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Comparative nutrient and sensory analysis of eight different commercial *Chlorella* powders

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A growing global population and climate change challenge conventional agriculture and global food safety. Microalgae are an emerging sustainable nutrient food resource, increasingly regarded as an important component for the human diet. The chlorophyte *Chlorella* sp. is regulated and commercialized for food applications. Commercial *Chlorella* preparations are either produced by heterotrophic fermentation or photoautotrophic cultivation in bioreactors or outdoor ponds. Products can differ significantly with regard to taxonomy of the production strain, changed genetic properties due to strain development, cultivation conditions, and downstream biomass processing methods. Notably, heterotrophic strains may be adapted to generate little or no photosynthetic pigments, resulting in yellow or white variant. In this study, eight different commercial *Chlorella* products from different EU suppliers were analyzed and compared regarding identity of the production strain, macro- and micronutrient profiles, sensory properties, as well as microbial load. The production processes featured different cultivation methods, production strains, genetic variants (yellow and white) and downstream processing methods. Data indicates significant variations between the *Chlorella* products. White and yellow *Chlorella* products showed different protein and taste profiles compared to green, photosynthetically competent wild type strains. We confirmed relatively high and stable protein concentrations but could detect variations in sugar and specifically lipid and vitamin profiles depending on strain, cultivation and downstream processing methods. The microbial load varied strongly between closed and open cultivation systems but was compliant with legislative (EFSA) specifications for all samples. The comparative nutrient- and sensory data set

presented in this study will aid in selecting algae products for the development of innovative foods, thereby accelerating adoption and differentiation of algae food products.

KEYWORDS

sensory analysis, nutritional profile, *Chlorella* powder, microalgae, innovative food

1 Introduction

Because of a rapidly growing global population global food demand will increase by 50% until 2050 (Nations, 2022a; Nations, 2022b). While food production is responsible for about 34% of all human-induced greenhouse gas emissions it also faces challenges because of climate change and the scarcity of arable land. To address these issues and to ensure food security for the world population, it is essential to explore more sustainable alternatives to conventional agriculture and livestock breeding (Crippa et al., 2021; Mirón et al., 2023). Microalgae build up biomass much faster than terrestrial crops and represent a rich source of high-quality protein, essential fatty acids, vitamins, minerals, and bioactive compounds supporting a healthy human diet (Andrade et al., 2018; Bhuvana et al., 2019; Yang et al., 2023). *Chlorella vulgaris*, for example, shows a projected market value of USD 413.3 million by 2028 with an annual biomass production of about 5,000 tons and has been recognized as the most valuable commercially produced green microalga (Levasseur et al., 2020). This species is regulated for food and feed applications from the food and drug administration (FDA) as well as European food safety authority (EFSA) and is generally recognized as safe (GRAS) (Additives and Food, 2015; Food and Administration, 2016). The reported intracellular protein content of *Chlorella* sp. biomass is between 40% and 70% and complies with the requirements of a high quality protein according to the essential amino acid index (EAAI), which is used to evaluate protein quality for human nutrition (Becker, 2007; Bito et al., 2020; Fu et al., 2021; Markou et al., 2012; Matard-Mann et al., 2017; Muys et al., 2019; Qin et al., 2020; Yang et al., 2023). In addition to its high protein content, *Chlorella* sp. biomass contains a variety of vitamins and other bioactive and potentially health promoting compounds (Bito et al., 2020; Markou et al., 2012; Matard-Mann et al., 2017; Qin et al., 2020).

The composition of the commercialized biomass is mainly dependent on the cultivation method, the production species/strain, their genetic variation and downstream processing (Hosseinzand et al., 2018; Kolackova et al., 2023; Muys et al., 2019; Nwoba et al., 2019; Silva et al., 2021; Vilatte et al., 2023). *Chlorella* sp. can be cultivated photoautotrophically in illuminated, closed photobioreactors or open ponds, with carbon dioxide as carbon source (phototrophic/autotrophic). Alternatively, algae biomass can be cultivated heterotrophically in conventional stirred tank fermenters using monomeric sugars (e.g., glucose) as

a carbon source. Moreover, both photoautotrophic and heterotrophic cultivation modes can be combined in mixotrophic cultivation modes using photobioreactor systems (Barros et al., 2019; Patel et al., 2021). The ability to grow *Chlorella* heterotrophically enables the development of genetically adapted strains with less or devoid of photosynthetic pigments. These yellow or white algae products have altered sensory profiles (Cabrol et al., 2023; Schüler et al., 2020), which offers new options for the development of food products. Nevertheless, microalgae biomass is currently predominantly used as an additive to products to increase their nutritional value, or as a dietary supplement due to its potentially health-promoting properties. Therefore, in addition to the contained nutrients color, taste, and odor can play a major role in the general consumer acceptance of microalgae in food. In this context, product quality and shelf life of microalgae biomass products is highly dependent on the microbial loads of microalgae preparations, which are significantly affected by the individual cultivation and downstream processing methods.

There is sporadic information on production strains, cultivation methods and nutritional profiles mainly provided by manufacturers of microalgae biomass. However, detailed and standardized analysis of micro- and macronutrient composition as well as sensory profiles are typically not reported. Moreover, data on microbial loads pertaining to these products are commonly not disclosed. This study used standardized analysis methods to compare macro- and micronutrient composition, sensory profiles, as well as microbial load of eight different dried commercial *Chlorella* sp. powders. Highlighting the impacts of different ways of microalgae production and processing may be of interest to food technologists to evaluate the potential for food development of different *Chlorella* products.

2 Materials and methods

2.1 *Chlorella* powders

Eight different types of *Chlorella* powders covering different suppliers, strains, cultivation methods, and downstream processing were analyzed for their nutrient and sensory profiles (Table 1).

2.2 Qualitative pigment analysis

For the pigment analysis 10–15 mg of the different biomass samples were extracted with 4 mL HPLC-grade acetone in 10 mL glass tubes with solvent-proof screw-top lids according to Paper et al. (Paper et al., 2022). and directly analyzed using a Shimadzu Prominence LC-20A HPLC-system (Shimadzu, Duisburg, Germany) equipped with an SPD-M20A diode array detector on

Abbreviations: FFA, free fatty acid; FAME, fatty acid methyl ester; EU, European Union; RT, room temperature; EDTA, Ethylenediaminetetraacetic acid; FA, folic acid, H4-THF, tetrahydrofolic acid, 5-CH3-H4-THF, 5-methyl-tetrahydrofolic acid, 5-CHO-H4-THF, 5-formyl-tetrahydrofolic acid, 10-CHO-FA, 10-formyl-folic acid; Fru, fructose, Xyl, xylose, Gal, galactose, Man, Mannose.

a Phenomenex Luna C8 LC column (250 mm × 4.6 mm I.D.; particle size 5 µm, Phenomenex Ltd. Deutschland, Aschaffenburg, Germany) according to (Paper et al., 2022). Pigment standards were purchased from CaroteNature (Lupsingen, Germany) and Sigma-Aldrich Chemie (Weinheim, Germany) in HPLC grade.

