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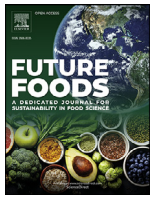

Potential of microalgae as flavoring agents for plant-based seafood alternatives

Bert Coleman[a,b,*], Christof Van Poucke[c], Bavo Dewitte[a], Ann Ruttens[f], Tanja Moerdijk-Poortvliet[d], Christos Latsos[d], Koen De Reu[c], Lander Blommaert[e], Barbara Duquenne[c], Klaas Timmermans[e], Jasper van Houcke[d], Koenraad Muylaert[b], Johan Robbens[a]

[a] *Aquatic Environment and Quality, Cell Blue Biotech and Food Integrity, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Ankerstraat 1, 8400 Ostend, Belgium*
[b] *Biology Department KULAK, KU Leuven Kulak, Etienne Sabbelaan 53, 8500 Kortrijk, Belgium*
[c] *Technology and Food Science Unit, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Brusselsesteenweg 370, 9090 Melle, Belgium*
[d] *HZ University of Applied Sciences, Department of Technology, Water and Environment, Research Group Marine Biobased Specialties, P.O. Box 364, 4380 AJ Vlissingen, The Netherlands*
[e] *Royal Netherlands Institute for Sea Research (NIOZ), Department of Estuarine and Delta Systems (EDS), P.O. Box 140, 4400 AC Yerseke, The Netherlands*
[f] *Sciensano, Rue Juliette Wytsmanstraat 17, 1050 Brussels, Belgium*

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ABSTRACT

The aroma and taste of eight different phototrophic microalgae species were investigated and compared with five seaweeds to evaluate their potential as flavor ingredients in plant-based seafood alternatives. To assess their performance, commercial seafood flavoring products were used as a reference during the sensory evaluation and their chemical odor-active and taste-active profiles were compared with those of the algae. Stronger seafood odor and taste were observed in microalgae *Rhodomonas salina, Tetraselmis chui* and *Phaeodactylum tricornutum* compared to seaweeds which could be explained by the presence of important seafood aroma compounds (dimethylsulfide, fatty acids-derived compounds and trimethylamine) and taste compounds (glutamic acid, alanine, arginine and 5′-ribonucleotides). *R. salina* has potential as a plant-based seafood flavoring because of its crab aroma. *P. tricornutum* possess a high umami taste and shellfish flavor, however, its bitterness could be undesirable. *T. chui* is less bitter and characterized by high umami and seafood (crab, fishy) flavor, however, it possesses a slightly higher grassy odor.

1. Introduction

Plant-based alternatives for meat and dairy products have received increased attention among consumers because of the raising awareness for animal welfare, intrinsic health benefits of plant-based food and ecological impact of consuming animal-based food (van Vliet et al., 2020). While numerous plant-based alternatives have entered the food market, the development of plant-based seafood products is still in its infancy (SPINS, 2020). Avoidance of seafood by consumers is based on sustainability (overfishing) as well as animal welfare concerns (in aquaculture) (FAO, 2020). A central issue in the development of plant-based seafood is mimicking the flavor of animal-based seafood products. Flavor is defined as the set of impressions perceived in the mouth and nose which includes aroma, taste (salty, sweet, sour, bitter and umami) and textural mouthfeel (Auvray and Spence, 2008). A first step in the development of plant-based seafood alternatives is understanding the key contributors that give rise to the seafood flavor.

The characteristic aroma of fish and shellfish is derived from a complex mixture of different odor-active volatile chemicals. A first class of typical odor-active volatiles in seafood are oxidation products of polyunsaturated fatty acids (PUFAs) which are formed by lipoxygenase activity or autoxidation (Josephson et al., 1991; Lindsay, 1990). These fatty acid-derived volatiles include aldehydes (e.g. hexanal, 4-heptenal, 2-octenal and 2,6-nonadienal), alcohols (e.g. 3,5-octadien-2-ol and 1-octen-3-ol) and ketones (e.g. 1-octen-3-one and 3,5-octadien-2-one). A second class of important contributors to seafood aroma, espe-

* Corresponding author at: Aquatic Environment and Quality, Cell Blue Biotech and Food Integrity, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Ankerstraat 1, 8400 Ostend, Belgium
E-mail address: bert.coleman@ilvo.vlaanderen.be (B. Coleman).

