine the efficacy of ultrasound. The efficacy is also dependent on the surface of food matrix and temperature (111). Moreover, other parameters interfere with efficacy, such as frequency and sonication treatment time (73). The peptidoglycan in the cell membrane of Gram-positive could be a reason behind the resistance to ultrasound by these bacteria compared to Gram-negative (112), and the susceptibility to ultrasound treatment may vary between strains from the same type. The resistance of cells on plates during *in vitro* experiments to sonication by ultrasound was higher in contrast to the susceptibility of both *Campylobacter* and Enterobacteriaceae in raw poultry (112).

It was highlighted that chicken breast subjected to high-intensity ultrasound promoted the growth of mesophilic, psychrophilic, and lactic acid bacteria compared to untreated samples, possibly resulting from the release of nutrients (113). However, it was pointed out that the presence of *E. coli* was lower for samples subjected to longer treatment (30–50 min) compared to non-treated, whereas for *S. aureus*, it significantly decreased after 50 min. The microbial reduction in previously contaminated chicken wings depends on both treatment time (3–6 min) and sonication environment solution where the treatment was in (1% solution of lactic acid or sterile distilled water). The combination of lactic acid and sonication (40 kHz, 2.5 W/cm^2) had a bactericidal effect on all the bacteria tested and was considered suitable for poultry carcass skin decontamination (73). However, combining ultrasound treatment (37 kHz, 380 W, 5 min) with 70% ethanol induced the highest microbial reduction from chicken skin for three types of attachment by *S. typhimurium* loosely, intermediately, and tightly attached by respectively 2.86, 2.49, and 1.63 log CFU/g (114).

Effect of Ultrasound on Physicochemical Properties of Poultry Products

Marinating is mostly used to increase meat tenderness, enhance flavor profile, reduce cooking time, and increase the shelf life of meat. Ultrasound (40 kHz, 22 W/cm^2) increased the marination efficiency of chicken breast when treated with 15 and 20 min (115). A similar increase in marination efficiency, cooking yield, and tenderization was reported when broiler chicken was treated for 20 min and 18 h marination (91% water, 6% NaCl, 3% sodium tripolyphosphate) (116). A positive influence of ultrasound frequency (25, 45, and 130 kHz) and treatment time (1, 3, 6, 16, and 24 h) on marination efficiency was also reported for chicken breast, giving higher uptake of sodium chloride (117). Ultrasound improved the marination properties of meat by breaking the integrity of muscle cell or by enhancing the enzymatic reactions in cell (11, 111). Thus, ultrasound can be used as an alternative to standard marination techniques used in the industry.

Combined Effect of Ultrasound and Natural Compounds on Poultry Products

The exposure of broiler drumstick skin to ultrasonic energy in water and submerged in 1% lactic acid did not show consistent effect in terms of reducing aerobic plate counts. The irregular characteristics of broiler skin surface were proposed as the reason behind the lack of microbial reduction by protecting bacteria in the skin crevices and avoidance of the cavitation (72). In contrast, other work showed the decontamination efficacy of sonication (40 kHz) of chicken wing skin in 1% lactic acid aqueous solution, where the reduction of *E. coli*, *Proteus* sp., *Salmonella anatum*, and *P. fluorescens* inoculated on the surface of the chicken skin significantly increased with treatment time rising from 3 to 6 min. Except for *E. coli*, the microbial reduction was higher when sonication was performed in an aqueous solution of lactic acid instead of water. This was explained by the presence of ions penetrating the cytoplasm due to the action of gradient pressure yielding from ultrasound and the presence of free radicals received in sonochemical reactions (73). Combining high-intensity ultrasound with 0.3% oregano EO treatment was the most appropriate combination to achieve the best reduction of lactic acid bacteria (2.30 $\log_{10}$ CFU ml^{-1}), mesophilic populations (3.36 $\log_{10}$ CFU ml^{-1}), and anaerobic bacteria (3.11 $\log_{10}$ CFU ml^{-1}) present in chicken breasts at day 0 of refrigeration. However, the treatment with ultrasound alone was ineffective to control microbial growth during chilled storage, where the release of nutrient was suggested as a reason permitting microbial growth (74).

Cold Atmospheric Plasma

Plasma is a quasi-neutral ionized gas composed of ions, free electrons, atoms, and molecules in their ground as well as the excited state. Plasma can be generated using any kind of energy, which can ionize the gas, and mostly electric or electromagnetic source are used for generation of plasma species. Plasma can be classified as a thermal plasma or non-thermal plasma. Thermal or non-thermal plasma processes can be designed to be delivered in a format that is cold or near room temperature at the point of application, which is of value for retaining quality and nutritional characteristics while providing efficient bio-decontamination resulting from reactive oxygen or nitrogen species, charged particles, electric field, UV as components of diverse mechanisms of action (118).

Effect of Cold Plasma on Microbial Decontamination of Poultry Products

It is well-documented that cold plasma (CP) has a large potential for controlling microbial quality, extending shelf life, and avoiding post-processing contamination (25). It not only induces bacterial decontamination but also inactivates a broad spectrum of microorganisms including fungi, viruses, and spores (119). Various plasma compounds have critical interventions in the microbial decontamination process like NO_2, NO, O, O_3, OH, H_2O_2, UV photons, charged particles, and electric fields (120). Bacterial cell etching, erosion, morphological alteration, nucleic acid damaging, protein oxidation, and loss of cell viability are the mechanisms of CP to retain microbial safety in foods (121). However, different parameters and mechanisms interfere with the gravity of damage occurring. The antimicrobial effects of CP are a function of process: duration of treatment, gas mixture, mode of exposure (direct or indirect), power source intensity, as well as intrinsic characteristics of product: surface topology, nature of samples treated (liquid, solid, or semisolid), and characteristics of the target cell (122).

In-package CP treatment configuration enhanced the microbial safety, avoided postprocess contamination, and retained toxicological safety of ready-to-eat chicken products (123). Using the *Salmonella* mutagenicity assay, no genotoxicity was seen in plasma-treated chicken breast (120). Tulane virus (1.08 ± 0.15 log CFU/cube), indigenous mesophilic bacteria (0.70 ± 0.12 log CFU/cube), and *Salmonella* (1.45 ± 0.05 log CFU/cube) from chicken samples were significantly reduced upon CP treatment (24 kV for 3 min), where increasing voltage (from 22 to 24 kV) and treatment time had a positive impact on the microbiological quality (123). However, the majority of cells showed morphological changes (cell flattened and other distortions) at 2,000 Hz, whereas at 1,000 Hz, only cell clumps appeared, and other cells were hollowed out (124). *S. typhimurium*, *E. coli* O157: H7, and *L. monocytogenes* populations on chicken breast reduced from 5.48, 5.84, and 5.88 log CFU/g, respectively, at 0 min to 2.77, 3.11, and 3.74 log CFU/g at 10-min plasma exposure (120). Other studies highlight the potential of in-package dielectric barrier discharge (DBD) (70 kV) in controlling poultry-related pathogens, namely, *Salmonella* and *Campylobacter*, and inhibiting the growth of spoiling bacteria (psychrophiles) from chicken breast treated and stored (5 days/4°C) (23), where increasing CP treatment time to beyond 60 s improved microbial reduction of psychrophiles, while no significant effect was seen against foodborne pathogens. The *in situ* decontamination potential of plasma-activated water (PAW) against *P. fluorescens* ATCC13525 previously inoculated on chicken skin pieces was associated with the plasma process parameters of plasma discharge frequency and treatment time (124), where the concentration of plasma-generated reactive species of nitrite, nitrate, peroxide, hydroxyl, and ozone increased with the discharge frequency (124).

Effect of Cold Plasma on Physicochemical Properties of Poultry Products

The reaction of myoglobin with hydrogen peroxide may produce choleglobin-inducing discoloration of meat. An increase in both L* and b* value was observed when chicken breast was treated with flexible thin-layer DBD (123); in contrast, application of DBD applied on chicken breast (110 kV, 60 kHz) showed a decrease in L* value mainly due to slime formation after 9 days of storage at 4°C (28). However, Zhuang et al. (25) did not report significant changes in L*, a*, and b value when chicken breast was treated with 70 kV. The reactive species generated from the plasma can induce lipid peroxidation. Zhuang et al. (2019), Lee et al. (2019), and Moutiq et al. (2020) reported no changes in lipid profile of chicken breast after CP treatment, attributed to plasma reactive species being less damaging on chicken breast as compared to red meat due to variation in fat content (25, 28, 120, 123).

Combined Effect of Cold Plasma and Natural Compounds on Poultry Products

Yeh et al. (2019) considered the combined treatment effect of rosemary (*Rosmarinus officinalis*) extract (1%) and in-package DBD-CP on poultry ground meat (**Table 1**) (67). The treatment induced a reduction of the diversity in bacterial communities, and by day 5 of storage at 4°C, the maximum population densities of treated samples were similar to those at day 0 (67). Rosemary extract had a significant effect in reducing the total microbial counts not only of previously plasma-treated chicken patties but also of non-plasma-treated chicken patties (16). Thyme oil/silk fibroin nanofibers treated with CP were proposed as an active packaging approach with antimicrobial potential against *S. typhimurium* inoculated on poultry meat (chicken meat and duck meat). The inhibition potential of thyme EO/silk fibroin nanofibers treated with CP was reported as higher compared to thyme EO/silk fibroin nanofibers, with an increase in the rate of thyme oil released (23.5–25%) upon plasma treatment clearly noted (57). Sahebkar et al. (2020) found that associating CP (10 min at 32 kHz) and EO (in marinade solutions) treatments of breast chicken filet inoculated with *S. aureus* and *E. coli* challenge populations leads to significant microbial reductions by up to 3–4 log CFU/g (68). A synergetic effect was identified by combining three different EOs (*Crocus sativus* L., *Allium sativum* L., and *Zataria multiflora* Boiss.) and CP treatment, where the advantage of combining the EOs with CP was retained after 14 days of storage (68).

FUTURE CHALLENGES FOR THE APPLICATION OF NON-THERMAL TECHNOLOGIES TO POULTRY PROCESSING

In recent years, there has been intensive research and development of non-thermal process technologies for application in fresh food processing. EU regulations refer to "fresh meat" as meat that has not undergone any preserving process other than chilling, freezing, or quick-freezing, including meat that is vacuum-wrapped or wrapped in a controlled atmosphere (125). The application of a novel non-thermal technology will be subject to these rules, which may on the one hand lead to consumer skepticism toward acceptance of these technologies while on the

other hand limit adoption of processes that can enhance safe and sustainable processing of food resources. To date, HPP and UV (with limitations in some countries) are approved. Thus, combining these approaches with other generally recognized safe approaches including approved natural compounds provides technical options to enhance poultry processing outcomes. The morphological characteristics of poultry products and the skin make the application of non-thermal technology very critical. For example, from a microbial safety perspective, the successful application of UV in poultry processing must consider density, and effects may be limited due to non-absorption and non-penetration of light in the chicken meat surface (104, 107).

While many plant EOs are considered generally safe by FDA, with increasing use, the daily dose intake remains a safety question (126). The adoption of EOs is controlled by a nexus of dosage level, antimicrobial efficacy (127), and the effect of organoleptic characteristics on consumer acceptability (128). The stability, strong smell, volatility, and limited solubility are technical issues to be considered from an efficacy perspective. These should also be considered in tandem with other processing features as the process or environment may stimulate the degradability of these compounds (129).

CONCLUSION

Effective interventions, based on the combinations of emerging process technologies with the well-understood efficacies of nature-based compounds, can be designed to enhance safety and quality and minimize food loss in poultry processing. Studies to date have demonstrated that the order or sequence of application can be variable to address the key risks at different process stages, providing great flexibility if being considered as effective replacements for thermal or conventional chemical strategies.

AUTHOR CONTRIBUTIONS

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

ACKNOWLEDGMENTS

The authors would like to acknowledge the Department of Agriculture, Food and Marine under grant number 17/F/275 and Science Foundation Ireland (SFI) under grant number SFI/16/BBSRC/3391 along with the BBSRC under grant reference BB/P008496/1.