2.3 Microbiological load

2.4. Microbiological analysis was performed according to standard ISO methodology in an ISO17025 accredited laboratory (STS 0093). All media were purchased from HiMedia (Lucerna-Chem AG, Lucerne, Switzerland). All media were performance tested according to ISO 11133:2014 before use. 10 g of powdered algae samples were reconstituted in 90 mL of Sodium Chloride Peptone Broth (SCPB; Millipore, Zug, Switzerland). Serial dilutions (1:10) were prepared for duplicate plating of chosen dilutions. Aerobe mesophilic microorganisms (ISO 4833-1:2013) were analyzed using Plate Count Agar (PCA) incubated at 30°C for 72 h. Total yeast and molds were enumerated following ISO 7954:1987, where samples were incubated for up to 5 days at 25°C on Yeast Extract Glucose Chloramphenicol Agar (YGC-A). Quantitative determination of presumptive *Bacillus cereus* was performed using Mannitol Egg Yolk Polymyxin Agar (MYP-A) as described in ISO 7932:2004/Amd 1:2020. Samples were incubated for 24 h at 30°C. On the medium, typical colonies of *B. cereus* appear rough and dry, set against a bright pink background with an egg yolk precipitate. Colonies exhibiting this characteristic appearance were examined microscopically for the typical morphology of *B. cereus*, which is characterized by large cells (3.5 µm) that sometimes form short chains or long strands, featuring central to terminal ellipsoidal spores that do not cause the cells to swell. For the enumeration of *Enterobacteriaceae*, Violet Red Bile Glucose Agar (VRBG-A) was used according to ISO 21528-2:2017. An overlay of VRBG-A was used to prevent swarming colonies and to provide semi-anaerobic conditions. Samples were incubated at 37°C for 24 h. Small (1–2 mm in diameter), round and violet to pink colonies with a halo surrounding the colony and a confirmed oxidase negative test were counted. After all ISO accredited methods were performed, the 1 g of powdered sample was reconstituted in 9 mL of SCPB and heated to 80°C for 12 min to eliminate all viable microorganisms (McHugh et al., 2017). The heat-treated samples were analyzed for mesophilic aerobic spores using Tryptic Soy Agar (TSA) incubated at 37°C for 48 h (Kyrylenko et al., 2023). The presence of total anaerobic spores was estimated by plating the heat-treated samples on Brain Heart Infusion Agar (BHI-A) and incubating at 37°C for 24 h in an anaerobic workstation (Whitley A85 Workstation, Meintrup DWS Laborgeräte, Herzlake, Germany) (Hu et al., 2021).

2.4 Determination of water content and water activity

The water content of the samples was determined by drying oven method according to the MEBAK method R-110.40.020 [2016-03] (Mebak, 2016a). The water activity (aw) of the samples was measured using a Novasina TH 500/RTD-502 AW SPRINT

(Novasina, Lachen, Switzerland) with the measurement chamber set to 25°C.

2.5 Determination of raw Protein content

The raw protein content of the samples was determined by Kjeldahl method according to the MEBAK method R-110.41.030 [2016-03] (Mebak, 2016b) taking into account the nitrogen-to-protein conversion factor (k_p) of 4.78 (Sägesser et al., 2023).

2.6 Determination of ash content

About 1 g of the different *Chlorella* powders ashed at 650°C in pre-weight crucibles for 4h. Afterwards, the crucibles were placed in a vacuum desiccator for cooling and weight again to determine the ash content. All experiments were carried out in biological triplicates.

2.7 Amino acid analysis

The amino acid composition after acidic hydrolysis was determined according to the literature (Wollmann and Hofmann, 2013). Aliquots (5 mg) of the *Chlorella* powders were suspended in aqueous hydrochloric acid (6 M; 3 mL) and incubated for 24 h at 110°C. Cooled and neutralized (NaOH; 12 M) solutions were further diluted to 5 mL with water. Aliquots (990 µL) thereof were then spiked with the internal standard (10 µL) and quantified by means of SIDA-HPLC-MS/MS (Hillmann et al., 2012).

2.8 Fatty acid analysis

The fatty acid profile was determined according to Engelhart-Straub et al. using 10 mg of the different *Chlorella* powders. The sample preparation, namely, the extraction and transesterification of the lipids, was performed using a Multi-Purpose Sampler MPS robotic (Gerstel, Linthicum Heights, MD, United States) with C12:0 TAG as internal standard for quantification (Engelhart-Straub et al., 2022). The samples were measured at an GC-2025 coupled to an AOC-20i Auto Injector and AOC-20s Auto Sampler (Shimadzu, Duisburg, Germany). Marine oil fatty acid methyl ester (FAME) mix (20 components from C14:0 to C24:1; Restek GmbH, Bad Homburg, Germany) was used as standard for the quantification of the fatty acid methyl esters (FAMES).

2.9 Sugar analysis

The *Chlorella* powders were chemically hydrolyzed using 2% sulfuric acid. For this, 50 mg of each powder was suspended in 19.6 mL water and incubated at 95°C for 1 h in a water bath. Afterwards, the solids were separated from the water phase by centrifugation (19,000 rcf, 10 min, RT). The supernatant was acidified to a final sulfuric acid concentration of 2% (v/v) with

concentrated acid and the pellet was resuspended in 2% (v/v) sulfuric acid. After autoclaving (60 min, 121°C) both acidic liquid phases were neutralized with solid calcium carbonate. 2 mL of each supernatant were frozen overnight, thawed, and filtered through a 0.2 µm Phenex-NY syringe filter (Phenomenex, Torrance, CA, United States). 3 µL of 0.5 M EDTA was then added to 197 µL of sample. The samples were analyzed in a Agilent HPLC according to Jurkowski et al. with 60 min runtime per sample (Jurkowski et al., 2022).

2.10 Determination of vitamins C, D, and E

Vitamin C concentrations were quantified using the Ascorbic Acid Assay Kit MAK074 (Sigma-Aldrich, St. Louis, MO, United States). By means of a coupled enzyme reaction, the resulting colorimetric product was measured at 570 nm, which forms proportionally to the ascorbic acid content in the sample. Vitamin D2 and D3 contents were quantified by means of the ELISA Kit E-EL-0012, purchased from Elabscience Biotechnology Inc. (Wuhan, China). The measurements were conducted at 450 nm. Vitamin E contents were quantified by the Colorimetric Assay Kit E-BC-K033 (Elabscience Biotechnology Inc.), measuring the reduction of Fe³⁺ to Fe²⁺ by Vitamin E and further colorimetric reaction with phenanthroline at 533 nm. All sample preparations were conducted completely according to the respective guidelines and spectrophotometric measurements were performed with a microplate reader (Infinite 200, Tecan Group AG, Männedorf, Switzerland) at individual wavelengths specified for each assay.

2.11 Folate quantification

2.11.1 Extraction

The algae samples were analyzed in triplicate according to Striegel et al. with regards of the five folate vitamers PteGlu, H₄folate, 5-CH₃-H₄folate, 5-CHO-H₄folate, and 10-CHO-PteGlu (Striegel et al., 2018). The complete information about chemicals, standards, and preparations of solutions as well as validation of the method can be obtained from this previous publication (Striegel et al., 2018). 5–10 mg of freeze-dried, ground algae samples were used for quantification. After equilibration for 15 min with 10 mL buffer, the respective internal standards [¹³C₅]-PteGlu, [¹³C₅]-H₄folate, [¹³C₅]-5-CH₃-H₄folate, [¹³C₅]-5-CHO-H₄folate, and [¹³C₅]-10-CHO-PteGlu were added in equal amounts of the expected concentration of the unlabeled analytes in the sample. Following 15 min equilibration time and boiling for 10 min, 900 mL chicken pancreas solution and 400 mL rat serum were added for deconjugation. After overnight incubation at 37°C, the samples were boiled for 10 min and 10 mL acetonitrile was added to the cooled sample. The supernatant of each sample was used for purification by solid phase extraction (SPE), filtered and measured by LC-MS/MS. All given results are based on dry biomass.

2.11.2 Instrumentation

The LC-MS/MS analysis was carried out as described in a previous publication with slight modifications (Striegel et al., 2018). The quantification was performed on a Shimadzu Nexera

X2 UHPLC system (Shimadzu, Kyoto, Japan) with a Raptor ARC 18 column (2.7 µm, 100 × 2.1 mm, Restek, Bad Homburg, Germany) and a Raptor ARC-18 precolumn (2.7 µm, 5 × 2.1 mm, Restek) as a stationary phase held at 30°C. The mobile phase for the binary gradient consisted of (A) distilled water with 0.1% (v/v) formic acid and (B) acetonitrile with 0.1% (v/v) formic acid at a flow rate of 0.4 mL/min. The LC system was coupled with a triple quadrupole mass spectrometer (LCMS-8050, Shimadzu, Kyoto, Japan), operated in positive ESI mode.