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cially to shellfish aroma, are sulfuric compounds such as dimethyl sulfide (DMS), dimethyl disulfide and methanethiol. These sulfuric compounds are derived from the degradation of sulfur-containing compounds such as dimethylsulfoniopropionate (DMSP), methionine and taurine (Varlet and Fernandez, 2010). The third class contributing to the seafood aroma involve nitrogen-containing compounds such as trimethylamine (TMA), which originate from the microbial reduction of the osmolyte trimethylamine oxide (TMAO) (Lindsay, 1990). In addition to these important volatiles, seafood is known for its umami taste. Umami is related to the presence of specific free amino acids (FAAs) glutamate (Glu) and aspartate (Asp), as well as the presence of salt and 5'-ribonucleotides such as inosine monophosphate (IMP), guanosine monophosphate (GMP) and adenosine monophosphate (AMP) (Fuke, 1994). Furthermore, FAAs glycine (Gly), alanine (Ala) and arginine (Arg) contribute to the sweet taste of seafood (Konosu et al., 1978; Finne, 1992).

Algae, both microalgae and seaweeds (macroalgae), are considered as a sustainable and healthy marine food source. Different species of seaweeds have been recently used as flavor ingredients in plant-based seafood alternatives (e.g. vegan shrimps, fish broth, caviar) for its natural flavor of the sea (Kazir and Livney, 2021). Indeed, many seaweeds contain odor-active volatiles that also occur in seafood, such as carbonyls, alcohols and sulfur compounds (Garicano Vilar et al., 2020). In addition, particular brown seaweeds from the Fucales and Laminariales orders are rich in free glutamate and aspartate, eliciting umami taste (Mouritsen et al., 2019). In contrast to seaweeds, relatively little is known about the flavor of microalgae, despite the fact that these have received ample attention in the past decade as a novel source of food. Some studies have investigated the addition of microalgae in food products such as bread, cookies, pasta, soup and yogurt and have reported fishy off-flavors, even at very low additions (0.25 - 2% w/w) (Batista et al., 2019; Nunes et al., 2020; Lafarga et al., 2019; Babuskin et al., 2014; Fradique et al., 2013; Robertson et al., 2016).

Whereas fishy flavors of microalgae may be unwanted in most food applications, they may be desirable for the imparting flavor in plant-based seafood alternatives. To use microalgae as a flavor ingredient, it is important that seafood aroma and taste compounds are present in sufficiently high concentrations and that off-flavors are limited. Phytochemical as well as ecological studies on microalgae have revealed the presence of volatile organic compounds (VOCs) that are shared with seafood aroma, such as fatty acid-derived aldehydes, ketones and alcohols (Pohnert and Boland, 2002; Fontana et al., 2007). Furthermore, when marine algae are damaged by grazers, DMSP is broken down emitting sulfuric volatiles compounds (Fredrickson and Strom, 2009). Other VOCs in microalgae such as carotenoids-derived compounds (e.g. ionones) have floral or fruity aromas which would probably be unwanted in plant-based seafood (Achyuthan et al., 2017). To our knowledge, no studies so far have explored the taste of microalgae. However, the high protein content (28-71% dry weight) of microalgae (Becker, 2007) could result in a high amount of free amino acids Glu and Asp, causing high umami taste.

The aim of this study was to assess the potential of phototrophic microalgae from different taxonomical origin to be used as flavor ingredients in plant-based seafood alternatives and to compare their performance with seaweeds. Therefore, eight species of microalgae and five species of seaweeds were evaluated for taste and aroma using a combination of sensory evaluation by a trained expert panel and chemical profiling. Commercial seafood flavoring products were used as taste and odor reference during the sensory evaluations. The chemical profiling included an analysis of the odor-active volatile compounds as well as taste-active chemicals (free amino acids, nucleotides and salts). Furthermore, these chemical profiles were compared with those of commercial seafood flavoring products to evaluate their potential in the development of plant-based seafood alternatives.