REFERENCES

1. Center for Disease Control. (CDC) 2015. Surveillance for Foodborne Disease Outbreaks — United States, 2009 – 2015. *MMWR Morb Mortal Wkly Rep*. (2018) 67:2009–15. doi: 10.15585/mmwr.ss6710a1
2. EFSA and ECDC. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA J*. (2018) 16:5500. doi: 10.2903/j.efsa.2018.5500
3. CDC. Surveillance for foodborne disease outbreaks - United States, 2009–2010. *Ann Emerg Med*. (2013) 62:91–3. doi: 10.1016/j.annemergmed.2013.04.001
4. CDC. *Campylobacter (Campylobacteriosis)*. Atlanta, GA: Center for Disease Control (CDC) (2019).
5. Safefood. *Consumer Preferences of Poultry Decontamination Methods on the Island of Ireland*. Dublin: Safefood (2017).
6. Buncic S, Sofos J. Interventions to control *Salmonella* contamination during poultry, cattle and pig slaughter. *Food Res Int*. (2012) 45:641–55. doi: 10.1016/j.foodres.2011.10.018
7. Han Y, Xu X, Jiang Y, Zhou G, Sun X, Xu B. Inactivation of food spoilage bacteria by high pressure processing: evaluation with conventional media and PCR–DGGE analysis. *Food Res Int*. (2010) 43:1719–24. doi: 10.1016/j.foodres.2010.05.012
8. Black EP, Setlow P, Hocking AD, Stewart CM, Kelly AL, Hoover DG. Response of spores to high-pressure processing. *Compr Rev Food Sci Food Saf*. (2007) 6:103–19. doi: 10.1111/j.1541-4337.2007.00021.x
9. Voigt DD, Kelly AL, Huppertz T. High-pressure processing of milk and dairy products. *Emerg Dairy Process Technol Oppor Dairy Ind*. (2015) 3:71–92. doi: 10.1002/9781118560471.ch3
10. Marušić Radovčić N, Ježek D, Markov K, Frece J, Ćurić D, Medić H. The effect of high pressure treatment on the quality of chicken breast meat. *Hrvat časopis za prehrambenu Tehnol Biotehnol i Nutr*. (2020) 14:76–81. doi: 10.31895/hcptbn.14.3-4.6
11. Xiong G, Fu X, Pan D, Qi J, Xu X, Jiang X. Influence of ultrasound-assisted sodium bicarbonate marination on the curing efficiency of chicken breast meat. *Ultrason Sonochem*. (2020) 60:104808. doi: 10.1016/j.ultsonch.2019
12. Lawrie RA, Ledward D. *Lawrie's Meat Science*. Cambridge: Woodhead Publishing (2014).
13. Al-Hilphy AR, Al-Temimi AB, Al Rubaiy HHM, Anand U, Delgado-Pando G, Lakhssassi N. Ultrasound applications in poultry meat processing: a systematic review. *J Food Sci*. (2020) 85:1386–96. doi: 10.1111/1750-3841.15135
14. Mostafavi HA, Fathollahi SMMH. *The Potential of Food Irradiation:Benefits and Limitations*. Tehran: Trends in Vital Food and Control Engineering (2012).
15. Calderón-Miranda ML, Barbosa-Cánovas GV, Swanson BG. Inactivation of *Listeria innocua* in liquid whole egg by pulsed electric fields and nisin. *Int J Food Microbiol*. (1999) 51:7–17. doi: 10.1016/S0168-1605(99)00070-7
16. Gao Y, Zhuang H, Yeh HY, Bowker B, Zhang J. Effect of rosemary extract on microbial growth, pH, color, and lipid oxidation in cold plasma-processed ground chicken patties. *Innov Food Sci Emerg Technol*. (2019) 57:102168. doi: 10.1016/j.ifset.2019.05.007
17. Huffman RD. Current and future technologies for the decontamination of carcasses and fresh meat. *Meat Sci*. (2002) 62:285–94. doi: 10.1016/S0309-1740(02)00120-1
18. Clemente I, Condón-Abanto S, Pedrós-Garrido S, Whyte P, Lyng JG. Efficacy of pulsed electric fields and antimicrobial compounds used alone and in combination for the inactivation of *Campylobacter jejuni* in liquids and raw chicken. *Food Control*. (2020) 107:106491. doi: 10.1016/j.foodcont.2019.01.017
19. Kure CF, Axelsson L, Carlehög M, Måge I, Jensen MR, Holck A. The effects of a pilot-scale steam decontamination system on the hygiene and sensory quality of chicken carcasses. *Food Control*. (2020) 109:106948. doi: 10.1016/j.foodcont.2019.106948
20. Silano V, Barat Baviera JM, Bolognesi C, Brüschweiler BJ, Chesson A, Cocconcelli PS, et al. Evaluation of the safety and efficacy of the organic acids lactic and acetic acids to reduce microbiological surface contamination on pork carcasses and pork cuts. *EFSA J*. (2018) 16:5482. doi: 10.2903/j.efsa.2018.5482
21. Cadena M, Kelman T, Marco ML, Pitesky M. Understanding antimicrobial resistance (AMR) profiles of *Salmonella* biofilm and planktonic bacteria challenged with disinfectants commonly used during poultry processing. *Foods*. (2019) 8:275. doi: 10.3390/foods8070275

22. Micciche AC, Feye KM, Rubinelli PM, Lee JA, Knueven CJ, Ricke SC. Comparison of acid sanitizers on *Salmonella* typhimurium inoculated commercial poultry processing reuse water. *Front Sustain Food Syst.* (2019) 2:90. doi: 10.3389/fsufs.2018.00090
23. Wang H, Qi J, Duan D, Dong Y, Xu X, Zhou G. Combination of a novel designed spray cabinet and electrolyzed water to reduce microorganisms on chicken carcasses. *Food Control.* (2018) 86:200–6. doi: 10.1016/j.foodcont.2017.11.027
24. Keener KM, Bashor MP, Curtis PA, Sheldon BW, Kathariou S. Comprehensive review of campylobacter and poultry processing. *Compr Rev Food Sci Food Saf.* (2004) 3:105–16. doi: 10.1111/j.1541-4337.2004.tb00060.x
25. Zhuang H, Rothrock MJ, Hiett KL, Lawrence KC, Gamble GR, Bowker BC, et al. In-package air cold plasma treatment of chicken breast meat: treatment time effect. *J Food Qual.* (2019) 2019:1837351. doi: 10.1155/2019/1837351
26. Wang J, Zhuang H, Hinton A, Zhang J. Influence of in-package cold plasma treatment on microbiological shelf life and appearance of fresh chicken breast fillets. *Food Microbiol.* (2016) 60:142–6. doi: 10.1016/j.fm.2016.07.007
27. Vester C, Kjeldgaard J, Ingmer H, Bisgaard M, Christensen H. International Journal of Food Microbiology Microbiota encompassing putative spoilage bacteria in retail packaged broiler meat and commercial broiler abattoir. *Int J Food Microbiol.* (2019) 300:14–21. doi: 10.1016/j.ijfoodmicro.2019.04.003
28. Moutiq R, Misra NN, Mendonça A, Keener K. In-package decontamination of chicken breast using cold plasma technology: microbial, quality and storage studies. *Meat Sci.* (2020) 159:107942. doi: 10.1016/j.meatsci.2019.107942
29. Tresse O. *Bacterial Contaminants of Poultry Meat : Sources, Species, and Dynamics*. Nantes: Microorganisms (2017).
30. Katiyo W, Kock HL De, Coorey R, Buys EM. LWT - Food Science and Technology Sensory implications of chicken meat spoilage in relation to microbial and physicochemical characteristics during refrigerated storage. *LWT Food Sci Technol.* (2020) 128:109468. doi: 10.1016/j.lwt.2020.109468
31. Argyri AA, Papadopoulou OS, Nisiotou A, Tassou CC, Chorianopoulos N. Effect of high pressure processing on the survival of Salmonella Enteritidis and shelf-life of chicken fi llets. *Food Microbiol.* (2018) 70:55–64. doi: 10.1016/j.fm.2017.08.019
32. Argyri AA, Papadopoulou OS, Sourri P, Chorianopoulos N, Tassou CC. Quality and safety of fresh chicken fillets after high pressure processing : survival of indigenous *Brochothrix thermosphacta* and inoculated *Listeria monocytogenes*. *Microorganisms*. (2019) 7:520. doi: 10.3390/microorganisms7110520
33. Pasquali F, De Cesare A, Meunier M, Guyard M, Rivoal K, Chemaly M, et al. *Current Challenges in Poultry Meat Safety*. Duxford: Elsevier Ltd. (2017). doi: 10.1016/B978-0-08-100763-1.00007-6
34. Sommers C, Sheen S, Scullen OJ, Mackay W. Inactivation of *Staphylococcus saprophyticus* in chicken meat and purge using thermal processing, high pressure processing, gamma radiation, and ultraviolet light (254 nm). *Food Control.* (2017) 75:78–82. doi: 10.1016/j.foodcont.2016.12.020
35. Udhayavel S, Thippichettypalayam Ramasamy G, Gowthaman V, Malmarugan S, Senthilvel K. Occurrence of Clostridium perfringens contamination in poultry feed ingredients: isolation, identification and its antibiotic sensitivity pattern. *Anim Nutr.* (2017) 3:309–12. doi: 10.1016/j.aninu.2017.05.006
36. Golić N, Veljović K, Popović N, Djokić J, Strahinić I, Mrvaljević I, et al. *In vitro* and *in vivo* antagonistic activity of new probiotic culture against *Clostridium difficile* and *Clostridium perfringens*. *BMC Microbiol.* (2017) 17:1–9. doi: 10.1186/s12866-017-1015-5
37. Guo S, Liu D, Zhang B, Li Z, Li Y, Ding B, et al. Two Lactobacillus species inhibit the growth and α-toxin production of Clostridium perfringens and induced proinflammatory factors in chicken intestinal epithelial cells *in vitro*. *Front Microbiol.* (2017) 8:1–12. doi: 10.3389/fmicb.2017.02081
38. Hamad GM, Abdelmotilib NM, Darwish AMG, Zeitoun AM. Commercial probiotic cell-free supernatants for inhibition of *Clostridium perfringens* poultry meat infection in Egypt. *Anaerobe.* (2020) 62:102181. doi: 10.1016/j.anaerobe.2020.102181
39. Bolton DJ. Campylobacter virulence and survival factors. *Food Microbiol.* (2015) 48:99–108. doi: 10.1016/j.fm.2014.11.017
40. Perez-arnedo I, Gonzalez-fandos E. Prevalence of *Campylobacter* spp. in poultry in three Spanish farms, a slaughterhouse and a further processing plant. *Foods*. (2019) 8:111. doi: 10.3390/foods8030111
41. Rossler E, Olivero C, Soto LP, Frizzo LS, Zimmermann J, Rosmini MR, et al. Prevalence, genotypic diversity and detection of virulence genes in thermotolerant *Campylobacter* at different stages of the poultry meat supply chain. *Int J Food Microbiol.* (2020) 326:108641. doi: 10.1016/j.ijfoodmicro.2020.108641
42. Berrang ME, Meinersmann RJ, Cox NA. Campylobacter subtypes detected in broiler ceca and livers collected at slaughter. *Poult Sci.* (2019) 98:5908–12. doi: 10.3382/ps/pez340
43. Gharajalar N, Sahar, Hassanzadeh P, Hosseinali Nejad N. Molecular detection of *Campylobacter* species and Cytolethal distending toxin isolated from chicken livers in Tabriz. *Comp Immunol Microbiol Infect Dis.* (2020) 71:101474. doi: 10.1016/j.cimid.2020.101474
44. Lamas A, Regal P, Vázquez B, Miranda JM, Cepeda A, Franco CM. Salmonella and *Campylobacter* biofilm formation : a comparative assessment from farm to fork. *J Sci Food Agric*. (2018) 98:4014–32. doi: 10.1002/jsfa.8945
45. Chlebicz A, Slizewska K. Campylobacteriosis, Salmonellosis, Yersiniosis, and Listeriosis as zoonotic foodborne diseases: a review. *Int J Environ Res Public Health.* (2018) 15:1–28. doi: 10.3390/ijerph15050863
46. WHO. *Salmonella (non-typhoidal)*. (2018). Available online at: https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal) (accessed June 9, 2020).
47. Jajere SM. A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. *Vet World*. (2019) 12:504–21. doi: 10.14202/vetworld.2019.504-521
48. Bouchrif B, Paglietti B, Murgia M, Piana A, Cohen N, Ennaji MM, et al. Prevalence and antibiotic-resistance of *Salmonella* isolated from food in Morocco. *J Infect Dev Ctries.* (2009) 3:35–40. doi: 10.3855/jidc.103
49. Tack DM, Ray L, Griffin PM, Cieslak PR, Dunn J, Rissman T, et al. Preliminary incidence and trends of infections with pathogens transmitted commonly through food - foodborne diseases active surveillance network, 10 U.S. Sites, 2016-2019. *MMWR Morb Mortal Wkly Rep*. (2020) 69:509–14. doi: 10.15585/mmwr.mm6917a1
50. Dantas STA, Camargo CH, Tiba-casas MR, Vivian RC, Pinto JPAN, Pantoja JCF, et al. Environmental persistence and virulence of *Salmonella spp*. Isolated from a poultry slaughterhouse. *Food Res Int.* (2020) 129:108835. doi: 10.1016/j.foodres.2019.108835
51. El-Sayed SM, Youssef AM. Potential application of herbs and spices and their effects in functional dairy products. *Heliyon.* (2019) 5:e01989. doi: 10.1016/j.heliyon.2019.e01989
52. Shah MA, Bosco SJD, Mir SA. Plant extracts as natural antioxidants in meat and meat products. *Meat Sci.* (2014) 98:21–33. doi: 10.1016/j.meatsci.2014.03.020
53. Zhang H, Wu J, Guo X. Effects of antimicrobial and antioxidant activities of spice extracts on raw chicken meat quality. *Food Sci Hum Wellness.* (2016) 5:39–48. doi: 10.1016/j.fshw.2015.11.003
54. Corrêa TQ, Blanco KC, Garcia ÉB, Perez SML, Chianfrone DJ, Morais VS, et al. Effects of ultraviolet light and curcumin-mediated photodynamic inactivation on microbiological food safety: a study in meat and fruit. *Photodiagnosis Photodyn Ther.* (2020) 30:101678. doi: 10.1016/j.pdpdt.2020.101678
55. Amerah AM, Ouwehand AC. *Use of Essential Oils in Poultry Production*. London: Elsevier Inc. (2016). doi: 10.1016/B978-0-12-416641-7.00010-9
56. Burt S. Essential oils: their antibacterial properties and potential applications in foods–a review. *Int J Food Microbiol.* (2004) 94:223–53. doi: 10.1016/j.ijfoodmicro.2004.03.022
57. Lin L, Liao X, Cui H. Cold plasma treated thyme essential oil/silk fibroin nanofibers against *Salmonella Typhimurium* in poultry meat. *Food Packag Shelf Life.* (2019) 21:100337. doi: 10.1016/j.fpsl.2019.100337
58. Chouliara E, Karatapanis A, Savvaidis IN, Kontominas MG. Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4°C. *Food Microbiol.* (2007) 24:607–17. doi: 10.1016/j.fm.2006.12.005