2.12 Metal ion analysis

For the measurement of metal content in algal biomass, between 0.5 g and 1 g of dry algal biomass were incubated in 10 mL of demineralized water under constant shaking. Afterwards, the samples were centrifuged at 10,000 rcf for 10 min and the supernatant was analyzed. ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) (Agilent 725 Series ICP Optical Emission Spectrometer, Agilent Technologies Inc., United States) was used to determine the metal content in the investigated solutions. As a calibration standard, a Certipur® ICP multi-element standard solution IV from Merck (Merck KGaA, Darmstadt, Germany) comprising 23 elements was used. The ICP Expert II Agilent 725-ES Instrument Software Version 2.0 (Agilent Technologies Inc., United States) was used to analyze the data.

2.13 Sensory analysis

2.13.1 General conditions and panel training

Sensory analysis was carried out by fifteen trained panelists (ten women and five men, age 21–45 years) from the Chair of Food Chemistry and Molecular Sensory Science at the Technical University of Munich using a quantitative descriptive analysis. Panelists had no history of known disorders and were trained weekly for a minimum of at least 2 years to be able to distinguish precisely between different aroma and taste qualities (Utz et al., 2022). Before the quantitative descriptive analyses (QDA) began, the panelists agreed upon a consistent panel language according to Stone and Sidel and available sensory lexicons (de Godoy et al., 2020; Stone et al., 2012; Stone et al., 2004). Thus, for orthonasal aroma evaluation, the following attributes including their reference compounds in brackets (20 mL; 100-fold odor thresholds in water) were chosen: metallic (Iron (II) sulfate), green (hexanal), hay-like (3-methyl-2,4-nonanedione), malty (3-methylbutanal), earthy (2,3,5-trimethyl-pyrazine), citrus-like (octanal), and sea breeze-like (without a reference substance) (Fischer and Schieberle, 2009; Kreissl et al., 2022; Mall and Schieberle, 2017). For taste evaluation, the panelists continuously wore nose clips to avoid cross-modal interactions with odorants. For training, aqueous solutions of sucrose for sweet taste, lactic acid for sour taste, monosodium glutamate for umami taste, sodium chloride for salty taste, caffeine for bitter taste, and tannic acid for astringency were used according to earlier investigations (Utz et al., 2021). For kokumi sensation, a reduced glutathione solution in model broth was utilized, respectively (Brehm et al., 2019). Before and between each test, the panelists rinsed their mouth

TABLE 1 Overview of tested *Chlorella* powders showing species, cultivation method, downstream processing and supplier according to the supplier's information.

Name	Species	Cultivation	Downstream	Supplier
A	<i>Chlorella vulgaris</i> (green)	Autotrophic, tubular photobioreactor, Germany	Spray dried	Algenfarm Klötze GmbH and Co. KG
B	<i>Chlorella sorokiniana</i> (white)	Heterotrophic, stainless steel bioreactor, Netherlands	Washed, spray dried	Aliga microalgae – Aliga ApS
C	<i>Chlorella sorokiniana</i> (white)	Heterotrophic, stainless steel bioreactor, Netherlands	Washed, high-pressure homogenized, spray dried	Aliga microalgae – Aliga ApS
D	<i>Chlorella sorokiniana</i> (white)	Heterotrophic, stainless steel bioreactor, Netherlands	Spray dried	Aliga microalgae – Aliga ApS
E	<i>Chlorella sorokiniana</i> (green)	Heterotrophic, stainless steel bioreactor, Netherlands	Spray dried	Aliga microalgae – Aliga ApS
F	<i>Chlorella pyrenoidosa</i> (green)	Autotrophic, freshwater pool, People's Republic of China	Mechanical cell disruption, spray dried	BIONUTRA - BTG Berlin Trade GmbH
G	<i>Chlorella vulgaris</i> (yellow)	Heterotrophic, stainless steel bioreactor, Portugal	Spray dried	ALLMICROALGAE - Natural Products, SA (Allma)
H	<i>Chlorella vulgaris</i> (white)	Heterotrophic, stainless steel bioreactor, Portugal	Spray dried	ALLMICROALGAE - Natural Products, SA (Allma)

for about 20 s with Evian water (Évian-les-Bains, France). All sensory data was collected in special sensory cabins with air-conditioning at 20–25°C.

2.13.2 Flavor profile analysis (FPA)

According to [Caul \(1957\)](#) a flavor profile analysis was performed ([Caul, 1957](#)). Thereby, each FPA of different *Chlorella* powders was divided into orthonasal aroma and taste experiments. 100 g/L *Chlorella* powder was suspended in Evian water (pH 5.5) and presented in closed sensory vials (45 mL) to the panelists, who evaluated the described aroma and taste qualities from 0 (not detectable) to 5 (very intense). Principal Component Analysis (PCA) was performed on the average scaled sensory data using the PCA() function from FactoMineR in R (R Core Team, Vienna, Austria). The first two principal components were visualized in a combined plot using the ggplot2 and ggrepelR packages for both individuals and variables.

2.14 Statistical analysis

Differences in biomass composition were statistically evaluated with a one-way ANOVA followed by a *post hoc* analysis according to Scheffé. A level of $p \leq 0.05$ was considered significant. Calculations were performed with Microsoft Excel® (365 MSO, Version 2501, Microsoft Corp., Redmond, United States).

3 Results

3.1 Pigment analysis

The qualitative HPLC-based pigment analysis showed signals for chlorophylls (e.g., chlorophyll A and B) as well as lutein in all green *Chlorella* powders (A, E, F). In addition, β -carotene was

detected in samples E and F, while neoxanthin was just present in sample E. The yellow *Chlorella* powder G showed a signal for lutein as dominant pigment, but zeaxanthin was also detected for this sample. For the white *Chlorella* powders there were no pigments detectable at all ([Supplementary Figure S2](#)).

3.2 Microbiological load

The photoautotrophically generated sample A (Algomed, *C. vulgaris* (green)) and F (Bionutra, *Chlorella pyrenoidosa* (green)), which are cultivated in closed photobioreactors or open ponds, had elevated counts of aerobic mesophilic microorganisms, aerobe mesophilic spores, anaerobe mesophilic spores, and *B. cereus* ([Table 2](#)). However, food is considered potentially hazardous only if levels emetic toxin producing *B. cereus* strain(s) exceed 10^5 cfu/g in foods ([Authority, 2005](#)). Furthermore, as all samples exhibited a water activity (a_w) below 0.4 sporulation and growth of all spores in the samples is unlikely. All tested *Chlorella* powders fulfilled the requirements for food applications in microbiological contaminations (EFSA).

3.3 Biomass composition

Protein content was calculated with Kjeldahl measurements using a conversion factor of 4.78 and was overall lower for the chlorophyll-deficient strains B-D and G-H ([Sägesser et al., 2023](#)). The carbohydrate content, determined by HPLC analysis after chemical hydrolysis to convert carbohydrates like cell wall components and starch to monomeric sugars, was higher for all heterotrophic cultivations (B-E, G-H) independent of the chlorophyll content. The lipid quantification according to FAME analysis resulted in lipid contents lower than 10% for all samples, with slightly lower results for the *Chlorella*

TABLE 2 Microbiological profiles of the tested *Chlorella* powders; A: Algomed, *Chlorella vulgaris* (green), phototrophic; B: Aliga, *Chlorella sorokiniana* (white), heterotrophic, washed, HPH; C: Aliga, *Chlorella sorokiniana* (white), heterotrophic, washed; D: Aliga, *Chlorella sorokiniana* (white), heterotrophic; E: Aliga, *Chlorella sorokiniana* (green), heterotrophic; F: Bionutra, *Chlorella pyrenoidosa* (green), phototrophic; G: Allma, *Chlorella vulgaris* (yellow), heterotrophic; H: Allma, *Chlorella vulgaris* (white), heterotrophic. All experiments were carried out in biological duplicates.