2. Materials and methods

2.1. Chemicals and reagents

Internal standards 2-methyl-3-heptanone (103128-5G) and methyl nonanoate (76368-1ML), and alkane standard solution C8-C20 (ANALYT&/04070-1ML) were used for aroma analysis, all purchased from Sigma-Aldrich. Methanol (CL00.1377.2500) was obtained from Chem-Lab Analytical bvba.

For free amino acid analysis, internal standards were purchased from Campro scientific: methionine-3,3,4,4-d4 (CS04-482_1380-250MG); histidine 13C615N3 (CS03-381_110); glutamine 13C5 (CS01-181_173); DL-lysine-1,2-C13 (CS01-183_540); aspartic acid 15Nd3 (CS01-185_154); L-aspargine-15N2 (CS01-185_154); L-alanine-2,3,3,3-d4 (CS01-182_128-1G). Both N-methyl-L-valine (90119) and homoarginine (H1007) were obtained from Merck Life Science bv. All amino acids standards were purchased from Merck Life Science bv: L-phenylalanine 78019; L-tyrosine T3754; L-leucine L8000; L-methionine M9625; L-isoleucine I2752; L-valine V0500; L-threonine T8625; L-serine 54500; L-alanine A7627; L-proline 81709; glycine G7126; L-glutamic acid G1251; aspartic acid A9256; L-cystine 30200; L-histidine H8000; L-arginine A8094; L-lysine L5501; glutamine G8540; asparagine A0884; tryptophan T0254.

5-sulfosalicyclic acid dehydrate (S2130-100G) was obtained from Sigma-Aldrich, ammonium formate (84884.180) and formic acid (1.00264.1000) from VWR international bv and acetonitrile (CL00.0194.2500) from Chem-Lab Analytical bvba. Cis-3-hexen-1-ol (H12900-10G) was obtained from Sigma-Aldrich and applied within sensory evaluation.

ICP-OES (Induced Coupled Plasma-Optical Emission Spectrometry) calibration standards for salt analysis were prepared from a 100 mg/L multi-element stock solution from Analytika (Prague, Czech Republic). Water used for making the dilutions was home produced doubly distilled water. Nitric acid (Suprapur, SpA 67–69%,) used in the salt analysis was purchased from Romil (Cambridge, UK).

2.2. Microalgae, seaweeds and seafood

Biomass of microalgae was obtained either as a frozen paste or freeze-dried power. *Tisochrysis lutea* (Haptophyta), *Skeletonema costatum* (Ochrophyta), *Tetraselmis chui* (Chlorophyta), *Phaeodactylum tricornutum* (Bacillariophyta) and *Nannochloropsis oceanica* (Ochrophyta) were obtained as a frozen paste (1kg) provided by Necton S.A. (Olhão, Portugal). The frozen paste was freeze-dried within 2 months of arrival. *Dunaliella salina* (Chlorophyta) was obtained from Monzón Biotech (Barcelona, Spain) and *Chlorella vulgaris* (Chlorophyta) from Bio-Life bvba (Isnes, Belgium) as a freeze-dried powder. Freeze-dried *Rhodomonas salina* (Cryptophyta), cultivated in an outdoor tubular photobioreactor, was obtained from the HZ University of Applied Sciences (Vlissingen, Netherlands). Freeze-dried microalgae were packaged in vacuum-sealed plastic-lined aluminum foil bags until analyses.

Biomass of seaweeds was obtained as dried thalli. *Palmaria palmata* (Rhodophyta), *Laminaria ochroleuca* (Phaeophyta) and *Undaria pinnatifida* (Phaeophyta) originated from the coast in Galicia (Spain) and were obtained from Porto-Muiños (Cerceda, Spain). *Ulva laetevirens/rigida* (Chlorophyta) cultivated in outdoor system tanks was acquired from NIOZ (Texel, Netherlands). *Saccharina latissima* (Phaeophyta) cultivated nearshore (Eastern Scheldt) was obtained from Zeewaar B.V. (Amsterdam, Netherlands).