59. Fratianni F, De Martino L, Melone A, De Feo V, Coppola R, Nazzaro F. Preservation of chicken breast meat treated with thyme and balm essential oils. *J Food Sci.* (2010) 75:M528–35. doi: 10.1111/j.1750-3841.2010.01791.x
60. Ntzimani A, Giatrakou V, Savvaidis I. Combined natural antimicrobial treatments (EDTA, lysozyme, rosemary and oregano oil) on semi cooked coated chicken meat stored in vacuum packages at 4°C: microbiological and sensory evaluation. *Innov food Sci Emerg Technol.* (2010) 11:187–96. doi: 10.1016/j.ifset.2009.09.004
61. Gurtler J, Mai TL. Traditional preservatives - organic acids. *Encycl Food Microbiol.* (2014) 3:119–30. doi: 10.1016/B978-0-12-384730-0.00260-3
62. Anyasi T, Jideani A, Edokpayi J, Anokwuru C. *Application of Organic Acids in Food Preservation*. New York, NY: Nova Publishers (2017). p. 45
63. Theron MM, Lues JFR. Organic acids and meat preservation : a review organic acids and meat preservation : a review. *Food Rev Int.* (2007) 23:141–58. doi: 10.1080/87559120701224964
64. FDA. Food additives for use in meat and poultry products: sodium diacetate, sodium acetate, sodium lactate and potassium. *Fed Regist.* (2000) 65:3121–3.
65. Zhang Z-H, Wang L-H, Zeng X-A, Han Z, Brennan CS. Non-thermal technologies and its current and future application in the food industry: a review. *Int J Food Sci Technol.* (2019) 54:1–13. doi: 10.1111/ijfs.13903
66. Wang L, Xia Q, Li Y. Synergistic effects of high pressure processing and slightly acidic electrolysed water on the inactivation of *Bacillus cereus* spores. *Int J Food Sci Technol.* (2017) 52:2429–35. doi: 10.1111/ijfs.13527
67. Yeh HY, Line JE, Hinton A Jr, Gao Y, Zhuang H. The effect of rosemary Extract and cold plasma treatments on bacterial community diversity in poultry ground meats. *Heliyon.* (2019) 5:e02719. doi: 10.1016/j.heliyon.2019.e02719
68. Sahebkar A, Hosseini M, Sharifan A. Plasma-assisted preservation of breast chicken fillets in essential oils-containing marinades. *LWT.* (2020) 131:109759. doi: 10.1016/j.lwt.2020.109759
69. Rodríguez-Calleja JM, Cruz-Romero MC, O'Sullivan MG, García-López ML, Kerry JP. High-pressure-based hurdle strategy to extend the shelf-life of fresh chicken breast fillets. *Food Control.* (2012) 25:516–24. doi: 10.1016/j.foodcont.2011.11.014
70. de Oliveira TLC, de Castro Leite BR, Ramos ALS, Ramos EM, Piccoli RH, Cristianini M. Phenolic carvacrol as a natural additive to improve the preservative effects of high pressure processing of low-sodium sliced vacuum-packed turkey breast ham. *LWT Food Sci Technol.* (2015) 64:1297–308. doi: 10.1016/j.lwt.2015.06.011
71. Chien S-Y, Sheen S, Sommers CH, Sheen L-Y. Modeling the inactivation of intestinal pathogenic *Escherichia coli* O157:H7 and uropathogenic *E. coli* in ground chicken by high pressure processing and thymol. *Front Microbiol.* (2016) 7:1–11. doi: 10.3389/fmicb.2016.00920
72. Sams AR, Feria R. Microbial effects of ultrasonication of broiler drumstick skin. *J Food Sci.* (1991) 56:247–8. doi: 10.1111/j.1365-2621.1991.tb08020.x
73. Kordowska-Wiater M, Stasiak DM. Effect of ultrasound on survival of gram-negative bacteria on chicken skin surface. *Bull Vet Inst Pulawy.* (2011) 55:207–10.
74. Piñon MI, Alarcon-Rojo AD, Renteria AL, Mendez G, Janacua-Vidales H. Reduction of microorganisms in marinated poultry breast using oregano essential oil and power ultrasound. *Acta Aliment.* (2015) 44:527–33. doi: 10.1556/066.2015.44.0024
75. Yordanov DG, Angelova GV. *High Pressure Processing for Foods Preserving*. Plodiv: Biotechnology & Biotechnological Equipment (2014). p. 2818.
76. Toldrá F. *Safety of Meat and Processed Meat*. New York, NY: Springer (2009). doi: 10.1007/978-0-387-89026-5
77. Gassiot M, Masoliver P. *Commercial High Pressure Processing of Ham and Other Sliced Meat Products at Esteban Espuña, S.A.* Oxford; Cambridge; Philadelphia, PA; New Delhi: Woodhead Publishing Limited (2010). doi: 10.1533/9780857090713.1.21
78. Bajovic B, Bolumar T, Heinz V. Quality considerations with high pressure processing of fresh and value added meat products. *MESC.* (2012) 92:280–9. doi: 10.1016/j.meatsci.2012.04.024
79. Tracz M. Effects of high hydrostatic pressure on *Campylobacter jejuni* in poultry meat. *Pol J Vet Sci.* (2015) 18:261–6. doi: 10.1515/pjvs-2015-0034
80. Sheen S, Cassidy J, Scullen B, Uknalis J, Sommers C. Inactivation of *Salmonella* spp. in ground chicken using high pressure processing. *Food Control.* (2015) 57:41–7. doi: 10.1016/j.foodcont.2015.04.005
81. Xu A, Scullen OJ, Sheen S, Liu Y, Johnson JR, Sommers CH. Inactivation of extraintestinal pathogenic *E. coli* suspended in ground chicken meat by high pressure processing and identification of virulence factors which may affect resistance to high pressure. *Food Control.* (2020) 111:107070. doi: 10.1016/j.foodcont.2019.107070
82. Liu Y, Betti M, Gänzle MG. High pressure inactivation of *Escherichia coli*, *Campylobacter jejuni*, and spoilage microbiota on poultry meat. *J Food Prot.* (2012) 75:497–503. doi: 10.4315/0362-028X.JFP-11-316
83. Dissing J, Bruun-Jensen L, Skibsted LH. Effect of high-pressure treatment on lipid oxidation in turkey thigh muscle during chill storage. *Eur Food Res Technol.* (1997) 205:11–3. doi: 10.1007/s002170050115
84. Kruk ZA, Yun H, Rutley DL, Jung E, Ji Y, Jo C. The effect of high pressure on microbial population, meat quality and sensory characteristics of chicken breast fi llet. *Food Control.* (2011) 22:6–12. doi: 10.1016/j.foodcont.2010.06.003
85. Orlien V, Hansen E, Skibsted LH. Lipid oxidation in high-pressure processed chicken breast muscle during chill storage: critical working pressure in relation to oxidation mechanism. *Eur Food Res Technol.* (2000) 211:99–104. doi: 10.1007/s002179900118
86. Yuste J, Pla R, Capellas M, Mor-Mur M. Application of high-pressure processing and nisin to mechanically recovered poultry meat for microbial decontamination. *Food Control.* (2002) 13:451–5. doi: 10.1016/S0956-7135(01)00071-8
87. Ogihara H, Yatuzuka M, Horie N, Furukawa S, Yamasaki M. Synergistic effect of high hydrostatic pressure treatment and food additives on the inactivation of *Salmonella enteritidis*. *Food Control.* (2009) 20:963–6. doi: 10.1016/j.foodcont.2008.11.004
88. Demirci A, Ngadi MO. *Microbial Decontamination in the Food Industry*. Pennsylvania: Woodhead Publishing (2012). doi: 10.1533/9780857095756
89. Bhat ZF, Morton JD, Mason SL, Bekhit AEDA. Current and future prospects for the use of pulsed electric field in the meat industry. *Crit Rev Food Sci Nutr.* (2019) 59:1660–74. doi: 10.1080/10408398.2018.1425825
90. Pillet F, Formosa-Dague C, Baaziz H, Dague E, Rols MP. Cell wall as a target for bacteria inactivation by pulsed electric fields. *Sci Rep.* (2016) 6:1–8. doi: 10.1038/srep19778
91. Espina L, Monfort S, Álvarez I, García-Gonzalo D, Pagán R. Combination of pulsed electric fields, mild heat and essential oils as an alternative to the ultrapasteurization of liquid whole egg. *Int J Food Microbiol.* (2014) 189:119–25. doi: 10.1016/j.ijfoodmicro.2014.08.002
92. Haughton PN, Lyng JG, Cronin DA, Morgan DJ, Fanning S, Whyte P. Efficacy of pulsed electric fields for the inactivation of indicator microorganisms and foodborne pathogens in liquids and raw chicken. *Food Control.* (2012) 25:131–5. doi: 10.1016/j.foodcont.2011.10.030
93. Álvarez I, Mañas P, Condón S, Raso J. Resistance variation of *Salmonella enterica* serovars to pulsed electric fields treatments. *J Food Sci.* (2003) 68:2316–20. doi: 10.1111/j.1365-2621.2003.tb05765.x
94. Mcdonnell C, Allen P, Chardonnereau F, Arimi J, Lyng J. The use of pulsed electric fields for accelerating the salting of pork. *Leb und-Technologie.* (2014) 59:1054–60. doi: 10.1016/j.lwt.2014.05.053
95. Arroyo C, Lascorz D, O'Dowd L, Noci F, Arimi J, Lyng JG. Effect of Pulsed Electric Field treatments at various stages during conditioning on quality attributes of beef longissimus thoracis et lumborum muscle. *Meat Sci.* (2015) 99:52–9. doi: 10.1016/j.meatsci.2014.08.004
96. Bekhit AE-DA, van de Ven R, Fahri F, Hopkins DL. Effect of pulsed electric field treatment on cold-boned muscles of different potential tenderness. *Food bioprocess Technol.* (2014) 7:3136–46. doi: 10.1007/s11947-014-1324-8
97. Ahmad RS, Imran A, Hussain MB. Nutritional composition of meat. *Meat Sci Nutr.* (2018) 61:77045. doi: 10.5772/intechopen.77045
98. Khan AA, Randhawa MA, Carne A, Mohamed Ahmed IA, Barr D, Reid M, et al. Quality and nutritional minerals in chicken breast muscle treated with low and high pulsed electric fields. *Food Bioprocess Technol.* (2018) 11:122–31. doi: 10.1007/s11947-017-1997-x
99. Khan AA, Randhawa MA, Carne A, Mohamed Ahmed IA, Al-Juhaimi FY, Barr D, et al. Effect of low and high pulsed electric field processing on macro