Product	Aerobe mesophilic microorganisms cfu/g	Aerobe mesophilic spores cfu/g	Anaerobe mesophilic spores cfu/g	<i>Bacillus cereus</i> Cf/g	Enterobacteriaceae Cf/g	Yeasts (Y)/ Molds (M) Cf/g
A	8.0×10^6	3.7×10^5	1.6×10^5	3.4×10^3	<10	<100
B	2.0×10^3	1.3×10^2	10	4.0×10^2	<10	<100
C	<1,000	<10	<10	1.0×10^2	<10	<100
D	<1,000	30	<10	6.0×10^2	<10	<100
E	<1,000	<10	<10	<100	<10	<100
F	3.5×10^4	2.0×10^3	2.5×10^2	1.3×10^3	<10	50 (M)
G	<1,000	50	15	<100	<10	<100
H	<1,000	30	<10	<100	<10	<100
Method	ISO 4833	Kyrylenko et al. (2023)	Hu et al. (2021)	ISO 7932	ISO 21528	ISO 7954

sorokiniana samples (B-D) (Table 3). In addition, the ash content was in a comparable range for all samples without washing step in the downstream processing (A, D-H) and lower with washing step (B, C). Proteins, carbohydrates, lipids, ash, and moisture did not sum up to 100 g, the difference (A: 23.7, B: 17.5, C: 7.0, D: 9.9, E: 2.7, F: 20.8, G: 15.4, H: 19.6) is mostly referred as fiber content.

3.3.1 Amino acid profile

Independent of the species, cultivation, or downstream processing all *Chlorella* powders had a comparable amino acid profile containing all essential amino acids except L-methionine and L-tryptophan. The highest fluctuation between samples was found in L-arginine ranging from 2.1% to 10.4% of the total amino acids. Also, the E/N-ratio ranging from 0.65 to 0.89 (A: 0.89, B: 0.76, C: 0.68, D: 0.69, E: 0.65, F: 0.80, G: 0.75, H: 0.75) is comparable over all tested samples (Figure 1A; Supplementary Table S1).

3.3.2 Carbohydrate composition

The sugar profile of the tested *Chlorella* powders mainly consists of glucose, rhamnose and a mixed peak of fructose, xylose, galactose, and mannose, representing the monomers of cellulose, pectin, and hemicellulose. While there is no significant difference in the sugar profile between the different species, heterotrophic cultivation resulted in a higher amount of glucose (A, F) compared to the photoautotrophically cultivated biomass (Figure 1B; Supplementary Table S2).

3.3.3 Fatty acid composition

In contrast to the amino acid profile, the fatty acid profile showed more pronounced differences between the products. While the amount of C16:0 is comparable for most samples, sample B showed a twofold higher C16:0 content. In general, the green powders (A, E, F) contained more C18:3 compared to the chlorophyll deficient strains (Figure 1C; Supplementary Table S3).

3.3.4 Vitamins C, D, and E

The photoautotrophic samples (A, F) showed a higher vitamin E content, but a lower vitamin C content compared to the heterotrophic samples (B-E, G-H). Among the *Chlorella sorokiniana* (B-E) strains, vitamin E was determined for the chlorophyll-containing sample E in the highest amount and at low quantities for the white *Chlorella* powders (B-D). In addition, the mechanical cell disruption using high-pressure homogenization led to an increase of detectable vitamin D and reduced vitamin C (samples B compared to C and D) (Figure 2A; Supplementary Table S4).

3.3.5 Folates

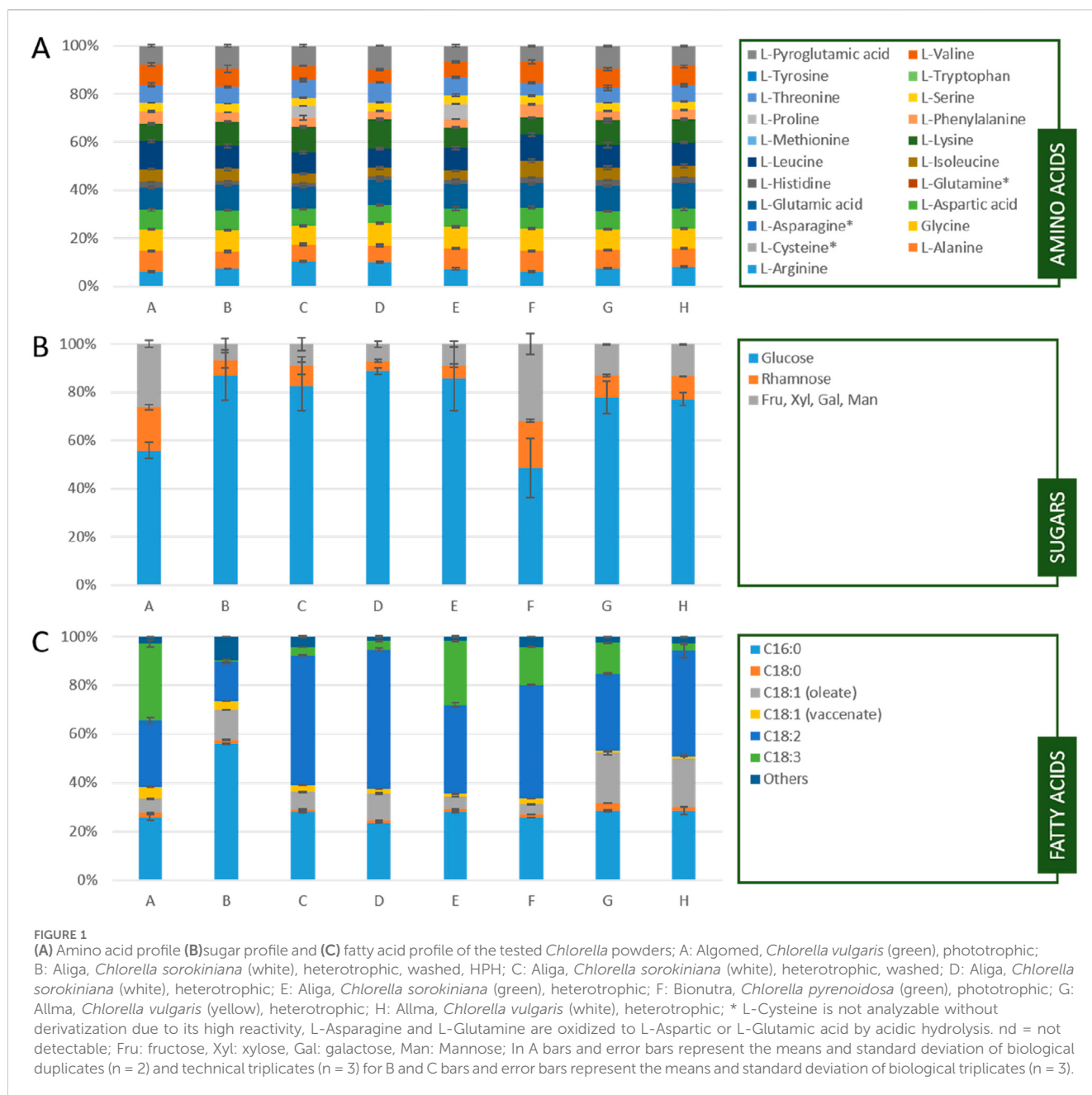
Folates were present in all tested samples, but the amount and proportion of the vitamers was different. While the folate content was higher for the *Chlorella vulgaris* strains (A, G, H) as well as *Chlorella pyrenoidosa* (F), it was much lower for the *C. sorokiniana* strains (B-E) (Figure 2B; Supplementary Table S5).

3.3.6 Metals

Potassium was the most prominent metal ion found in all samples. The samples from Aliga (B-E) also showed a significant amount of sodium in the powder, which is most probably a residue from the cultivation medium. Magnesium was present in all samples, while calcium was just found in traces in the Aliga samples (B-E). Washing, or washing combined with mechanical cell disruption led to a decrease in metal ion content. (B, C compared to D) (Figure 2C; Supplementary Table S6).