Commercial seafood flavorings were obtained from Flandor Flavours International (Zulte, Belgium). The flavors were made from (frozen) seafood products without any additions of spices, vegetables or preservatives. The seafood flavorings tested included shrimp extract powder (*Pandalus borealis*), coalfish powder (*Pollachius virens* meat), codfish powder (*Gadus morhua* meat), lobster and lobster extract powder (*Homarus gammarus*), crab extract powder (*Cancer pagurus*) and mussel powder (*Mytilus edulis*).

2.3. Aroma analysis

Volatile organic compounds (VOCs) were determined using automated headspace solid-phase microextraction (HS-SPME) – gas chromatography-mass spectrometry (GC-MS) using a Gerstel MPS sampler coupled to an Agilent 7890A GC and 5975C inert XL mass spectrometer. Various extraction parameters, including extraction time (5, 10, 15, 20, 25, 30 min), extraction temperature (30, 40 and 60°C), sample concentration (1, 10, 50, 100 and 200 mg dried sample/mL), sample volume (1, 3, 6 and 12 ml) and SPME fiber (Polydimethylsiloxaan (PDMS) and Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS)) were studied carefully in order to obtain the highest sensitivity. The best extraction conditions were selected as follows: 30 min of extraction time, 40°C extraction temperature, 100 mg DW/mL and 12 ml sample volume using Supelco 50/30μm DVB/CAR/PDMS fiber.

To avoid photodegradation, 20ml amber colored headspace vials were filled with 1.2 g dried sample and 12 ml Millipore water (100 g/L). To improve reproducibility, samples were kept refrigerated until 10 minutes before the start of the incubation to avoid oxidation and microbial activity prior to extraction. Within these 10 minutes, the refrigerated samples were spiked with 10μL internal standard (IS) mixture of 8.16 ng/μL 2-methyl-3-heptanone and 87.5 ng/μL methyl nonanoate in HPLC-grade methanol and hermetically sealed. Next, the vial was incubated for 30 minutes at 40°C, followed by extraction at the same temperature using agitation. The loaded SPME fiber was desorbed in splitless mode (250°C, 3 min) and compounds were separated on a DB-5MS column (30m x 250 μm x 1 μm) using a helium flow rate of 1 mL/min. The oven temperature program was set as follows: start at 35°C, hold for 3 minutes, then raised to 220°C at a rate of 3.5°C/min. Mass spectra in the electron impact ionization (EI) mode were generated at 70 eV, and recording was performed in full scan mode (35 – 250 m/z).

To identify the aroma compounds, Unknown analysis of Masshunter (Agilent) was used. Identification of the aroma compounds was based on (1) spectral match, compared to the NIST and an in-house database, and (2) retention index (RI), compared to the aroma office (Gerstel) and an in-house database. RI calibration of the chromatogram was done using an alkane standard solution (C8-C20). Aroma compounds were identified if following conditions were met: (1) peak height is more than 10^3 (counts), (2) signal-to-noise ratio is higher than 3, (3) spectrum match probability higher than 75%, and (4) calculated experimental RI differs less than 15 from library RIs. Experimental Kovats RI, library RI and match factor information used for identification can be found in Supplementary Information (SI).

Semi-quantitative determination of the volatiles was done by spiking 10μL of the internal standard mix to each sample prior to HS-SPME-GC-MS. A stock solution was prepared at 8.16 ng/μL 2-methyl-3-heptanone and 87.5 ng/μL methyl nonanoate in methanol. Similar to Isleten Hosoglu (2018), the area of the chromatographic peak of each identified volatile was divided by the area corresponding to the internal standard 2-methyl-3-heptanone. Areas corresponding to identified esters were divided by the area of internal standard methyl nonanoate. To semi-quantitatively calculate the concentrations of each volatile, the obtained responses were multiplied with the concentration of internal standard in the samples (6.8μg/L for 2-methyl-3-heptanone and 72.9μg/L for methyl nonanoate), assuming that all of the response factors were equal to one. Subsequently, these concentrations were divided by their individual odor threshold value (OTV) determined in water to calculate the odor activity values (OAVs) of each volatile. Since both concentration in the sample and odor threshold are taken into account, OAVs provide more insight on which compounds play an role in the flavor. The used odor threshold value (OTV) were obtained from literature and can be found in Supplementary Information (SI). This approach for calculating semi-quantitative results is comparable to the approach described by Giri et al. (2010). Three replicate analyses were performed on each sample analyzed on separate days. A blank sample with 12mL Millipore water was used to subtract background volatiles from the lab.