and micro minerals in beef and chicken. *Innov Food Sci Emerg Technol.* (2018) 45:273–9. doi: 10.1016/j.ifset.2017.11.012
100. Mandal R, Mohammadi X, Wiktor A, Singh A, Pratap Singh A. Applications of pulsed light decontamination technology in food processing: an overview. *Appl Sci.* (2020) 10:3606. doi: 10.3390/app10103606
101. Bintsis T, Litopoulou-Tzanetaki E, Robinson RK. Existing and potential applications of ultraviolet light in the food industry - a critical review. *J Sci Food Agric.* (2000) 80:637–45. doi: 10.1002/(SICI)1097-0010(20000501)80:6<637::AID-JSFA603>3.0.CO;2-1
102. Chun HH, Kim JY, Lee BD, Yu DJ, Song KB. Effect of UV-C irradiation on the inactivation of inoculated pathogens and quality of chicken breasts during storage. *Food Control.* (2010) 21:276–80. doi: 10.1016/j.foodcont.2009.06.006
103. Isohanni PMI, Lyhs U. Use of ultraviolet irradiation to reduce *Campylobacter jejuni* on broiler meat. *Poult Sci.* (2009) 88:661–8. doi: 10.3382/ps.2008-00259
104. McLeod A, Hovde Liland K, Haugen JE, Sørheim O, Myhrer KS, Holck AL. Chicken fillets subjected to UV-C and pulsed UV light: reduction of pathogenic and spoilage bacteria, and changes in sensory quality. *J Food Saf.* (2018) 38:12421. doi: 10.1111/jfs.12421
105. Haughton PN, Lyng JG, Cronin DA, Morgan DJ, Fanning S, Whyte P. Efficacy of UV light treatment for the microbiological decontamination of chicken, associated packaging, and contact surfaces. *J Food Prot.* (2011) 74:565–72. doi: 10.4315/0362-028X.JFP-10-356
106. Yang S, Sadekuzzaman M, Ha SD. Reduction of *Listeria monocytogenes* on chicken breasts by combined treatment with UV-C light and bacteriophage ListShield. *LWT Food Sci Technol.* (2017) 86:193–200. doi: 10.1016/j.lwt.2017.07.060
107. Holck AL, Liland KH, Drømtorp SM, Carlehö GM, McLeod A. Comparison of UV-C and pulsed UV light treatments for reduction of salmonella, listeria monocytogenes, and enterohemorrhagic *Escherichia coli* on eggs. *J Food Prot.* (2018) 81:6–16. doi: 10.4315/0362-028X.JFP-17-128
108. Begum M, Hocking AD, Miskelly D. Inactivation of food spoilage fungi by ultra violet (UVC) irradiation. *Int J Food Microbiol.* (2009) 129:74–7. doi: 10.1016/j.ijfoodmicro.2008.11.020
109. Silva-Espinoza BA, Palomares-Navarro JJ, Tapia-Rodriguez MR, Cruz-Valenzuela MR, González-Aguilar GA, Silva-Campa E, et al. Combination of ultraviolet light-C and clove essential oil to inactivate *Salmonella Typhimurium* biofilms on stainless steel. *J Food Saf.* (2020) 40:12788. doi: 10.1111/jfs.12788
110. Feng H, Barbosa-Cánovas GV, Weiss J. *Ultrasound Technologies for Food and Bioprocessing*. New York, NY: Springer New York (2011). doi: 10.1007/978-1-4419-7472-3
111. Turantaş F, Kiliç GB, Kiliç B. Ultrasound in the meat industry: general applications and decontamination efficiency. *Int J Food Microbiol.* (2015) 198:59–69. doi: 10.1016/j.ijfoodmicro.2014.12.026
112. Haughton PN, Lyng JG, Morgan DJ, Cronin DA, Noci F, Fanning S, et al. An evaluation of the potential of high-intensity ultrasound for improving the microbial safety of poultry. *Food Bioprocess Technol.* (2012) 5:992–8. doi: 10.1007/s11947-010-0372-y
113. Piñon MI, Alarcon-Rojo AD, Renteria AL, Carrillo-Lopez LM. Microbiological properties of poultry breast meat treated with high-intensity ultrasound. *Ultrasonics.* (2020) 102:1. doi: 10.1016/j.ultras.2018.01.001
114. Seo MK, Jeong HL, Han SH, Kang I, Ha SD. Impact of ethanol and ultrasound treatment on mesophilic aerobic bacteria, coliforms, and *Salmonella Typhimurium* on chicken skin. *Poult Sci.* (2019) 98:6954–63. doi: 10.3382/ps/pez486
115. Leal-Ramos MY, Alarcon-Rojo AD, Mason TJ, Paniwnyk L, Alarjah M. Ultrasound-enhanced mass transfer in Halal compared with non-Halal chicken. *J Sci Food Agric.* (2011) 91:130–3. doi: 10.1002/jsfa.4162
116. Smith DP. Effect of ultrasonic marination on broiler breast meat quality and Salmonella contamination. *Int J Poult Sci.* (2011) 10:757–9. doi: 10.3923/ijps.2011.757.759
117. Inguglia ES, Burgess CM, Kerry JP, Tiwari BK. Ultrasound-assisted marination: role of frequencies and treatment time on the quality of sodium-reduced poultry meat. *Foods.* (2019) 8:473. doi: 10.3390/foods8100473
118. Thirumdas R, Sarangapani C, Annapure US. Cold plasma: a novel non-thermal technology for food processing. *Food Biophys.* (2014) 10:1–11. doi: 10.1007/s11483-014-9382-z
119. Fernández A, Thompson A. The inactivation of *Salmonella* by cold atmospheric plasma treatment. *Food Res Int.* (2012) 45:678–84. doi: 10.1016/j.foodres.2011.04.009
120. Lee H, Yong HI, Kim HJ, Choe W, Yoo SJ, Jang EJ, et al. Evaluation of the microbiological safety, quality changes, and genotoxicity of chicken breast treated with flexible thin-layer dielectric barrier discharge plasma. *Food Sci Biotechnol.* (2016) 25:1189–95. doi: 10.1007/s10068-016-0189-1
121. Ulbin-Figlewicz N, Jarmoluk A, Marycz K. Antimicrobial activity of low-pressure plasma treatment against selected foodborne bacteria and meat microbiota. *Ann Microbiol.* (2015) 65:1537–46. doi: 10.1007/s13213-014-0992-y
122. Lu H, Patil S, Keener KM, Cullen PJ, Bourke P. Bacterial inactivation by high-voltage atmospheric cold plasma: influence of process parameters and effects on cell leakage and DNA. *J Appl Microbiol.* (2014) 116:784–94. doi: 10.1111/jam.12426
123. Lee ES, Cheigh CI, Kang JH, Lee SY, Min SC. Evaluation of in-package atmospheric dielectric barrier discharge cold plasma treatment as an intervention technology for decontaminating bulk ready-to-eat chicken breast cubes in plastic containers. *Appl Sci.* (2020) 10:1–21. doi: 10.3390/app10186301
124. Mai-Prochnow A, Alam D, Zhou R, Zhang T, Ostrikov K, Cullen PJ. The effect of rosemary E and cold plasma treatments on bacterial community diversity in poultry ground meats. Microbial decontamination of chicken using atmospheric plasma bubbles. *Plasma Process Polym.* (2020) 2020:52. doi: 10.1002/ppap.202000052
125. The European Parliment and the council of Eurpean union. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs. *Off J Eur Union.* (2004) 139:1–155.
126. Llana-Ruiz-Cabello M, Pichardo S, Maisanaba S, Puerto M, Prieto AI, Gutiérrez-Praena D, et al. *In vitro* toxicological evaluation of essential oils and their main compounds used in active food packaging: a review. *Food Chem Toxicol.* (2015) 81:9–27. doi: 10.1016/j.fct.2015.03.030
127. Jayasena DD, Jo C. Essential oils as potential antimicrobial agents in meat and meat products: a review. *Trends Food Sci Technol.* (2013) 34:96–108. doi: 10.1016/j.tifs.2013.09.002
128. Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Front Microbiol.* (2012) 3:1–24. doi: 10.3389/fmicb.2012.00012
129. Fernández-López J, Viuda-Martos M. Introduction to the special issue: application of essential oils in food systems. *Foods.* (2018) 7:56. doi: 10.3390/foods7040056

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.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 20 August 2021
doi: 10.3389/fnut.2021.663584

The Antifungal Activity of Loquat (*Eriobotrya japonica* Lindl.) Leaves Extract Against *Penicillium digitatum*

Yuting Shen[1†], Chuying Chen[1†], Nan Cai[1], Ruopeng Yang[1], Jinyin Chen[1,2], İbrahim Kahramanoğlu[3], Volkan Okatan[4], Kannan R. R. Rengasamy[5] and Chunpeng Wan[1*]*

[1] Jiangxi Key Laboratory for Postharvest Technology and Nondestructive Testing of Fruits, Vegetables/Collaborative Innovation Center of Postharvest Key Technology and Quality Safety of Fruits and Vegetables in Jiangxi Province, College of Agronomy, Jiangxi Agricultural University, Nanchang, China, [2] College of Materials and Chemical Engineering, Pingxiang University, Pingxiang, China, [3] Faculty of Agricultural Sciences and Technologies, European University of Lefke, Gemikonagi, Turkey, [4] Department of Horticulture, Faculty of Agriculture, Eskişehir Osmangazi University, Eskişehir, Turkey, [5] Green Biotechnologies Research Centre of Excellence, University of Limpopo, Mankweng, South Africa

OPEN ACCESS

Edited by:
Susana Ferreira,
University of Beira Interior, Portugal

Reviewed by:
Mónica Gandía,
University of Valencia, Spain
Paloma Sánchez-Torres,
Institute of Agrochemistry and Food Technology (IATA), Spain

***Correspondence:**
Jinyin Chen
jinyinchen@126.com
Chunpeng Wan
chunpengwan@jxau.edu.cn

[†] 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: 03 February 2021
Accepted: 19 July 2021
Published: 20 August 2021

Citation:
Shen Y, Chen C, Cai N, Yang R, Chen J, Kahramanoğlu İ, Okatan V, Rengasamy KRR and Wan C (2021) The Antifungal Activity of Loquat (Eriobotrya japonica Lindl.) Leaves Extract Against Penicillium digitatum. Front. Nutr. 8:663584. doi: 10.3389/fnut.2021.663584

This study was performed to determine the antifungal activity of loquat (*Eriobotrya japonica* Lindl) leaf extract (LLE) against the citrus postharvest pathogen *Penicillium digitatum* (*P. digitatum*). The LLE exhibited an antifungal activity against *P. digitatum*, with a minimum inhibitory concentration (MIC) of 0.625 mg/ml and a minimum fungicidal concentration (MFC) of 1.25 mg/ml. Significant inhibitory effects of LLE on mycelial growth and spore germination of *P. digitatum* were seen in a dose-dependent manner. Simultaneously, to investigate possible antifungal mechanisms by LLE, we analyzed their influence on morphological changes, cell membrane permeability, cell wall and cell membrane integrity, and adenosine phosphates (ATP, ADP, and AMP) levels. Alterations, such as sunken surface and malformation, occurred in the LLE-treated *P. digitatum* spores. Furthermore, intracellular inclusion content decreased after LLE treatment, indicating an increase in cell membrane permeability. Besides, the LLE treatment induced a significant decline in the level of adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP) with a noticeable addition of extracellular ATP, ADP, and AMP during the entire treatment period. Overall, the results manifested that the antifungal activity of LLE against *P. digitatum* can be attributed to the derangement of cell membrane permeability and disordered energy metabolism. This is the first report on the mechanism of antifungal activity of LLE and could be useful in the development of targeted fungicides from natural origin.

Keywords: loquat leaves, *Penicillium digitatum*, membrane permeability, energy metabolism, antifungal activity

INTRODUCTION

Massive postharvest losses in citrus fruits during storage, transportation, and selling are mainly caused by green (*Penicillium digitatum*) and blue (*Penicillium italicum*) mold fungus. Moreover, the sour rot and stem-end rot diseases caused by *Geotrichum citri-aurantii* and *Diaporthe citri*, respectively, may also contribute to postharvest losses in citrus fruits (1). *P. digitatum* is the most common pathogen reported to have caused about 90% of total postharvest loss in citrus fruits (2). Currently, postharvest fungal diseases are controlled and prevented using chemical fungicides, such

as imazalil, prochloraz, thiabendazole, and many others (3–5). However, excessive use of chemical fungicides causes environmental problems and potential health issues in humans and animals. This also leads to the development of resistant fungal strains, which leads to devastating results. Therefore, the current demand is to explore and develop natural and effective antifungal agents as alternatives to chemical fungicides.

Plant-derived extracts, such as essential oils, are generally recognized as safe (GRAS) components (6). The application of several plant extracts (e.g., pomegranate peel, pompia leave, *Ficus hirta* Vahl. Fruit, and *Sapindus saponaria* L. fruit) has been reported to reduce postharvest fungal diseases in citrus fruits and other horticultural commodities (7–10). For instance, the essential oil extracted from pompia leaves effectively controlled the growth of *P. digitatum* (8). Moreover, the results showed that the activity of the ethanol extract of *Sapindus saponaria* L. fruit against *Colletotrichum Musae* was similar to that of thiabendazole at a 500-μg/ml concentration (10). Pomegranate peel extract was also very effective against plant diseases by inducing and enriching the fruit critical defense pathways and antibiotic biosynthesis (7). Also, postharvest fruit loss due to fungal pathogen during storage was effectively controlled by the *F. hirta* Vahl. fruit extract (9).

Loquat (*Eriobotrya japonica* Lindl) is a subtropical perennial fruit tree widely distributed in southeastern China and highly consumed because of its soft and juicy pulp, delicious taste, and health-related properties (11). Moreover, the leaves of loquat, commonly known by the name of "Pí Pá Yè" in Chinese pharmacopeia, possess enormous biological activities and are extensively used to treat cough ailments and pulmonary diseases, chronic bronchitis, inflammation, and diabetes (11, 12). The leaves of loquat are particularly rich in phenolics and have a potent antioxidant activity (12, 13). To date, no information is available on the antifungal effect of loquat leaf extract on postharvest citrus fruits against fungal pathogens. Therefore, this work aimed to investigate the *in vitro* antifungal activity of loquat leaf extract (LLE) on postharvest citrus fruit pathogens, namely, *P. digitatum*, *P. italicum*, *D. citri*, and *G. citri-auranti*. Moreover, the effect of LLE treatments on mycelia growth, spore germination, morphology alteration, and membrane lipid peroxidation of *P. digitatum* was explored to provide a mechanistic overview of antifungal activity.

MATERIALS AND METHODS

Chemicals and Preparation of Loquat Leaf Extract

Ethanol, n-butanol, glucose, and agar powder were procured from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Air-dried loquat leaves (purchased from Zhangshu medicinal materials market and authenticated as leaves of *Eriobotrya japonica* Lindl) were finely pulverized and extracted with 50% ethanol (100 g of dry powder per 1 L) at 60°C for 1 h. The extracts were then filtered and concentrated under reduced pressure using a rotary evaporator (Rotavapor R-3, Buchi, Flawil, Switzerland) at 45°C. After that, the extracts were suspended in water and sequentially extracted with ethyl acetate and n-butanol. The n-butanol extract was filtrated, combined, and evaporated to obtain dried LLE. The dried LLE was stored in a cryovial at −20°C until further analysis.

Fungal Preparation

The phytopathogenic fungi *P. digitatum* (CGMCC 3.15410) and *P. italicum* (CGMCC 3.4040) were bought to the laboratory from the center of China General Microbiological Culture Collection (Beijing, China). *G. citri-auranti* was provided by the Department of Plant Protection in Jiangxi Agricultural University (Nanchang, China) and identified by Prof. Junxi Jiang. *D. citri* was isolated from decayed citrus fruits with a representative disease symptom of *Phomopsis* stem-end rot and identified by DNA sequencing (Qingke Biotech, Changsha, China). Each fungus was cultured on a potato dextrose agar (PDA) medium (leaching solution of potato 200 g, glucose 20 g, agar powder 20 g, and deionized water, 1 L) at 25 ± 1°C for a reactivation period of 7 days. The spore suspension of *P. digitatum* was prepared based on the protocol of previous studies (1, 14).

Antifungal Activity of LLE Against *P. digitatum*

Evaluation of MIC and MFC

Both the minimal inhibitory concentration (MIC) and the minimal fungicidal concentration (MFC) of loquat leaf extract (LLE) against *P. digitatum* were evaluated *in vitro* using the method of Chen et al. (15). The dried LLE was dissolved with double-distilled water (ddH_2O) and mixed with a PDA medium; then, the final concentrations of LLE ranged from 0 to 1.25 mg/ml per disc. Using a punch to transfer the mycelial plugs at the central of the PDA dish, After the cultivation of 25°C for 2 and 6 days to evaluate its antifungal activity. The values of MIC and MFC were defined as the lowest concentration of LLE that inhibited *P. digitatum* growth after incubation for 2 and 6 days, respectively.