3.4 Sensory analysis

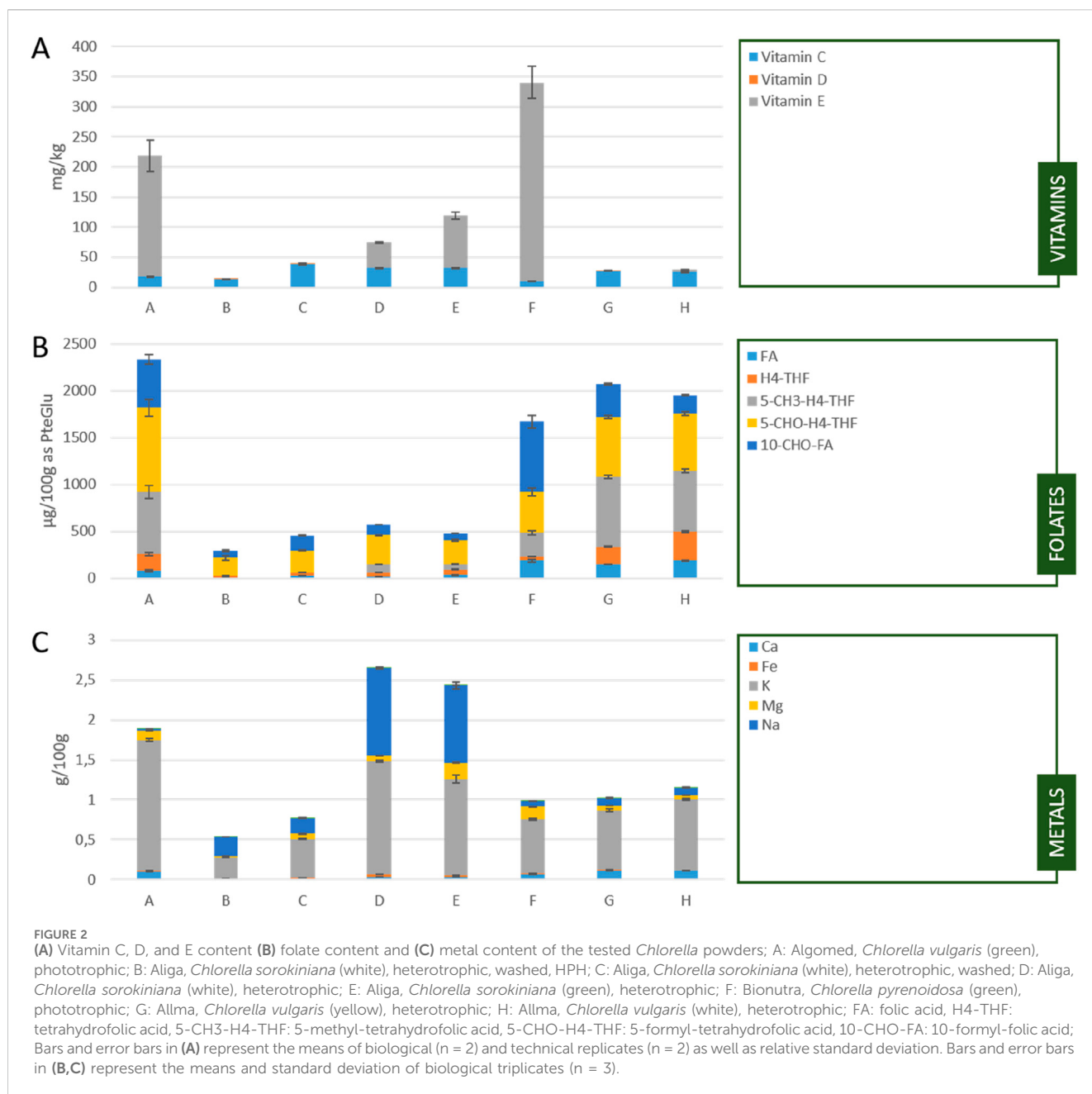
The quantitative descriptive analysis of the orthonasal aroma evaluation of the microalgae samples revealed similar aroma profiles among the different products (Figure 3A). For all samples except B-E (Aliga), hay-like was the most dominant aroma. Sample A (Algomed) exhibited the highest intensity of the green and hay-like aromas, scoring



2.8 and 3.2, respectively, suggesting a pronounced grassy and straw-like note. In contrast, sample D (Aliga) had the lowest green aroma score (1.1). Samples G and H both received similar scores in most aroma categories, suggesting a comparable sensory profile between the two Allma samples. Regarding other aroma attributes, sample D (Aliga) stood out with the highest malty aroma score of 2.1. Allma samples (G, H) received lower scores for earthy aroma than the other samples. In general, all the samples were attributed with lower and similar values for metallic, citrus-like, and sea breeze-like aromas. The prevailing scents in photoautotrophic samples (A, F) were green, hay-like, and earthy, whereas in heterotrophic samples, especially Allma (G, H), the dominant aromas were hay-like and malty. Overall, only smaller differences were found between the samples. The biggest distinction was observed for the green and hay-like aromas. The sensory analysis of taste attributes among the different microalgae samples

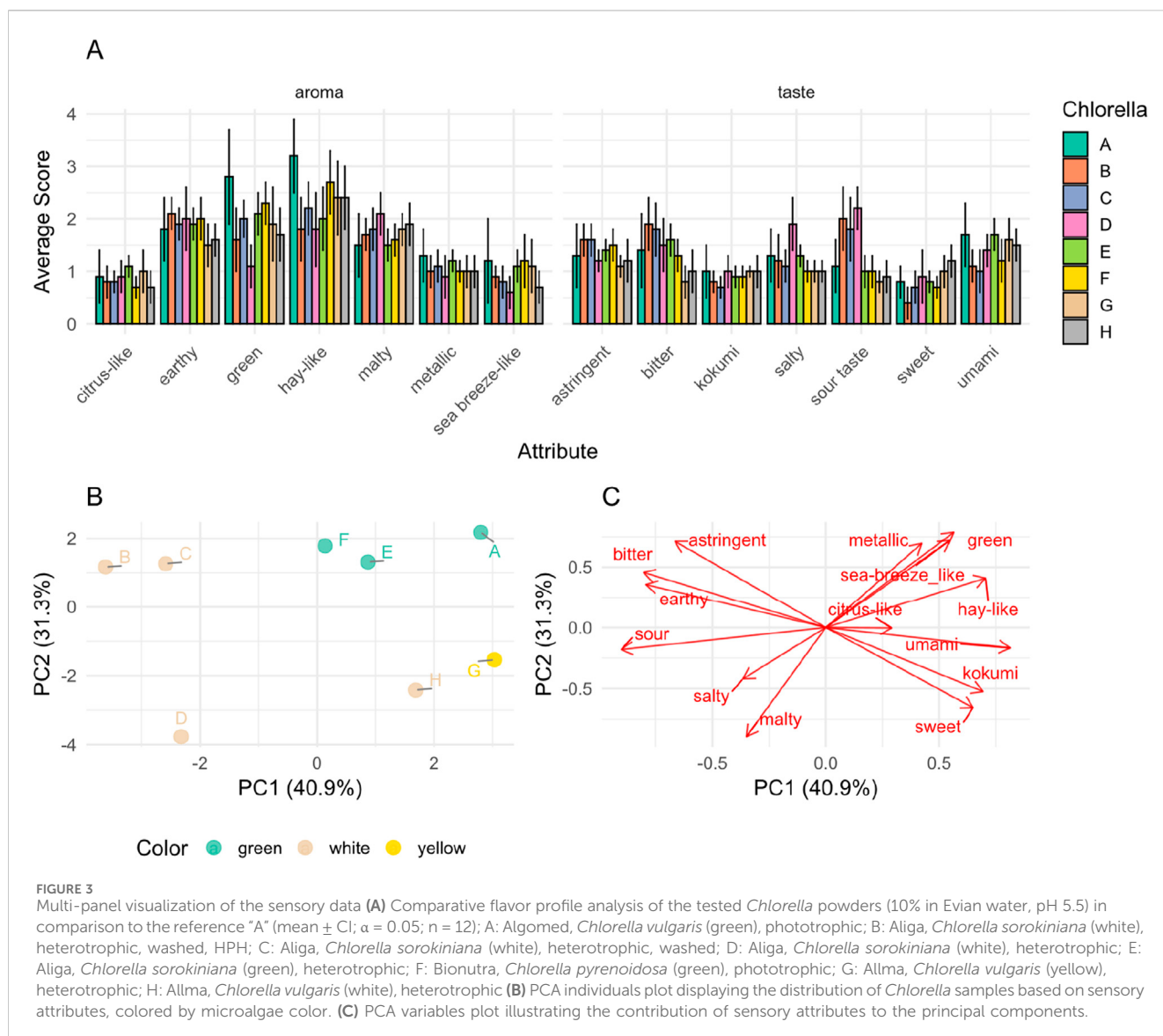
revealed some differences (Figure 3). In terms of sweetness, sample B (Aliga) exhibited the lowest score among the samples, the other ones being in a similar range as for sourness, samples B to D exhibited the most pronounced characteristics compared to the others. Concerning saltiness, sample D displayed notably higher levels than the other samples, which presented similar scores. Sample G exhibited the lowest score for bitterness. For umami, astringency, and kokumi, all the samples presented similar scores. Umami also was the dominant taste in both Allma samples (G, H), which were also characterized by reduced bitterness.