2.4. Free Amino acid analysis

Free amino acids (FAAs) were extracted based on Mæhre et al. (2014), by dissolving 0.2g dried sample in 8.5 mL UHPLC-MS water using a vortex for 15s. Prior the extraction, 0.5ml of the internal standard mixture was added containing 134.9 μg/ml methionine-3,3,4,4-d4; 34.1 μg/ml N-methyl-L-valine; 146.3 μg/ml histidine 13C615N3; 282.7 μg/ml glutamine 13C5; 1259.2 μg/ml L-glutamic acid C13; 294.4μg/ml DL-Lysine-1,2-C13; 435.7 μg/ml aspartic acid 15Nd3; 634.1 μg/ml L-aspargine-15N2; 316.8 μg/ml L-alanine-2,3,3,3-d4 and 430.9 μg/ml homoarginine in UHPLC-MS water. FAAs were separated from proteins and peptides by precipitation using 1 mL 35% sulfosalicylic acid. After 15 min of extraction at room temperature, samples were centrifuged (4000g for 10 min) and aliquots of 500 μL of the supernatants were diluted with 100μL acidified (1%v/v formic acid) ammonium formate buffer (4M) and 400μL acetonitrile (ACN). Amino acids were separated according to van 't Land (2019) by hydrophilic interaction chromatography (HILIC) using a LC-MS system. 3 μL of aqueous extract was injected onto an Intrada HILIC column (100mm x 3mm; 3μm) maintained at 37°C. A binary gradient at a flow rate of 0.6 mL/min was used, consisting of acidified (0.3%v/v formic acid) ACN and 80/20 (ACN (≥ 99.95%)/ammonium formate (≥ 99%)). Amino acids were detected using a Shimadzu triple quadrupole mass spectrometer (LCMS-8040) equipped with an electrospray ionization source (ESI). Nitrogen was used as nebulizer and argon as collision gas. Most amino acids were ionized in positive ESI and measured in multiple reaction monitoring (MRM), except for glycine in selected-ion monitoring (SIM). Aspartic acid was detected after negative ESI and SIM. Details on binary gradient, MRM and SIM can be found in SI. The quantification was performed using calibration curves of 20 amino acids at 6 concentrations close to their taste threshold. To obtain the taste activity values (TAVs), the FAAs concentrations were divided by their individual taste threshold found in the literature (in SI) in which a TAV above 1 was considered a contribution to the overall taste.

2.5. Free Nucleotides analysis

Free nucleotides were extracted and analyzed according to Moerdijk-Poortvliet et al. (2022). In brief, 50 mg dried sample and 5 mL Milli-Q were homogenized and extracted for 15 minutes at 35 °C followed by centrifugation (3700 g, 20 min). The supernatant of the samples was supplied with 125 μL concentrated H_2SO_4 for acid precipitation followed by centrifugation (3700 g, 20 min). The supernatant was analyzed by means of High Performance Liquid Chromatography (HPLC) using a DIONEX Ultimate 3000 HPLC system equipped with a SIELC PrimeSep D mixed-mode column (150×4.6 mm; 5 μm) with a corresponding guard column (10×4.6 mm; 5 μm) and detected by Ultraviolet (UV) (DAD 3000). The injection volume was 10 μL and elution was performed isocratically at 0.8 mL/min, with 10 mM H_2SO_4 (pH 1.95) as the mobile phase; the detection wavelength was 260 nm. Quantification of nucleotides was achieved using a 7-point external calibration curve (5 to 1000 μM).