Mycelium Growth

The antifungal activity of loquat leaf extract (LLE) against *P. digitatum* was determined using the protocol of Chen et al. (14) and defined as percent growth inhibition. Briefly, the discs (5 mm) of *P. digitatum* were placed in the center of Petri dishes containing 20 ml PDA with different LLE concentrations of 0 (set as the control), 0.156, 0.313, 0.625, 1.25, and 2.5 mg/ml. After incubation at 25°C for 6 days, the colony diameters (mm) of the control and LLE treated fungal growth were examined using a vernier caliper. The data were duplicated three times and expressed as the means ± standard deviation (SD).

Spore Germination

The spore germination assay determined the antifungal ability of LLE against *P. digitatum* following the method of Tao et al. (16). Briefly, the LLE was dissolved in potato dextrose broth (PDB) to acquire five different concentrations of 0 (set as the control), 0.156, 0.313, 0.625, and 1.25 mg/ml. Subsequently, 5 μl of *P. digitatum* spore suspension (1×10^6 CFU/ml) was added

to different LLE-treated slides. After 13 h of incubation at 25°C, nearly 100 spores per replicate were observed using an optical microscope, and the inhibitory germination rate of *P. digitatum* spore was estimated using the following formula:

$$IGR = \frac{GS_C - GS_{LLE}}{GS_C} \times 100$$

where IGR is inhibitory germination rate, GS_C is the mean amount of germinated spore in the control slide, and GS_{LLE} is the mean amount of germinated spore in the LLE-treated slide. The experiments were conducted twice with three replicates for each treatment.

Scanning Electron Microscopy Observations

The effect of LLE on microscopic morphological alterations in *P. digitatum* spore was observed by SEM (15). *P. digitatum* spores, which included the control and LLE-treated samples, were washed with a PBS buffer three times and subsequently fixed with 2.5% (v/v) glutaraldehyde at 4°C. After fixation for 48 h, the samples were rinsed three times in distilled water for 20 min and then dehydrated with sequential graded cold ethanol (30, 50, 70, and 90%) for 20 min, and finally with absolute ethanol for 45 min. The specimens were then sputter-coated with gold and observed with a scanning electron microscope (FEI Quanta 250 FEG, Hillsboro, OR, United States).

Assay of LLE on Cell Membrane Permeability of *P. digitatum*

The effects of LLE on the cell membrane permeability of *P. digitatum* were investigated by assaying extracellular conductivity, cell lysis rate, and leakages of protein and nucleic acids. Briefly, 200 μl of a *P. digitatum* suspension was evenly added in 100 ml of PDB and incubated in a shaker for 2 days. About 2 g of fresh mycelium was re-suspended in 50 ml PDB having different LLE concentrations of 0 (set as the control), MIC (0.625 mg/ml), and MFC (1.25 mg/ml). The samples were taken at various time intervals (0, 30, 60, 90, 120, and 240 min). Extracellular conductivity was detected as reported by Tao et al. (16) with a conductivity meter (model ST3100C, Ohaus Co., Parsipanny, NJ, United States). Cell lysis rate was evaluated according to the method of Chen et al. (1). Leakages of protein and nucleic acid from *P. digitatum* hyphae were determined based on the method described by Huang et al. (17) to measure the optical density (OD) of the supernatant at 260 and 280 nm. The experiments were conducted twice with three replicates for each treatment.

Measurement of Loss in Intracellular Constituents

The loss in intracellular constituents into the supernatant was measured according to the method described previously (18), with minor modifications. Soluble protein, reducing sugar, total lipid, and ergosterol contents in both the supernatants and the hyphae of *P. digitatum* were measured using bovine serum albumin standards, glucose, and cholesterol, respectively. The experiments were conducted twice with three replicates for each treatment.

Propidium Iodide Fluorescence Staining and Membrane Lipid Peroxidation

The effect of LLE on the plasma membrane integrity of *P. digitatum* was determined following the method described by Xin et al. (19). Briefly, *P. digitatum* hyphae were treated with LLE at 0 and 1.25 mg/ml (MFC) for 120 min. Then, the control and LLE-treated mycelia were dyed with propidium iodide (PI) at 25°C in the dark. After staining for 30 min, the control and LLE-treated mycelia were washed three times with PBS and observed with a Ni-U fluorescence microscope (Nikon Corporation, Tokyo, Japan).

The lipid peroxidation of *P. digitatum* cell was quantitatively determined in terms of malondialdehyde (MDA) content using the thiobarbituric acid method described by Pasquariello et al. (20), and MDA content was calculated using the equation below:

$$\text{lipid peroxidation} = [6.45 \times (OD532 - OD600) - 0.559 \times OD450] \times Vt/FW$$

where Vt is the total volume of the extract (ml), and FW is the frozen weight of *P. digitatum* (g).

The experiments were conducted twice with three replicates for each treatment.

Assays of LLE on the Activities of β-1,3-Glucanase and Alkaline Phosphatase

The 2-day-old mycelia from 100 ml PDB were collected and resuspended in 50 ml PDB with LLE at various concentrations (0.625, and 1.25 mg/ml). β-1,3-glucanase (β-Glu) activity, after exposure to LLE for 0, 30, 60, 90, 120, and 240 min, was measured according to the previously developed method (1). According to the instructions of the manufacturer, the extracellular AKP activity of *P. digitatum* hyphae after exposure to LLE treatments was determined using a commercial AKP kit (Jiancheng Bioengineering Research Institute Co., Nanjing, China) (21), and enzyme activities were expressed as U/mg prot. The experiments were conducted twice with three replicates for each treatment.

Assays of LLE on ATP, ADP, and AMP Content

The hyphae of *P. digitatum* were incubated according to the method for both β-Glu and AKP activity assays mentioned above. ATP, ADP, and AMP contents were determined following the method of Zheng et al. (22), and expressed as $mg \cdot kg^{-1}$ on a dry weight basis. The value of energy charge (EC) was calculated using the formula below:

$$EC = ([ATP] + 1/2 \times [ADP])/([ATP] + [ADP] + [AMP])$$

The experiments were conducted twice with three replicates for each treatment.

Statistical Analysis

Comparisons of data from different groups were analyzed by one-way analysis of variance (ANOVA) at 5% significance level with

TABLE 1 | Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of loquat leaf extract (LLE) against *P. digitatum*, *P. italicum*, *D. citri*, and *G. citri-auranti*.

Concentration (mg/ml)	*P. digitatum*	*P. italicum*	*D. citri*	*G. citri-auranti*
MIC	0.625	0.625	0.625	0.625
MFC	1.25	1.25	1.25	2.50

the SPSS 22.0 software (SPSS Inc. Chicago, IL, United States). The levels of significance were assessed as significant ($p < 0.05$) and highly significant ($p < 0.01$) using Prime and Excel 2010 software.

RESULTS

MIC and MFC

The treatments of LLE completely inhibited the mycelial growth of *P. digitatum*, *P. italicum*, and *D. citri* at the concentration of 0.625 mg/ml when incubated for 2 days. LLE (1.25 mg/ml) completely inhibited mycelial growth on the sixth day (**Table 1**). The results revealed that the MIC and MFC values of LLE against *P. digitatum*, *P. italicum*, and *D. citri* were 0.625 and 1.25 mg/ml, respectively. Moreover, LLE at the concentration of 0.625 and 2.5 mg/ml completely inhibited *G. citri-auranti* growth on the second and sixth days of incubation, respectively (**Table 1**). Hence, the MIC and MFC values of LLE against *G. citri-auranti* were 0.625 and 2.5 mg/ml, respectively.

Effects of LLE on Mycelial Growth and Spore Germination of *P. digitatum*

Different LLE concentrations showed potent inhibitory effects on the mycelial growth of *P. digitatum* on the PDA culture, compared with the control group (**Figure 1**). After 2 days, the diameter of the colony decreased significantly ($p < 0.05$) with increased LEE concentration, and the extension of the *P. digitatum* colony disappeared completely at LLE concentrations ≥0.625 mg/ml (**Figure 1**). Besides, the mycelial growth rate, after 48 h of culture with LLE concentrations of 0.625 and 1.25 mg/ml, decreased to 57.5 and 20.3%, respectively, relative to the mycelial growth in the control group (**Figure 1**). Furthermore, the mycelial growth of *P. digitatum* was negligible over the incubation period when treated with LEE concentrations of ≥1.25 mg/ml.

As shown in **Table 2**, a significant inhibitory effect of LLE on the spore germination of *P. digitatum* in the PDB medium was seen in a dose-dependent manner, where higher LLE concentration resulted in a lower spore germination rate. LLE at a concentration of 0.625 mg/ml could significantly ($p < 0.05$) suppress the spore germination of *P. digitatum* by 78.95 ± 0.41% compared with the control group (**Table 2**); whereas at an LLE concentration of 0.156 mg/ml, only 17.62 ± 0.45% of inhibitory percentage was recorded. As the LLE concentration increased up to 1.25 mg/ml, *P. digitatum* spore germinated rate was <5%. The linear regression of the inhibitory percentage of *P. digitatum* (Y) on the log-transformed LLE-treated concentrations (X) was determined as $Y = 1.75X - 1.491$, $R^2 = 0.987$, with the half-maximal effective concentration (EC_{50} refers to the LLE dose causing 50% inhibition of the spore germination) of LLE against *P. digitatum* being 0.317 mg/ml.

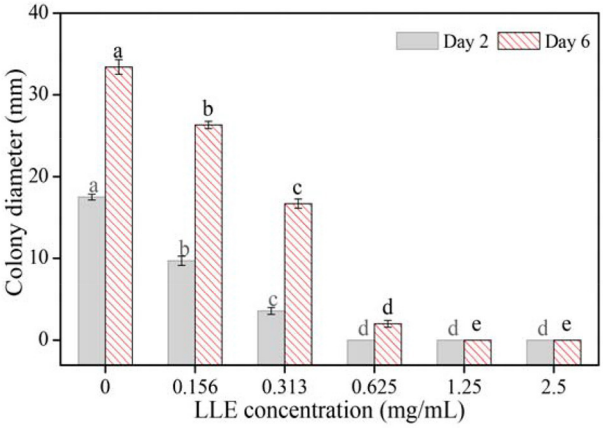

FIGURE 1 | Mycelial growth of *P. digitatum* on potato dextrose agar (PDA) exposure to loquat leaf extract (LLE) after 2 and 6 days. Different letters above the columns of the same day represent significant differences ($p < 0.05$) in colony diameter among the LLE concentrations.

TABLE 2 | Effect of LLE on spore germination of *P. digitatum*.

Concentration (mg/ml)	Spore germination rate (%)	Inhibitory percentage (%)
1.25	2.13 ± 0.81	97.79 ± 0.84a
0.625	20.27 ± 0.35	78.95 ± 0.41b
0.313	54.50 ± 0.46	43.40 ± 0.62c
0.156	79.33 ± 0.31	17.62 ± 0.45d
0 (control)	96.3 ± 0.26	0.00 ± 0.00e

Different letters (a–e) represent significant differences ($p < 0.05$).

Effect of LLE on Microscopical Morphology of *P. digitatum* by SEM

The scanning electron microscopy images showed that the microscopical morphology of the *P. digitatum* spores was severely affected by LLE treatment. The untreated spores had an engorged globular shape, with a smooth surface (**Figure 2A**). However, LLE treatment at the MFC (1.25 mg/ml) altered the microscopical morphology of the *P. digitatum* spores, including sunken surface, loss of linearity, and malformation (**Figure 2B**).

Effect of LLE on Cell Membrane Permeability of *P. digitatum*

The effect of LLE treatment on the cell membrane permeability of *P. digitatum* is presented in **Figure 3**. The extracellular conductivity in the 1.25 mg/ml LLE-treated *P. digitatum* cell suspension increased from 259.1 ± 1.03 μs/cm to 722.2 ± 11 μs/cm after 120 min of incubation (**Figure 3A**, $p < 0.05$). The cell lysis rate in LLE-treated *P. digitatum* suspensions significantly increased ($p < 0.05$), whereas the control group remained

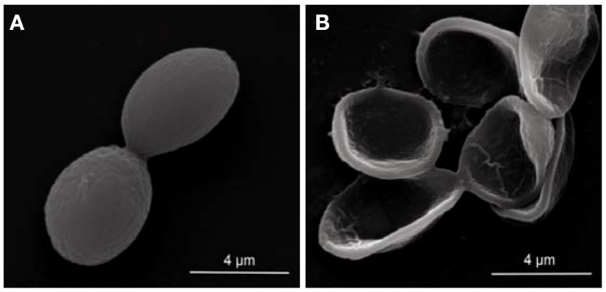

FIGURE 2 | Scanning electron microscopy (SEM) observation (×25,000 magnification) of the morphology alteration of *P. digitatum* spores exposed to 1.25 mg/ml (minimum fungicidal concentration, MFC) loquat leaf extract (LLE): **(A)** untreated spores and **(B)** LLE-treated spores.

stable. After 240 min of incubation, the cell lysis rate in the *P. digitatum* suspension treated with 0.625 and 1.25 mg/ml LLE was 50.54 ± 1.71 and 74.23 ± 2.54%, respectively, compared with 20.35 ± 0.92% in the control group (**Figure 3B**). As illustrated in **Figures 3C,D**, LLE treatment significantly induced the leakages of nucleic acid and protein in *P. digitatum* hypha ($p < 0.05$). After 240 min of incubation, both nucleic acid leakage and protein leakage in *P. digitatum* suspensions treated with 1.25 mg/ml LLE were 3.22 times and 4.01 times higher than that of the control. These results might indicate that cell membrane permeability was likely to be one of the vital antifungal mechanisms for LLE in *P. digitatum* growth.

The fluorescence microscopy images of *P. digitatum* hypha treated with MIC and MFC LLE are shown in **Figure 4A**, and are consistent with the cell membrane permeability results described above. No visible red fluorescence was observed in the control *P. digitatum* hypha after 30 min of incubation. In contrast, robust red fluorescence was observed in the MIC and MFC LLE-treated samples, respectively.