The principal component analysis (PCA) of sensory data revealed further clustering patterns among the *Chlorella* powders in the multivariate space, which are partially connected to cultivation location (along with supplier) and color (Figure 3B). The first two principal components explained 40.9% (PC1) and 31.3% (PC2) of the



variance, suggesting differences in sensory perception across the samples. An influence of sample color (green, white, yellow) was observed in PCA clustering (Figure 3B). Green-colored samples (A, E, F) clustered in the positive PC1/PC2 region, suggesting a shared sensory profile which is associated with the sensory variables green, hay-like, and sea-breeze-like attributes (Figure 3C). In contrast, some white samples (B, C, D) are positioned in the negative PC1 region and associated with sour, earthy, and bitter notes (Figures 3B,C). The yellow sample (G) and the white sample H positioned towards the positive PC1 and negative PC2 regions were associated with sweet, umami, and kokumi flavors (Figures 3B,C). These two samples cluster differently from white samples B, C and D and from green samples F, E, and A. This difference suggests that other variables may be playing a role in addition to color. In this case, samples G and H are produced by the same supplier in the same geographical region and with the same

processing conditions. Samples cultivated under autotrophic conditions (A, F) and heterotrophically cultivated samples (B, C, D, E, G, H) exhibited more significant variability along PC1 and PC2. This suggests that cultivation conditions and species differences do not seem to contribute to sensory characteristics uniformly. Among heterotrophic samples, *Chlorella sorokiniana* (B, C, D) formed a subgroup from *Chlorella vulgaris* (G, H). However, *C. sorokiniana* sample E and *C. vulgaris* sample A seem to follow the opposite pattern. Further examination of downstream processing methods indicated that high-pressure homogenization does not seem to explain variance. Sample C resulted in a sensory profile like sample B (same supplier, same color, but no high-pressure homogenization was applied), distinct from other samples. These two samples correlate with bitter, astringent, and earthy notes, which other variables may explain in addition to those presented in Table 1.



4 Discussion

4.1 Pigment analysis

The analysis of photosynthetic pigments gave results that correlated well with the color of the respective products. In accordance to the literature dominant pigments in all green samples were chlorophyll A and B followed by one of the most prominent carotenoids in *Chlorophyceae* lutein, while in the yellow sample chlorophylls were lacking and lutein was the main remaining pigment (Batista et al., 2013; Khairunnisa et al., 2024). Moreover, all white products did not show any photosynthetic pigments correlating with the sample color.

4.2 Microbiological load

While all samples had microbial loads below the food grade certification limits, there were significant differences between the

autotrophic and heterotrophically cultivated samples. Generally, the autotrophic samples had a higher microbial load than the heterotrophically grown *Chlorella* powders, as latter were generated by precision fermentation using axenic starter cultures in sterilized stainless steel bioreactors. The detected microbial load will most likely be due to the post-harvesting processes that are not conducted under sterile conditions. In this context, washing steps tend to reduce the microbial load, while additional mechanical processing, such as high-pressure homogenization tends to increase microbial loads. The latter may be due to remaining biomass between processing batches, which adds to carry over in the following processing steps. By contrast, in the autotrophic systems the algae cultures contain a symbiotic microbial flora, which is propagated together with the algae biomass in the photobioreactor or open ponds (Almalki et al., 2024). In this context, the autotrophically generated products can be compared to conventional agricultural food products, which all contain a microbial accompanying flora. Biomass processing can significantly impact microbial contamination levels and hence product quality and shelf life.

TABLE 3 Protein, carbohydrate, lipid, ash and moisture content of the tested *Chlorella* powders; A: Algomed, *Chlorella vulgaris* (green), phototrophic; B: Aliga, *Chlorella sorokiniana* (white), heterotrophic, washed, HPH; C: Aliga, *Chlorella sorokiniana* (white), heterotrophic, washed; D: Aliga, *Chlorella sorokiniana* (white), heterotrophic; E: Aliga, *Chlorella sorokiniana* (green), heterotrophic; F: Bionutra, *Chlorella pyrenoidosa* (green), phototrophic; G: Allma, *Chlorella vulgaris* (yellow), heterotrophic; H: Allma, *Chlorella vulgaris* (white), heterotrophic; Numbers represent the means and standard deviation of biological triplicates ($n = 3$). Statistically significant differences in biomass composition were determined in a one-way ANOVA ($p \leq 0.05$), followed a Scheffé *post hoc* test. Different superscript letters (a–f) in the one row correspond to significant differences ($p < 0.05$). For detailed significant differences see [Supplementary Tables S7, S13](#).

Biomass composition [g/100g]	A	B	C	D	E	F	G	H
Proteins	40.1 ^a	16.8 ^b	21.7 ^c	30.2 ^d	43.1 ^e	44.1 ^e	27.2 ^f	27.1 ^f
	±0.3	±0.1	±0.1	±1.1	±0.1	±0.2	±0.3	±0.1
Carbohydrates	18.6 ^a	52.8 ^{bc}	57.7 ^b	43.7 ^{cd}	39.6 ^d	15.3 ^a	38.7 ^d	37.8 ^d
	±0.4	±1.4	±8.7	±1.9	±2.4	±0.5	±1.2	±0.4
Lipids	6.0 ^a	1.8 ^b	2.6 ^c	2.4 ^{bc}	2.8 ^c	7.8 ^d	7.2 ^d	5.7 ^a
	±0.3	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.4
Ash	6.9 ^a	2.6 ^b	3.8 ^c	6.4 ^d	6.3 ^d	6.5 ^d	6.0 ^e	5.1 ^f
	±0.0	±0.1	±0.0	±0.0	±0.0	±0.1	±0.2	±0.1
Moisture	4.7 ^a	8.5 ^b	7.2 ^c	7.4 ^c	5.5 ^d	5.5 ^d	5.5 ^d	4.7 ^a
	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1

4.3 Biomass composition

The determined biomass compositions align with the information provided by the suppliers as well as the ranges reported in the literature. The deviations between the tested samples in proteins, carbohydrates, and lipids might be explained by different batches of biomass and different biomass cultivation and processing methods. Specifically, the composition of *Chlorella* biomass may vary significantly even within the same strain depending on the cultivation conditions, including light intensity, temperature, pH value, nutrient availability, and salinity (Kolackova et al., 2023; Muys et al., 2019). In addition to the cultivation parameters, the downstream processing can affect biomass composition depending on parameters like temperature, drying time, light, oxidation, and drying load (Hosseinzand et al., 2018; Silva et al., 2021; Vilatte et al., 2023). The detailed individual process parameters used for the biomass processing were not available.