2.6. Equivalent umami concentration

The intensity of the umami taste of microalgae can be estimated by measuring the equivalent umami concentration (EUC) expressed as monosodium glutamate (g MSG/100 g). The synergy effect between the umami amino acids and 5'-nucleotides is represented by the following equation (Yamaguchi et al., 1971):

$$Y = \Sigma a_i b_i + 1218\ (\Sigma a_i b_i)\ (\Sigma a_j b_j)$$

where Y is the EUC of the mixture expressed in g MSG/100 g; 1218 is a synergistic constant; a_i is the concentration (g/100 g) of each umami

Table 1
Sensory attributes with descriptions and reference products for quantitative descriptive analysis

Sensory attributes	Description	Reference product
Grassy odor	The odor associated with freshly cutted grass	0.2 g/L 1-hexen-3-ol solution
Floral odor	The odor associated with violet, sweet odor	Violet aroma from Kit Le Nez du Vin[a]
Earthy odor	The odor associated with beetroot, stale/musty odor	Pure beet root
Hay odor	The odor associated with old dried grass	Hay aroma from Kit Le Nez du Vin[a]
Rancid odor	The odor associated with rancid/sour/oxidized butter odor	No reference
Fishy odor/taste	The odor/taste of fish	10 g/L coalfish powder
Crab odor/taste	The odor/taste of crab	10 g/L crab extract powder
Mussel odor/taste	The odor/taste of mussel	10 g/L mussel powder
Salt	The taste on the tongue associated with salt	1.19 g/L NaCl
Bitter	The taste on the tongue associated with caffeine	0.195 g/L caffeine
Umami	The taste on the tongue associated with monosodium glutamate (MSG)	0.595 g/L MSG
Sweet	The taste on the tongue associated with sucrose	0.195 g/L sucrose

[a]Set of 54 small scent phials created by Jean Lenoir

amino acid, Asp or Glu; a_j is the concentration (g/100 g) of each umami 5′-nucleotide, IMP, GMP, or AMP; b_i is the relative umami equivalent concentration (RUC) for each umami amino acid compared to MSG (1 for Glu and 0.077 for Asp) and b_j is the RUC for each umami 5′-nucleotide compared to IMP (1 for IMP; 2.3 for GMP and 0.18 for AMP).

2.7. Salt analysis

Analysis of Na and K was performed by ICP-OES (Varian 720, Varian, Mulgrave, Australia) after acid mineralization with HNO_3 in a microwave oven (CEM MARS Xpress, Matthews, USA). Aliquots of 0.250 g dry sample were weighed in triplicate in polytetrafluoroethene (PTFE) microwave vessels. After the addition of 4 ml nitric acid and 4 ml bidistilled water, the vessels were closed and placed into a microwave system. The samples were heated to 180°C in 15 min and maintained at that temperature for 30 min. After cooling, samples were diluted on a weight basis (2 g sample solution + 8 g bidistilled water) in analytical 15 ml tubes. Quantification of the elements in the digests was performed using an 8-point external calibration curve (calibration range: 0.01 ppm - 100 ppm). Reported results are on λ= 568.820 for Na, and on λ= 766.491 for K.

2.8. Ethical approval

All sensory research performed in this study was in accordance with Ethical Standards of the Commission Flavour and Odour of ILVO (ECSG-ILVO). Prior to the taste sessions, samples were tested on microbial quality and safety (yeast and molds enumerations, coagulase positive *S. aureus* enumerations, *E. coli* enumerations, *Salmonella* detection, *Listeria spp.* and *Listeria monocytogenes* detection), trace element analysis (Cd, Pb, Hg, total As, inorganic As and I), polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). Afterwards, a risk evaluation was made and evaluated by the ECSG-ILVO. Informed consent was obtained from all participants of the sensory evaluation.

2.9. Sensory evaluation

To evaluate seafood features and typical flavors in the algae samples, an expert panel consisting of 16 assessors (5 males, 11 females, aged between 24 and 39 years) was selected and trained in a taste lab according to the ISO8586:2012 standard. The taste lab is equipped according to the ISO standard 8589:2007 including 9 cubicles provided with touchscreens running Fizz (Fizz Biosystèmes) software, a table with faucet and sink and standardized LED lights, air-conditioning to standardize the temperature and ventilation of the lab. Prior to the sensory evaluation, the assessors were introduced to the different microalgae and seaweeds to identify sensory attributes. Afterwards, attributes were selected based on a panel discussion and sensory vocabulary found in the literature. The selected attributes, listed in Table 1, were familiarized using seven odor references: (1) grassy; (2) floral; (3) earthy; (4) hay; (5) fishy; (6) mussel and (7) crab odor. For taste, following seven references were used: (1) salt; (2) bitter; (3) umami; (4) sweet; (5) fishy; (6) mussel and (7) crab taste. Finally, each panelist was trained to assess the intensity of the attributes using a scale ranging from 0 (absent) to 10 (very strong).