Effect of LLE on Intracellular Constituents of *P. digitatum*

Changes in intracellular reducing sugar, protein, total lipid, and ergosterol contents of *P. digitatum* exposed to LLE treatment at 0 mg/ml, MIC, and MFC are presented in **Figure 5**. A significant difference in intracellular reducing sugar content was observed after 60 min of exposure and gave a declining trend with increasing LLE concentrations (**Figure 5A**). After 120 min of incubation, the reducing sugar content in *P. digitatum* hypha was 15.95 ± 1.05 and 13.45 ± 0.27 mg/g at LLE concentrations 0.625 and 1.25 mg/ml, respectively, which was significantly lower than that in the control samples (21.7 ± 0.72 mg/g, $p < 0.05$). As demonstrated in **Figure 5B**, the protein content of control *P. digitatum* hypha was found to maintain a stable level during an incubation period of 0–240 min. In contrast, in the LLE-treated groups, the protein content significantly decreased. For instance, at 240 min after the treatment, the intracellular protein content in *P. digitatum* hypha treated with the MIC and MFC LLE was 17.95 and 34.62% lower than that of the control, respectively. In addition, the total lipid content in LLE-treated *P. digitatum* hypha decreased with increasing exposure time. In contrast, the total lipid content in the MIC and MFC LLE-treated samples after 240 min of incubation was 91.65 ± 0.62 and 63.69 ± 2.27 mg/g, respectively, which was significantly lower than that in the control group (121.78 ± 3.64 mg/g, $p < 0.05$, **Figure 5C**). Similarly, a continuous decrease in the ergosterol content of *P. digitatum* hypha after LLE treatment was observed throughout the whole incubation period, whereas the ergosterol content in the control group remained stable (**Figure 5D**). After 120 min of incubation, the ergosterol content in *P. digitatum* hypha treated with the MIC and MFC LLEs were 0.79 ± 0.06 and 0.54 ± 0.08 mg/g, respectively, which was significantly lower ($p < 0.05$) than that observed in the control group (1.2 ± 0.08 mg/g).

Effect of LLE on Lipid Peroxidation of *P. digitatum*

The effect of loquat leaf extract LLE treatment on the malondialdehyde (MDA) content of *P. digitatum* hypha is shown in **Figure 4B**. After 60 min of LLE treatment, a striking difference between the control and LLE-treated samples was observed, with the intracellular MDA content in the MIC and MFC LLE-treated groups being 2.61 ± 0.13 and 2.88 mmol ± 0.06 mmol/g, respectively, which was significantly higher than that in the control group (2.34 ± 0.06 mmol/g, $p < 0.05$). As shown in **Figure 4B**, the β-Glu activity in the control *P. digitatum* hypha was found to maintain a stable level during an incubation period of 0–240 min. In contrast, it rapidly increased in the LLE-treated groups. For instance, at 120 min of incubation, the β-Glu activity in *P. digitatum* hypha treated with the MIC and MFC LLEs were 1.47 times and 1.92 times higher than that of the control, respectively (**Figure 4C**, $p < 0.05$). The AKP activity of the MIC and MFC LLE-treated *P. digitatum* hypha was.27 ± 0.019 and 0.31 ± 0.015 U/mg, respectively, at 90 min after treatment, which was higher ($p < 0.05$) than that in the control samples. As the treatment time increased to 240 min, the AKP activity of *P. digitatum* hypha treated with LLE at MIC and MFC were 1.39 times and 1.68 times, respectively, higher than that of the control group (**Figure 4D**, $p < 0.05$).

Effect of LLE on ATP, ADP, and AMP Contents of *P. digitatum*

The effects of LLE treatment on the ATP, ADP, and AMP contents in *P. digitatum* hypha are presented in **Figure 6**. As shown in **Figure 6A**, the ATP content of *P. digitatum* hypha treated with LLE at MIC and MFC is 110.1 ± 2.25 and 77.9 ± 1.05 mg/kg, respectively, which is much lower than that in the control samples after 120 min of incubation (150.2 ± 3.03 mg/kg), indicating that the ATP supply of *P. digitatum* hypha is hindered by LLE treatment. **Figure 6D** shows the effect of ATP content release when the *P. digitatum* cell suspension is treated with LLE at MIC and MFC. The ATP content of the *P. digitatum* cell suspension treated with MFC LLE was dramatically increased after 120 min of exposure, which was significantly higher ($p < 0.05$) than that in MIC LLE (36.7%) or the control (98.6%). Similarly, both the ADP and AMP contents of *P. digitatum* hypha significantly decreased

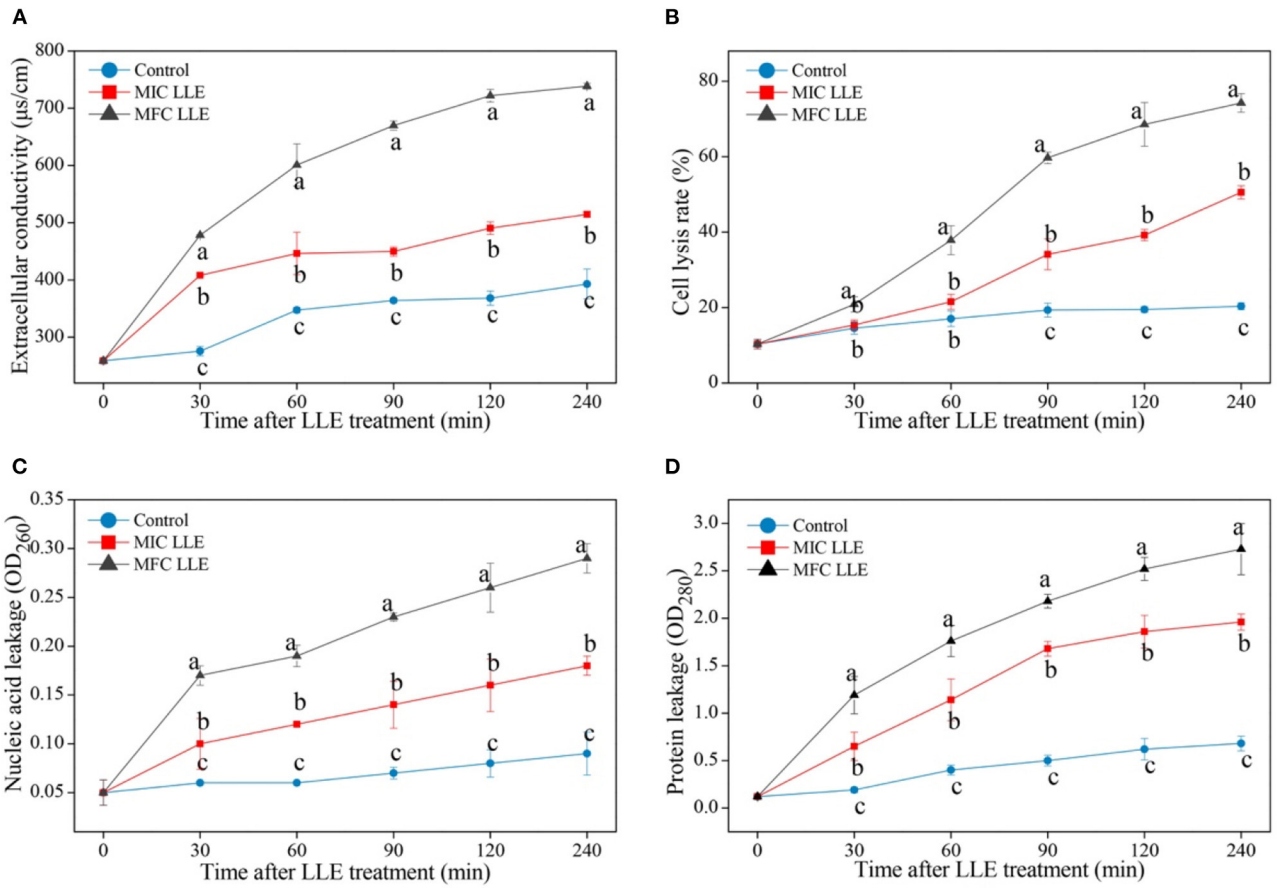

FIGURE 3 | Effect of loquat leaf extract (LLE) on cell membrane permeability of *P. digitatum*. **(A)** Change in extracellular conductivity of *P. digitatum* exposed to LLE treatment; **(B)** change in cell lysis rate of *P. digitatum* exposed to LLE treatment; **(C)** change in nucleic acid leakage of *P. digitatum* exposed to LLE treatment; and **(D)** change in protein leakage of *P. digitatum* exposed to LLE treatment. Different letters above the lines represent significant differences ($p < 0.05$) among treatments simultaneously.

with the MIC and MFC LLE treatments (**Figures 6B,C**, $p < 0.05$). In comparison, the release of ADP and AMP of the *P. digitatum* cell suspension significantly increased with the MIC and MFC LLE treatment (**Figures 6E,F**, $p < 0.05$). Those results suggested that the intracellular energy source of *P. digitatum* was infiltrated into the cell suspension after LLE treatment. Thus, the extracellular ATP, ADP, and AMP levels in the *P. digitatum* cell suspension exposed to LLE treatment was much higher than those of the control.

DISCUSSION

As we all know, loquat leaves have been proven to have good anti-inflammatory effects (23). Similarly, Cynanchum atratum (24), Mentha (25), and Ficus hirta Vahl (26, 27) showed anti inflammatory effects, along with strong antifungal effects [Cynanchum atratum (28), Ficus hirta Vahl (1, 29, 30), Mentha essential oil (31, 32)]. Therefore, the antifungal effect of loquat leaves needs to be verified, and then, its natural antifungal substances should be studied to provide the basis for developing new pesticides-botanical fungicides. In this study, the antifungal effects of LLE against *P. digitatum*, *P. italicum*, *D. citri*, and *G. citri-auranti* were evaluated *via* the agar dilution culture method (33). The results showed that the MIC value of LLE against these four fungi was 0.625 and 1.25 mg/ml of MFC against *P. digitatum*, *P. italicum*, and *D. citri* (**Table 1**). Furthermore, *P. digitatum* mycelial growth and spore germination inhibition were LLE dose-dependent (**Figure 1**; **Table 2**). These results showed that LLE has a strong inhibitory effect on *P. digitatum* and has a potential application prospect.

The antifungal mechanism of loquat leaf extract (LLE) was directly reflected by the morphological changes in *P. digitatum* spores, causing surface shrinkage, empty spores, and overflow of cell contents, which damaged the cell wall and cell membrane structure. The cell membrane lysis of *P. digitatum* was seen by changes in electrical conductivity (**Figure 3A**), nucleic acid leakage (**Figure 3C**), and protein leakage (**Figure 3D**). Thus, LLE caused disorder in the transmembrane electromotive force of the cell membrane, thereby damaging the function of the cell membrane, and the cell wall of *P. digitatum*.

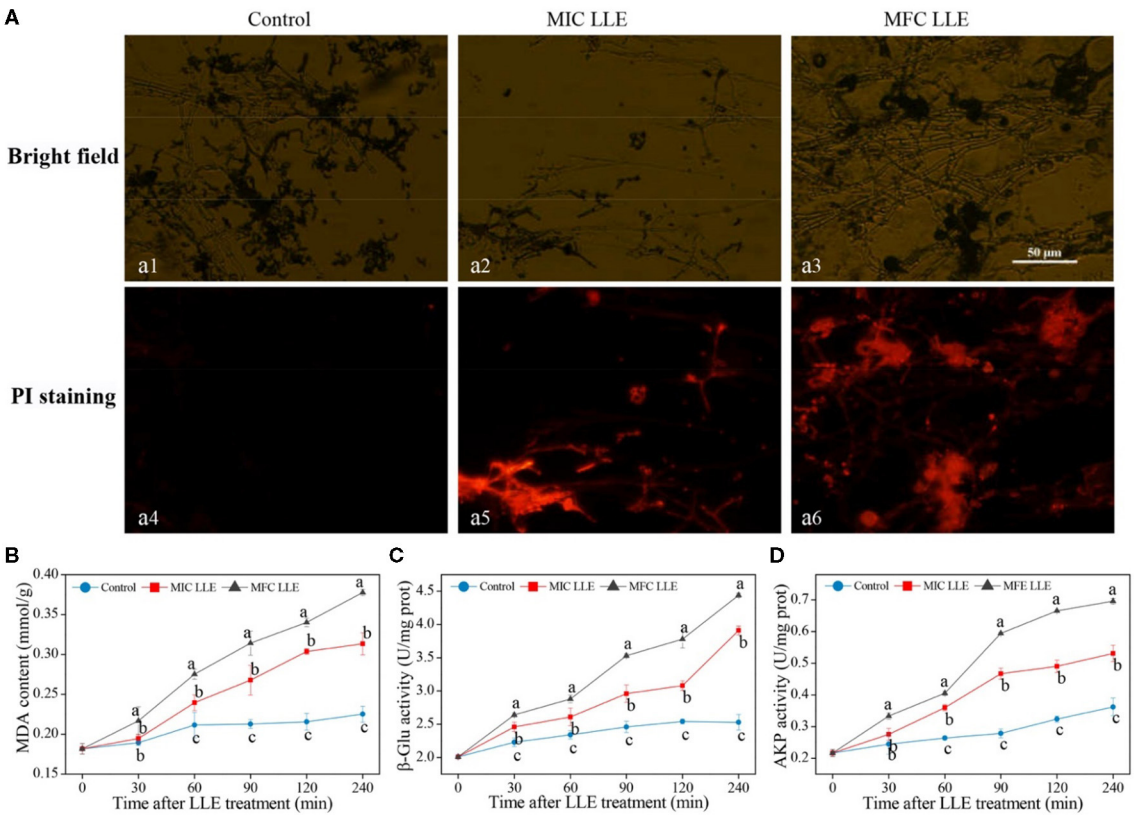

FIGURE 4 | Effect of loquat leaf extract (LLE) on cell membrane permeability and lipid peroxidation of *P. digitatum*. **(A)** Propidium iodide (PI) staining, a_1, a_4: control treatment (0 mg/ml LLE.); a_2, a_5: minimum inhibitory concentration (MIC treatment) (0.625 mg/ml LLE); a_3, a_6: minimum fungicidal concentration (MFC) treatment (1.25 mg/ml LLE); **(B)** change in malondialdehyde (MDA) content of *P. digitatum* exposed to LLE treatment; **(C)** change in the β-1,3-glucanase (β-Glu) activity of *P. digitatum* exposed to LLE treatment; **(D)** change in the alkaline phosphatase (AKP) activity of *P. digitatum* exposed to LLE treatment. Different letters above the lines represent significant differences ($p < 0.05$) among treatments at the same time after treatment.