The protein content was calculated from the nitrogen content determined by the Kjeldahl method using a nitrogen-to-protein conversion factor (k_p) of 4.78. This is a more conservative conversion factor compared to other reports, resulting in the calculation of lower protein contents (Sägesser et al., 2023). The Kjeldahl method is a general quantification method for the determination of organic and inorganic nitrogen including non-protein components, such as photosynthetic pigments (i.e., chlorophyll), cell wall components (i.e., glucosamine) as well as nitrogen-containing media components used for algae cultivation. This non-protein related nitrogen has to be considered when defining a conversion factor, next to the amino acid composition of the contained protein, otherwise protein content may be overestimated. For microalgal biomass different factors ranging from 3.59 to 6.35 are used, (López et al., 2010; Safi et al., 2013). The Kjeldahl method is the commonly used method for raw protein quantification in food and feed, but for a better comparison between different types of biomasses, especially between chlorophyll containing and deficient strains, individual

nitrogen-to-protein conversion factors for each sample are needed for accurate protein quantification. In addition, another *Chlorella vulgaris* powder from Allma showed a higher protein content calculated with a conversion factor of 6.25 but with a high *in-vitro* digestibility of the biomass including the protein fraction (Niccolai et al., 2019).

The amino acid profile is quite comparable between all tested samples independent of strain, cultivation condition or downstream processing. *Chlorella* generally has more leucine, lysine, and valine compared to meat like beef, mutton or pork. Reportedly, animal proteins tend to have higher levels of methionine and cysteine as well as glycine. Compared to soybean and egg, *Chlorella* contains lower levels of glutamic acid, lysine and Isoleucine. Overall, the data highlights the nutritional value of *Chlorella* sp. as a valuable protein source. Its amino acid profile makes it a promising candidate for supplementing human diets, particularly for individuals seeking plant-based protein alternatives (Table 4).

The carbohydrate content as well as the sugar profile of the tested *Chlorella* powders was comparable between the photoautotrophic (A, F) and the heterotrophic samples. In general, the heterotrophic samples showed a higher carbohydrate content and a higher share of glucose. The species, detailed cultivation conditions, and downstream processing seem to have a minor effect on the carbohydrate content or sugar profile. *C. sorokiniana* and *C. vulgaris* belong to the glucosamine group of the *Chlorella* sp., which means that the rigid cell is mostly composed of glucosamine together with glucose, mannose, rhamnose, and galactose (Takeda, 1988; Takeda, 1991). Therefore, the mixed peak of fructose, xylose, mannose, and galactose should be mainly composed of mannose and galactose. The higher share of glucose in the heterotrophic samples might also be due to the chemical hydrolysis of intracellular starch used as carbon storage in *Chlorella* (Takeshita et al., 2014). In addition, there are different methods to determine the carbohydrate and/or fiber content in food. While in some cases carbohydrates represent the residual biomass after determination on the other macronutrients, in this study

TABLE 4 Amino acid composition in *Chlorella vulgaris*, meat samples and legumes. Ala, L-alanine; Gly, glycine; Asp, L-aspartic acid; Glu, L-glutamic acid; His, L-histidine; Ile, L-isoleucine; Leu, L-leucine; Lys, L-lysine; Phe, L-phenylalanine; Pro, L-proline; Ser, L-serine; Thr, L-threonine; Val, L-valine; Pyr, L-pyroglutamic acid; Met, methionine; Tyr, tyrosine; Trp, tryptophan, Cys, cysteine. References: A: average of all *Chlorella* powders tested in this study; B: (Koyande et al., 2019); C: (Jorfi et al., 2012); D: (Joint, 1977); E (Becker, 2007).

Amino acids	<i>Chlorella</i> powders	<i>C. vulgaris</i>	Beef	Mutton	Chevon	Pork	FAO/WHO	Egg	Soybean
Ile	4.8%	3.9%	3.5%	3.1%	3.6%	3.4%	4.0%	6.6%	5.3%
Leu	9.7%	9.1%	7.1%	5.8%	7.1%	7.3%	7.0%	8.8%	7.7%
Val	7.3%	5.7%	4.3%	5.6%	4.5%	4.1%	5.0%	7.2%	5.3%
Lys	9.5%	8.7%	8.1%	8.4%	8.4%	8.2%	5.5%	5.3%	6.4%
Phe + Tyr	4.1%	8.7%	5.2%	5.4%	5.7%	5.4%	6.0%	10.0%	8.7%
Met + Cys	—	3.7%	4.3%	4.8%	5.0%	4.8%	3.5%	5.5%	3.2%
Trp	—	2.2%	—	—	—	—	1.0%	1.7%	1.4%
Thr	7.0%	4.9%	3.0%	2.9%	3.4%	2.8%	—	5.0%	4.0%
Ala	7.7%	8.1%	6.9%	6.5%	6.6%	6.7%	—	—	5.0%
Arg	7.8%	6.6%	8.7%	10.0%	8.4%	8.5%	—	6.2%	7.4%
Asp	7.9%	9.3%	2.8%	2.9%	3.0%	2.9%	—	11.0%	1.3%
Glu	10.1%	12.0%	7.8%	7.7%	8.3%	7.7%	—	12.6%	19.0%
Gly	8.8%	6.0%	20.2%	18.0%	18.5%	18.9%	—	4.2%	4.5%
His	2.0%	2.1%	5.9%	6.2%	5.3%	6.7%	—	2.4%	2.6%
Pro	5.6%	4.9%	8.2%	8.7%	8.5%	8.2%	—	4.2%	5.3%
Ser	3.5%	4.2%	4.1%	4.2%	4.2%	4.2%	—	6.9%	5.8%
Reference	A	B	C	C	C	C	D	E	E

carbohydrates like starch and most cell wall components were chemically hydrolyzed and quantified by HPLC (Grácio et al., 2024). The residual biomass after determination of the macronutrients including carbohydrates is commonly considered as fiber content. Depending of the methods used for quantification of total dietary fibers there is a wide range in literature covering the non-defined amount of the tested *Chlorella* powders between 7.0 and 23.7 (Gao et al., 2024; Niccolai et al., 2019; Nova et al., 2019)

The cultivation conditions play a crucial role in shaping the fatty acid profile of microalgae. For instance, using hetero- or photoautotrophic cultivation, nutrient availability, light intensity, temperature, as well as pH can affect the synthesis of specific fatty acids (Alkhamis et al., 2022; Bajwa et al., 2019; Cheirsilp et al., 2023; Gómez-De la Torre et al., 2023; Yun et al., 2020). Interestingly, the fatty acid profile of sample B was quite different compared to sample C and D, even though they are the same strain and only underwent different downstream processing methods. While the washing step did not affect the fatty acid profile significantly, the high-pressure homogenization step led to a decrease in unsaturated fatty acid and in total lipid content. This could be explained by strong mixing as well as an increase in temperature during the process leading to oxidation of unsaturated fatty acids. This effect was analyzed by Canelli et al. observing higher lipid oxidation with the mechanical cell disruption (Canelli et al., 2021).

The vitamin C, D and E content varies in the published range over the tested *Chlorella* powders (Bito et al., 2020; Sandgruber et al., 2023). While the content of vitamin C was in a comparable range for

all powders, the vitamin D content was quite low but higher for sample B with mechanical cell disruption. This increase might be caused by a better accessibility of the lipid soluble vitamin after cell disruption. On the other hand, this behavior was not detectable for sample F with mechanical cell disruption but other supplier, species, and cultivation. The highest vitamin E content was determined for the photoautotrophic samples, followed by the chlorophyll containing, heterotrophic sample (E). Interestingly, vitamin E was detected in the chlorophyll-deficient *Chlorella sorokiniana* but only without the washing step in downstream processing.

Notably, *Chlorella* has been recognized for its high folate levels (Bito et al., 2020; Edelman et al., 2019; Woortman et al., 2020). Folate content and distribution of the different oxidation levels are reported to be dependent on the cultivation conditions as well as downstream processing (Blancquaert et al., 2015; Fitzpatrick et al., 2012; Woortman et al., 2020). The results for the tested *Chlorella* powders indicate that the species seems to have the highest impact on the folate content and distribution. The three different species *C. vulgaris* (A, G, H), *C. sorokiniana* (B-E) and *Chlorella pyrenoidosa* (F) showed similar results within the group independent of the supplier, cultivation, downstream processing or chlorophyll deficiency. The folate content of *C. vulgaris* and *C. pyrenoidosa* was in the published range of *C. vulgaris* between 1,200 and 3,600 µg/100 g (Bito et al., 2020), *C. sorokiniana* showed significant lower levels between 267 and 530 µg/100 g. While the content of vitamin C, D, and E in the tested *Chlorella* powders was quite low, *Chlorella*, especially *C. vulgaris*, seems to be a suitable source of folates in human diet.