An acceptable concentration of 10 g dried sample/L was determined for the sensory evaluation of all algae. Dried algae samples were homogenized with a Thermomix TM 31. Fresh solutions were prepared for each algae using mineral water (Cristaline). Small amounts (10ml) were presented in randomly coded and closed 30 mL screw-capped amber colored glass vials. A different sensory session was organized with the trained panel for seaweeds and microalgae, respectively. Afterwards, a combined session was performed with those microalgae and seaweed (10 g dried sample/L) that have the highest scores on seafood attributes (fish, crab, mussel) to compare their potential for plant-based seafood alternatives.

2.10. Statistical analysis

Using R (version 4.0.5), sensory data were tested for homogeneity of variance (Levene's test, "car" package) and normal distribution (Shapiro-Wilk normality test, "dplyr" package). To determine differences between samples, one-way ANOVA was conducted followed by post hoc Duncan test ("DescTools" package) for normal distributed sensory attributes. For non-normal distributed attributes, Kruskal-Wallis test and post hoc Wilcox test were performed. Pearson correlation test was performed in R to find correlations between chemical data and sensory attributes. Significant differences were established at an α risk of 5%.

3. Results and discussion

Section 3.1 comprises (1) the odor evaluation of the different microalgae species, (2) a comparison between the microalgae and seaweed scoring the highest on the seafood attributes, using coalfish, crab and mussel flavorings as references and (3) a comparison of the chemical aroma profiles of microalgae and seaweeds with those of seafood flavorings. Section 3.2 follows the same structure discussing the taste aspect of algae.

3.1. Seafood aroma in algae

3.1.1. Odor evaluation of microalgae and seaweeds

Figures 1A and 1B summarize all average odor scores obtained from the odor evaluation of eight different microalgae by the expert panel. Seafood odor attributes such as fishy, mussel and crab are observed in different microalgae, especially in *R .salina, P. tricornutum* and *T. chui*. Interestingly, *R. salina* (4.8) is characterized with the highest crab odor