The fungal cell wall is composed of polysaccharide macromolecules, such as dextran and chitin. The cell wall plays a vital role in maintaining normal cell morphology, controlling the transport of vital substances, and providing a defensive mechanism. β-1, 3-glucanase hydrolyzes β-1,3-glucans, which are the primary polymers within the fungal cell wall. AKP is also a hydrolytic enzyme that causes dephosphorylation from nucleotides, proteins, alkaloids, and other molecules. In this study, the β-1, 3-glucanase activity (**Figure 4C**) of the mycelium and the AKP activity (**Figure 4D**) of the extracellular after LLE treatment was increased. The LLE was supposed to exert its antifungal activity by causing physical damage to the cell wall of *P. digitatum* and also by increasing the cell wall degradation enzymatic activity, thereby destroying cell wall biosynthesis and integrity, leading to cytoplasmic collapse and eventually causing cell death.

The cell membrane is a selective semi-permeable membrane whose integrity, fluidity, and selective permeability can control the movement of various substances, which are vital for microbial growth and pathogenicity. Moreover, the PI fluorescence staining results (**Figure 4A**) showed that the PI entered the cell and intercalated into the DNA, and emitted a large amount of red fluorescence, reflecting loss of cell wall integrity. These results were also shown in other studies (34). Polyphenols and triterpenes are abundant in the loquat plant (35), which may be involved in the impairment of the cell membrane (36, 37), thereby exerting antifungal activity. Besides, in physiological metabolism, it has been reported that lipid peroxidation is also part of the antifungal mechanism (38). MDA is the main product of lipid peroxidation, which can be used as an index to measure the damage of membrane lipid peroxidation. In this study, the mycelium MDA content of *P. digitatum* treated with loquat leaves was remarkably higher than that of the untreated group (**Figure 4B**). This illustrated that the extract could accelerate the membrane lipid peroxidation of *P. digitatum*, cause fluidity of the cell membrane, and increase cell wall permeability.

Lipid and ergosterol are essential components of the cell membrane (39). Ergosterol plays a magnificent role in maintaining cell viability, membrane integrity, and fluidity (40). Xin et al. (19) showed that the antifungal mechanism of Baiwei extract was due to its capability to destroy the integrity of the cell membrane, which is mostly influenced by the content of total lipid and ergosterol in the cell membrane. The effect of extract treatment on cell membrane composition was studied by measuring total lipid and ergosterol content in the cell membrane. As shown in **Figure 5C**, the LLE extract can reduce

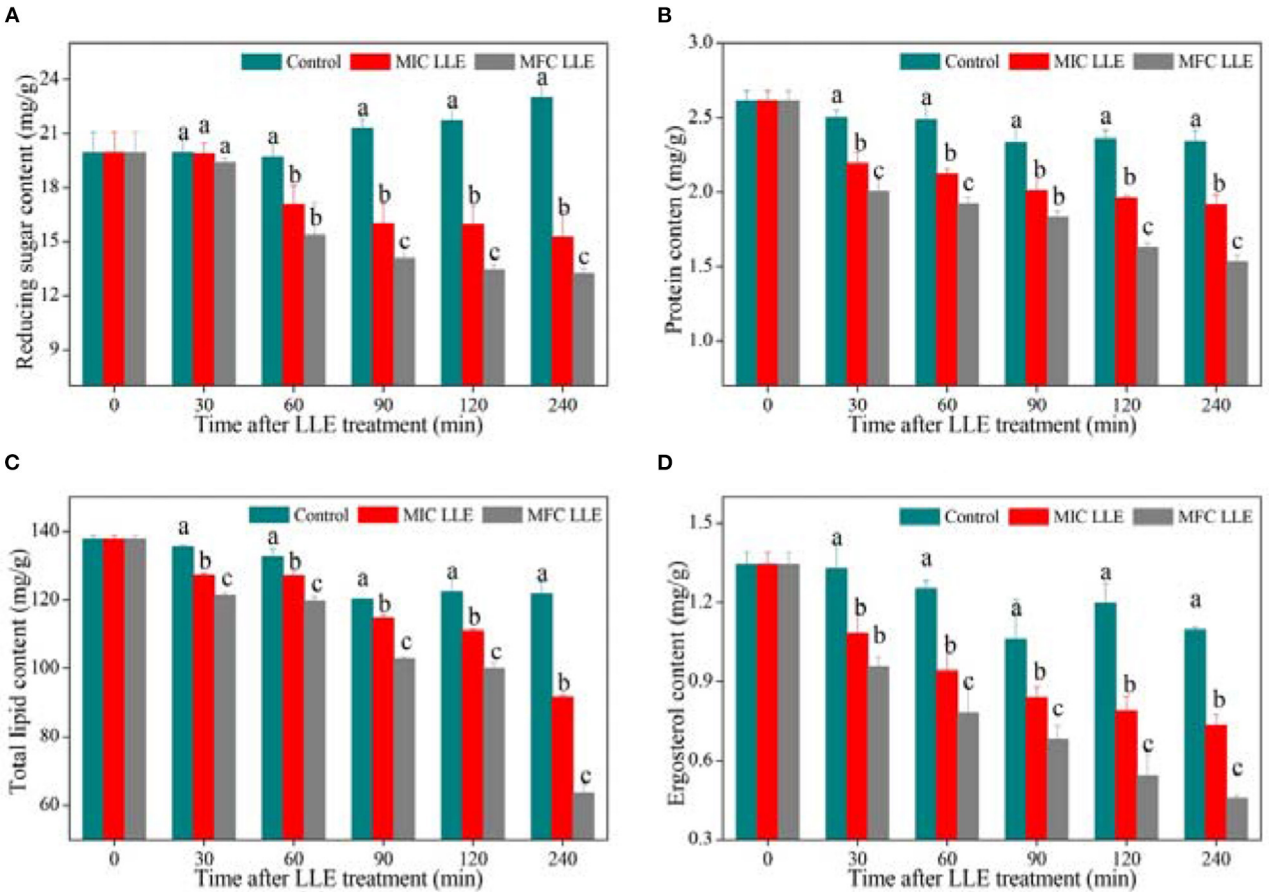

FIGURE 5 | Effect of loquat leaf extract (LLE) on intracellular constituents of *P. digitatum*. **(A)** Change in reducing sugar content of *P. digitatum* exposed to LLE treatment; **(B)** change in protein content of *P. digitatum* exposed to LLE treatment; **(C)** change in total lipid content of *P. digitatum* exposed to LLE treatment; and **(D)** change in ergosterol content of *P. digitatum* exposed to LLE treatment. Different letters above the columns represent significant differences ($p < 0.05$) among treatments simultaneously after treatment.

total lipid content in the membrane in a dose-dependent manner, affecting the stability of the cell membrane and increasing its fluidity. Helal et al. (40) proposed that a decrease in lipids will hinder the transport of fat-soluble substances and destroy cell-selective permeability. **Figure 5D** shows that LLE can significantly reduce ergosterol content, indicating that LLE could act on ergosterol and inhibit its synthesis. The decrease in lipid and ergosterol content reflected the irreversible damage of the cell membrane (40), thus we strongly postulated that the cell membrane might be the site of the LLE target.

Cells need nutrients to produce the energy for their growth and activities; hence nutrients are essential to cells. However, after the destruction of the cell membrane, pathways for the synthesis and transport of substances in cells may also be affected, thus preventing normal physiological metabolism of cells (41). The results of this study showed that after treatment with LLE, the contents of reducing sugar and soluble protein of *P. digitatum* cell suspensions were markedly higher than those of the untreated group (**Figure 5**). This may be due to increased membrane permeability with LLE, which causes an increase in the outflow of reducing sugar and soluble protein.

In the process of physiological metabolism, energy metabolism is also one of the essential metabolic pathways. ATP is the center of energy storage and utilization, and biochemical reactions must ensure various activities. Therefore, the change in its content in cells can directly affect the physiological activities of cells (42). Moreover, the ratio of various adenosine phosphates (ATP, ADP, and AMP) reflects cell energy charge (EC). Many metabolic activities in cells depend on energy charge changes, such as glycolysis, tricarboxylic acid cycle, electron transport system, and oxidative phosphorylation (43). Therefore, the extract of loquat leaves can significantly affect the changes in intracellular energy substances in *P. digitatum*, resulting in decreased intracellular ATP, ADP, and AMP content (**Figures 6A–C**). Moreover, the extract of loquat leaves leads to increased extracellular energy substances (**Figures 6D–F**). These phenomena may hinder the ATP synthesis pathway; hence the intracellular ATP has a significant downward trend, followed by ADP and AMP. The decrease in the intracellular synthesis of

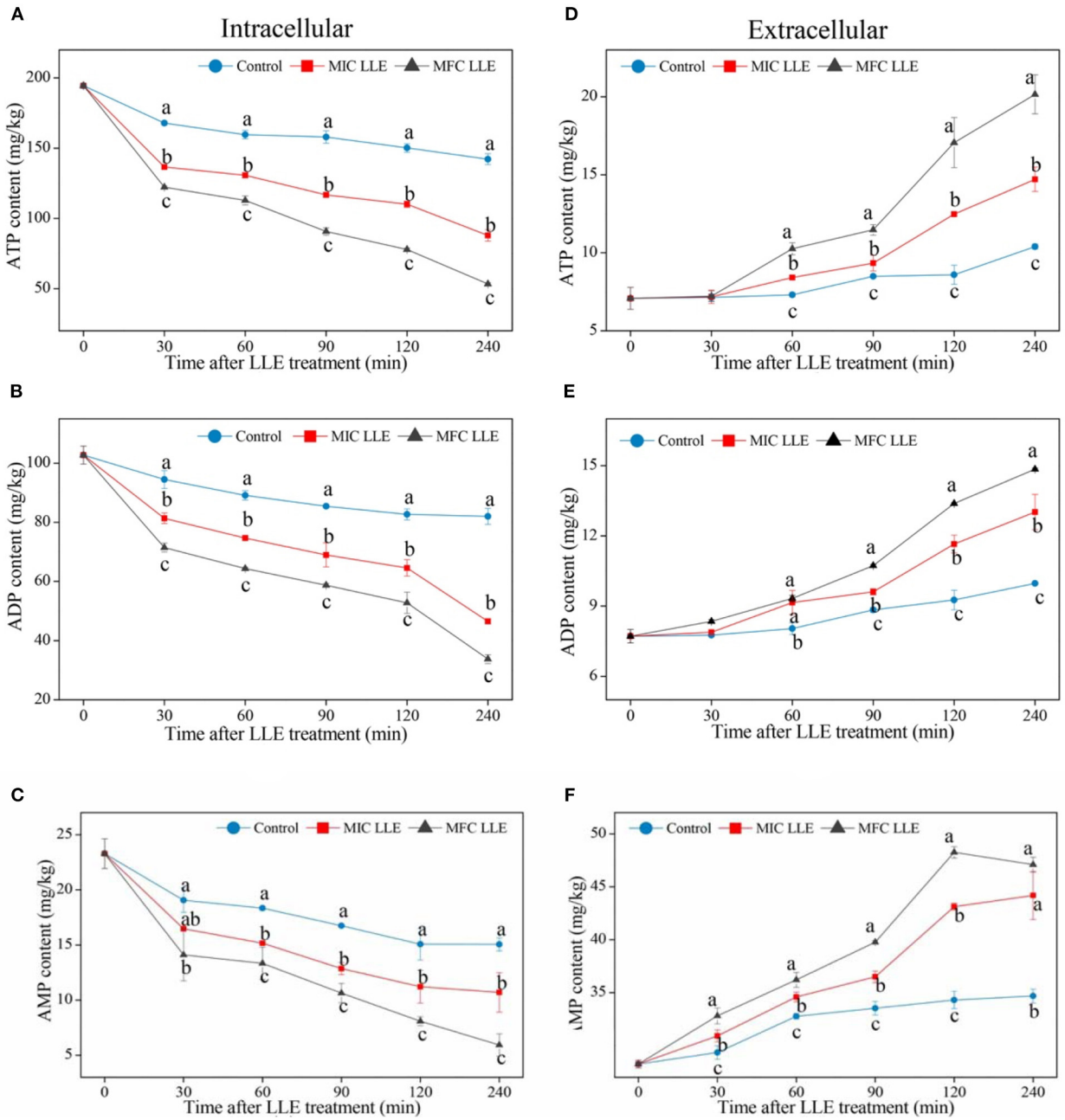

FIGURE 6 | Effect of loquat leaf extract (LLE) on adenosine triphosphate (ATP) **(A,D)**, adenosine diphosphate (ADP) **(B,E)**, and adenosine monophosphate (AMP) **(C,F)** contents in intracellular (left) and extracellular (right) *P. digitatum*. Different letters above the lines represent significant differences ($p < 0.05$) among treatments at the same time after treatment.

storage molecules by LLE leads to cell apoptosis and thus exerted its antifungal activity.