Microalgae are known to adsorb and bioaccumulate metals, due to negatively charged functional groups of their cell walls (Kumar et al., 2015; Paper et al., 2023; Sandgruber et al., 2023). The main source of metals in the *Chlorella* powder is most probably the media composition used for cultivation. Washing the cells in water or buffer has demonstrated the effective removal of salts from microalgae (Khaw et al., 2021; Zhu and Lee, 1997). This can be seen by the significant reduction in metal content for samples B and C compared with the unwashed sample D. This reduction in metals aligns with the ash content determined for the tested samples, representing the total mineral amount.

4.4 Sensory analysis

The sensory profile of microalgae can be affected by various compositional factors (Van Durme et al., 2013). For instance, the presence of sulfuric compounds correlates with typical seafood flavors (sea breeze like variables) while shorter chain aldehydes contribute to green, fruity, and vegetable flavors (green and hay like attributes). Food manufacturers may use these intrinsic attributes to incorporate microalgae into alternative protein food products as a nutritious and valuable ingredient (Coleman et al., 2022). Our results point to differences among the tested *Chlorella* powders; these differences must be taken into account by the food manufacturer when choosing the raw material ingredient to be integrated into the production process.

Amino acid composition, particularly the content of phenylalanine, can lead to distinctively higher levels of benzaldehyde, impacting the overall aroma. Furthermore, alanine, glycine, proline, serine, and threonine may provide a sweet taste, whereas histidine, allo-isoleucine, isoleucine, leucine, methionine, phenylalanine, tryptophan, and valine are related to a bitter taste; aspartic acid and glutamic acid are correlated to the umami taste (Zamuz et al., 2019). Additionally, the concentration of PUFAs plays a role, where species with higher PUFA concentrations tend to exhibit more linear aldehydes, due to enzymatic processes and fatty acid oxidation. The presence of carotenoids in microalgae can also lead to the production of ionones, well-known for their floral sensory properties (Van Durme et al., 2013). Few studies have explored the sensory traits of *C. vulgaris*. One study by Van Durme focused on evaluating the aroma of phototrophic *C. vulgaris* while another study by Isleten Hosoglu examined a heterotrophic grown *C. vulgaris* (Hosoglu, 2018; Van Durme et al., 2013). They agree that *Chlorella* has a rather bland flavor profile dominated by 'grassy, vegetable, cucumber' aromas (Hosoglu, 2018; Van Durme et al., 2013) with less fishy, shrimp and marine odors, making *Chlorella* less suitable for alternative seafood solutions (Francezon et al., 2021). Similar results were observed for the eight tested *Chlorella* powders in this study with a decrease in green and hay-like odor for the chlorophyll deficient strains (B-D, G, H) as well as heterotrophically cultivated green *Chlorella* to a smaller extent (E). While the sour taste was higher for all chlorophyll-deficient samples from Aliga (B-D), the salty flavor was reduced by washing in the downstream processing (D compared to B, C). Overall, from the dimensionality reduction of the sensory data, the color difference, alongside production location and supplier, seemed to emerge as relevant variables. Downstream processing, including drying, washing, and high-pressure homogenization, does not seem to

influence the sensory profile of the studied samples. This may be due to limited information on the detailed cultivation and processing steps adopted for the studied samples. Green *Chlorella* samples A, E, F which clustered together based on their sensory profile (Figure 3B) were also found to contain high levels of C18:3 fatty acids compared to the other samples (Figure 1C). This sample cluster is associated the most with green, hay-like sensory notes which are, attributes associated with oxidation of unsaturated fatty acids and consequent production of aldehydes and ketones in alternative proteins (Mittermeier-Kleßinger et al., 2021).

These results represent key information for food suppliers to select the most suitable *Chlorella* powder for the specific application. For instance, a vegan replacement for seafood may look at powders with a higher intensity of umami, kokumi, and sea breeze-like sensory notes while minimizing bitter and sour notes. A deeper mechanistic understanding of the variables influencing each descriptor must still be obtained to fine-tune the cultivation and production process. This mechanistic understanding cannot be based on commercial products but has to be studied and controlled from cultivation to storage and consumption (Zhou et al., 2017).

5 Summary

The eight different types of *Chlorella* powders covering different suppliers, strains, cultivation methods, and downstream processing showed a comparable biomass composition with slightly lower protein contents for the chlorophyll deficient strains, slightly higher sugar contents for the heterotrophic samples, and slightly lower lipid contents for *C. sorokiniana*. The amino acid profile was comparable between all tested samples, in the sugar profile the glucose concentration was higher for the heterotrophic samples and the highest deviation was shown in the fatty acid profile. The vitamin levels were in a similar range with a higher vitamin D content for sample B, a higher vitamin E content for the phototrophic samples A and F as well as a lower folate content for the *C. sorokiniana* strains (B-E). The presence of metals was assumed to be linked to the media formulation used for cultivation and was overall reduced by washing during downstream processing. The taste and odor profiles of the tested samples were comparable, with a reduced hay-like and green odor for the chlorophyll-deficient strains. In conclusion, the biomass composition showed mostly differences on a micronutrient level. The cellular capacity to do photosynthesis, such as the presence of chlorophyll, seemed to have the highest impact on taste and odor. Notably, the microbial loads of the different *Chlorella* product preparations varied significantly, which may be due to both cultivation and post-harvesting biomass processing methods. Heterotrophically produced biomass under sterile cultivation conditions had the lower loads. Biomass processing can significantly impact microbial contamination levels and hence product quality and shelf life, while all examined products were within EFSA specifications about microbial loads.

In summary, the comparative nutrient and sensory evaluation presented in this study will aid in selection of microalgae products for food development, fostering a broadening of applications in the food space. Our study could confirm the high nutritional density of algae biomass, with the high protein content being beneficial for generation of protein-rich food, including shakes, bakery products, and meat substitutes. While this study provides an overview of the

nutritional value and sensory perception of commercial *Chlorella* preparation, bioavailability of these nutrients for consumers was not assessed. In this context, human intervention studies to explore nutrient accessibility and effects are currently underway. As a first indicator, the balanced amino acid profile being independent from cultivation and processing conditions together with an abundance of vitamins (specifically vitamin C) may potentially aid digestibility and metabolic valorization of algae protein fractions (Said, 2023). Further studies with focus on isolation, concentration, and processing of algae protein fractions are ongoing in the Protein4Singapore consortium addressing urgent market needs and consumer demand.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#), further inquiries can be directed to the corresponding authors.

Author contributions

FM: Conceptualization, Formal Analysis, Methodology, Writing – review and editing, Data curation, Investigation, Visualization, Writing – original draft. TP: Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft, Validation, Writing – review and editing. DiG: Formal Analysis, Validation, Investigation, Methodology, Visualization, Writing – original draft, Data curation. FU: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. MP: Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review and editing. AS: Data curation, Formal analysis, Methodology, Validation, Visualization, Software, Writing – review and editing. LO: Data curation, Formal Analysis, Investigation, Methodology, Validation, Writing – original draft. JS: Data curation, Formal Analysis, Investigation, Methodology, Validation, Writing – original draft. MK: Data curation, Formal Analysis, Investigation, Methodology, Validation, Writing – original draft. DaG: Funding acquisition, Project administration, Resources, Writing – review and editing, Data curation, Methodology, Supervision. TN: Formal Analysis, Methodology, Project administration, Resources, Supervision, Validation, Writing – review and editing. TBe: Formal Analysis, Methodology, Project administration, Resources, Supervision, Validation, Writing – review and editing. MR: Formal Analysis, Methodology, Project administration, Resources, Supervision, Validation, Writing – review and editing. CD: Formal Analysis, Methodology, Project administration, Resources, Supervision, Validation, Writing – review and editing. WB: Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing. NS: Conceptualization, Formal Analysis, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review and editing, Data curation, Methodology. TBR:

Conceptualization, Formal Analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review and editing.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/frfst.2025.1534438/full#supplementary-material>

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