CONCLUSIONS

The current study revealed a strong antifungal activity of LLE against the citrus postharvest pathogen *P. digitatum*. The LLE exhibited strong antifungal activity against *P. digitatum*, with a minimum inhibitory concentration of 0.625 mg/ml and a minimum fungicidal concentration of 1.25 mg/ml, respectively. Sunken surface and malformation occurred at the LLE-treated *P. digitatum* spores. Besides, a higher increase of cell death was observed in propidium iodide (PI) fluorescent staining in the presence of LLE. Furthermore, LLE treatment induced a significant decline of the intracellular energy substances (ATP,

ADP, and AMP) content during the entire treatment period. Those results manifest that the antifungal activity of LLE against *P. digitatum* can be attributed to the derangement of cell membrane permeability and the disordered energy metabolism. The present study is proving a new mechanism of LLE extract against *P. digitatum,* which could be further tested at molecular level along with field trial. The study results could be helpful to use LLE extract as a natural antifungal product for preventing the growth and activity of *P. digitatum* and thus prevented the postharvest citrus fruit losses in a more sustainable manner.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

CW and JC: conceptualization, resources, supervision, and project administration. YS, CC, NC, and RY: methodology and investigation. YS and CC: software, validation, formal analysis, data curation, and writing—original draft preparation. CW, İK, VO, KR, and JC: writing—review and editing. İK, YS, and CC: visualization. CW: funding acquisition. All authors have read and agreed to the published version of the manuscript.

FUNDING

This research was funded by the Advantage Innovation Team Project of Jiangxi Province, Grant No: 20181BCB24005.

REFERENCES

1. Chen C, Chen J, Wan C. Pinocembrin-7-Glucoside (P7G) reduced postharvest blue mold of navel orange by suppressing *Penicillium italicum* growth. *Microorganisms.* (2020) 8:536. doi: 10.3390/microorganisms8040536
2. Yang Q, Qian X, Dhanasekaran S, Boateng NAS, Yan X, Zhu H, et al. Study on the infection mechanism of *Penicillium digitatum* on postharvest citrus (*Citrus Reticulata* Blanco) based on transcriptomics. *Microorganisms.* (2019) 7:672. doi: 10.3390/microorganisms7120672
3. Erasmus A, Lennox CL, Korsten L, Lesar K, Fourie PH. Imazalil resistance in *Penicillium digitatum* and *P. italicum* causing citrus postharvest green and blue mould: impact and options. *Postharvest Biol Technol.* (2015) 107:66-76. doi: 10.1016/j.postharvbio.2015.05.008
4. Liu J, Wang S, Qin T, Li N, Niu Y, Li D, et al. Whole transcriptome analysis of *Penicillium digitatum* strains treatmented with prochloraz reveals their drug-resistant mechanisms. *BMC Genomics.* (2015) 16:855-67. doi: 10.1186/s12864-015-2043-x
5. Kellerman M, Erasmus A, Cronjé PJR, Fourie PH. Thiabendazole residue loading in dip, drench and wax coating applications to control green mould and chilling injury on citrus fruit. *Postharvest Biol Technol.* (2014) 96:78-87. doi: 10.1016/j.postharvbio.2014.05.008
6. Palou L, Ali A, Fallik E, Romanazzi G. GRAS, plant- and animal-derived compounds as alternatives to conventional fungicides for the control of postharvest diseases of fresh horticultural produce. *Postharvest Biol Technol.* (2016) 122:41-52. doi: 10.1016/j.postharvbio.2016.04.017
7. Belgacem I, Pangallo S, Abdelfattah A, Romeo FV, Cacciola SO, Li Destri Nicosia MG, et al. Transcriptomic analysis of orange fruit treated with pomegranate peel extract (PGE). *Plants.* (2019) 8:101. doi: 10.3390/plants8040101
8. Danzi D, Ladu G, Veltkamp Prieto C, Garitas Bullon A, Petretto GL, Fancello F, et al. Effectiveness of essential oil extracted from pompia leaves against *Penicillium digitatum*. *J Sci Food Agric.* (2020) 100:3639-47. doi: 10.1002/jsfa.10394
9. Chen C, Nie Z, Wan C, Chen J. Preservation of xinyu tangerines with an edible coating using *Ficus hirta* Vahl. Fruits extract-incorporated chitosan. *Biomolecules.* (2019) 9:46. doi: 10.3390/biom9020046
10. Wan C, İbrahim K, Volkan O. Application of plant natural products for the management of postharvest diseases in fruits. *Folia Horticult.* (2021) 33:203-15. doi: 10.2478/fhort-2021-0016
11. Liu Y, Zhang W, Xu C, Li X. Biological activities of extracts from loquat (*Eriobotrya japonica* Lindl.): a Review. *Int J Mol Sci.* (2016) 17:1983. doi: 10.3390/ijms17121983
12. Zar PPK, Morishita A, Hashimoto F, Sakao K, Fujii M, Wada K, et al. Anti-inflammatory effects and molecular mechanisms of loquat (*Eriobotrya japonica*) tea. *J Funct Foods.* (2014) 6:523-33. doi: 10.1016/j.jff.2013.11.019
13. Mogole L, Omwoyo W, Mtunzi F. Phytochemical screening, antioxidant activity and α-amylase inhibition study using different extracts of loquat (*Eriobotrya japonica*) leaves. *Heliyon.* (2020) 6:e04736. doi: 10.1016/j.heliyon.2020.e04736
14. Chen C, Cai N, Chen J, Wan C. UHPLC-Q-TOF/MS-based metabolomics approach reveals the antifungal potential of Pinocembroside against citrus green mold phytopathogen. *Plants.* (2020) 9:17. doi: 10.3390/plants9010017
15. Chen C, Wan C, Peng X, Chen J. A flavonone pinocembroside inhibits *Penicillium italicum* growth and blue mold development in 'Newhall' navel oranges by targeting membrane damage mechanism. *Pestic Biochem Phys.* (2020) 165:104505. doi: 10.1016/j.pestbp.2019.11.025
16. Tao N, Chen Y, Wu Y, Wang X, Li L, Zhu A. The terpene limonene induced the green mold of citrus fruit through regulation of reactive oxygen species (ROS) homeostasis in *Penicillium digitatum* spores. *Food Chem.* (2019) 277:414-22. doi: 10.1016/j.foodchem.2018.10.142
17. Huang F, Kong J, Ju J, Zhang Y, Guo Y, Cheng Y, et al. Membrane damage mechanism contributes to inhibition of trans-cinnamaldehyde on *Penicillium italicum* using Surface-Enhanced Raman Spectroscopy (SERS). *Sci Rep.* (2019) 9:490-9. doi: 10.1038/s41598-018-36989-7
18. Chen C, Qi W, Peng X, Chen J, Wan C. Inhibitory effect of 7-Demethoxytylophorine on *Penicillium italicum* and its possible mechanism. *Microorganisms.* (2019) 7:36. doi: 10.3390/microorganisms7020036
19. Xin Z, Ouyang Q, Wan C, Che J, Li L, Chen J, et al. Isolation of antofine from *Cynanchum atratum* BUNGE *(Asclepiadaceae)* and its antifungal activity against *Penicillium digitatum*. *Postharvest Biol Technol.* (2019) 157:110961. doi: 10.1016/j.postharvbio.2019.110961
20. Pasquariello MS, Di Patre D, Mastrobuoni F, Zampella L, Scortichini M, Petriccione M. Influence of postharvest chitosan treatment on enzymatic browning and antioxidant enzyme activity in sweet cherry fruit. *Postharvest Biol Technol.* (2015) 109:45-56. doi: 10.1016/j.postharvbio.2015.06.007
21. Ouyang Q, Duan X, Li L, Tao N. Cinnamaldehyde exerts its antifungal activity by disrupting the cell wall integrity of *Geotrichum citri-aurantii*. *Front Microbiol.* (2019) 10:55. doi: 10.3389/fmicb.2019.00055
22. Zheng SJ, Jing GX, Wang X, Ouyang QL, Tao NG. Citral exerts its antifungal activity against Penicillium digitatum by affecting the mitochondrial morphology and function. *Food Chem.* (2015) 178:76–81. doi: 10.1016/j.foodchem.2015.01.077
23. Kuraoka-Oliveira AM, Radai JAS, Leitao MM, Cardoso CAL, Silva-Filho SE, Kassuya CAL. Anti-inflammatory and anti-arthritic activity in extract from the leaves of Eriobotrya japonica. *J Ethnopharmacol.* (2020) 249:112418. doi: 10.1016/j.jep.2019.112418
24. Hu G, Hong D, Zhang T, Duan H, Wei P, Guo X, et al. Cynatratoside-C from Cynanchum atratum displays anti-inflammatory effect via suppressing TLR4 mediated NF-ÈB and MAPK signaling pathways in LPS-induced mastitis in mice. *Chem Biol Interact*. (2018) 279:187–95. doi: 10.1016/j.cbi.2017.10.017
25. Kumar P, Mishra S, Malik A, Satya S. Insecticidal properties of mentha species: a review. *Ind Crop Prod.* (2011) 34:802-17. doi: 10.1016/j.indcrop.2011.02.019
26. Cheng J, Yi X, Chen H, Wang Y, He X. Anti-inflammatory phenylpropanoids and phenolics from *Ficus hirta* Vahl. *Fitoterapia.* (2017) 121:229-34. doi: 10.1016/j.fitote.2017.07.018

27. Ye X, Tian W, Wang G, Zhang X, Zhou M, Zeng D, et al. Phenolic glycosides from the roots of *Ficus hirta* Vahl. and their antineuroinflammatory activities. *J Agric Food Chem.* (2020) 68:4196-204. doi: 10.1021/acs.jafc.9b07876
28. Chuying C, Xuan P, Jinyin C, Chunpeng W. Antifungal activity of *Cynanchum atratum* alkaloids against citrus postharvest blue mould. *J Fruit Sci.* (2019) 36:94-102. doi: 10.13925/j.cnki.gsxb.20180152
29. Wan C, Han J, Chen C, Yao L, Chen J, Yuan T. Monosubstituted benzene derivatives from fruits of Ficus hirta and their antifungal activity against phytopathogen *Penicillium italicum*. *J Agric Food Chem*. (2016) 64:5621–24. doi: 10.1021/acs.jafc.6b02176
30. Wan C, Chen C, Li M, Yang Y, Chen M, Chen J. Chemical constituents and antifungal activity of *Ficus hirta* Vahl. fruits. *Plants.* (2017) 6:44-53. doi: 10.3390/plants6040044
31. Talibi I, Boubaker H, Boudyach EH, Ait Ben Aoumar A. Alternative methods for the control of postharvest citrus diseases. *J Appl Microbiol.* (2014) 117:1-17. doi: 10.1111/jam.12495
32. Samber N, Khan A, Varma A, Manzoor N. Synergistic anti-candidal activity and mode of action of Mentha piperitaessential oil and its major components. *Pharm Biol.* (2015) 53:1496-504. doi: 10.3109/13880209.2014.989623
33. Wilkinson JM, Hipwell M, Ryan T, Cavanagh HM. Bioactivity of *Backhousia citriodora*: antibacterial and antifungal activity. *J Agric Food Chem.* (2003) 51:76-81. doi: 10.1021/jf0258003
34. Li L, Hu X, Xia Y, Xiao G, Zheng P, Wang C. Linkage of oxidative stress and mitochondrial dysfunctions to spontaneous culture degeneration in *Aspergillus nidulans*. *Mol Cell Proteomics.* (2014) 13:449-61. doi: 10.1074/mcp.M113.028480
35. Zhang L, Saber FR, Rocchetti G, Zengin G, Hashem MM, Lucini L. UHPLC-QTOF-MS based metabolomics and biological activities of different parts of *Eriobotrya japonica*. *Food Res Int.* (2021) 143:110242. doi: 10.1016/j.foodres.2021.110242
36. Sanzani SM, Schena L, Ippolito A. Effectiveness of phenolic compounds against citrus green mould. *Molecules.* (2014) 19:12500-8. doi: 10.3390/molecules190812500
37. Farzaneh M, Kiani H, Sharifi R, Reisi M, Hadian J. Chemical composition and antifungal effects of three species of Satureja (*S. hortensis*, *S. spicigera*, and *S. khuzistanica*) essential oils on the main pathogens of strawberry fruit. *Postharvest Biol Technol.* (2015) 109:145-51. doi: 10.1016/j.postharvbio.2015.06.014
38. Dou S, Liu S, Xu X, Ouyang Q, Tao N. Octanal inhibits spore germination of *Penicillium digitatum* involving membrane peroxidation. *Protoplasma.* (2016) 254:1539-45. doi: 10.1007/s00709-016-1046-z
39. Tao N, Jia L, Zhou H. Anti-fungal activity of Citrus reticulata Blanco essential oil against *Penicillium italicum* and *Penicillium digitatum*. *Food Chem.* (2014) 153:265-71. doi: 10.1016/j.foodchem.2013.12.070
40. Helal GA, Sarhan MM, Abu Shahla ANK, Abou El-Khair EK. Effects of *Cymbopogon citratus* L. essential oil on the growth, morphogenesis and aflatoxin production of *Aspergillus flavus* ML2-strain. *J Basic Microbiol.* (2007) 47:5-15. doi: 10.1002/jobm.200610137
41. Sisler HD. Control of fungal diseases by compounds acting as antipenetrants. *Crop Prot.* (1986) 5:306-13. doi: 10.1016/0261-2194(86)90108-0
42. Okayama S, Kopelovich L, Balmus G, Weiss RS, Subbaramaiah K. p53 protein regulates Hsp90 ATPase activity and thereby Wnt signaling by modulating Aha1 expression. *J Biol Chem.* (2014) 289:6513-25. doi: 10.1074/jbc.M113.532523
43. Saquet AA, Streif J, Bangerth F. Energy metabolism and membrane lipid alterations in relation to brown heart development in 'Conference' pears during delayed controlled atmosphere storage. *Postharvest Biol Technol.* (2003) 30:123-32. doi: 10.1016/S0925-5214(03)00099-1

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.

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