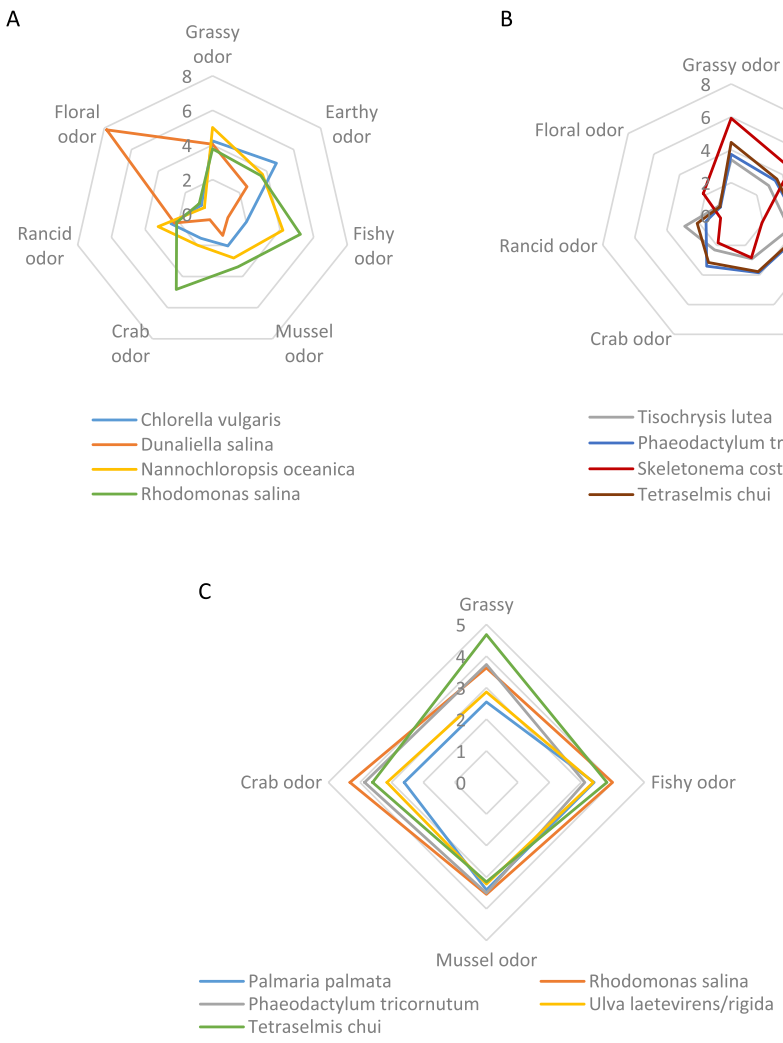

Figure 1. Spider diagrams of odor evaluation of 8 microalgae (A & B). Odor evaluation of microalgae *T. chui*, *P. tricornutum* and *R. salina* compared to seaweeds *P. palmata* and *U. laetevirens/rigida* (C). Average scores are shown after quantitative descriptive sensory evaluation.

score, being significantly higher than all microalgae (p value < 0.05), except for *P. tricornutum* (3.4) and *T. chui* (3.2). Furthermore, *R. salina* (5.2) is characterized with the highest fishy odor followed by *P. tricornutum* (4.5), *T. chui* (4.4), *N. oceanica* (4.2) and *T. lutea* (3.6). The scores for mussel odor are highest in *P. tricornutum* (3.9) and *T. chui* (3.8). In contrast, microalgae *C. vulgaris* (2.0), *S. costatum* (1.9) and *D. salina* (0.9) have significant lower seafood odors (p value < 0.05), compared to the other microalgae, making them less useful for seafood alternatives.

Beside the presence of seafood odor characteristics, it is important to avoid overpowering off-flavors in plant-based seafood alternatives such as grassy, floral, earthy or rancid odors. *N. oceanica* and *T. lutea* possess seafood odors, but are also characterized by a higher unpleasant rancid odor. Furthermore, a significantly higher floral odor score was observed in *D. salina* (7.8) compared to all other microalgae (p value < 0.01). Earthy and grassy odors are commonly found in microalgae, however, overpowering grassy odors are observed in *S. costatum* (5.9) and *N. oceanica* (5.0). Finally, the earthy scores were found to be the highest in *S. costatum* (4.6) and *C. vulgaris* (4.7). Based on these odor profiles, microalgae *R. salina, P. tricornutum* and *T.chui* have the highest potential for plant-based seafood flavoring because of their higher odor score for seafood attributes, while having lower scores for floral, rancid, grassy and earthy odors. Within odor evaluation of seaweeds, which can be found in Supplementary Information (SI), *U.laetevirens/rigida* and *P. palmata* are characterized with the highest scores on seafood odor attributes compared to brown seaweeds *L. ochroleuca, U. pinnatifida* and *S. latissima*. These brown seaweeds have generally lower odor scores and are characterized with hay-like odors. These findings on odor characteristics of seaweeds are in line with the study of López-Pérez et al. (2017).

Figure 1C shows the combined odor evaluation of microalgae *R. salina, P. tricornutum* and *T. chui* and seaweeds *U. laetevirens/rigida* and *P. palmata*. In general, stronger seafood odors were observed in the three microalgae *R. salina, T. chui* and *P. tricornutum* compared to the investigated seaweeds. The crab odor is significantly higher in the three microalgae compared to seaweed *P. palmata* (p value < 0.05), whereas only *R. salina* has a significantly higher crab score compared to *U. laetevirens/rigida* (p value < 0.05). The fishy odor score is slightly higher in *R. salina* and *T. chui* compared to both seaweeds. The evaluated microalgae have higher grassy intensities compared to seaweeds, especially *T. chui* (p value < 0.05).

3.1.2. Comparison of odor-active seafood compounds microalgae and seaweeds

Within the volatile analysis, a total of 189 volatiles were identified in the investigated microalgae, seaweeds and seafood flavor samples belonging to several chemical classes. From these, 64 volatiles were eliminated for further evaluation due to their unknown odor description and high odor threshold, including halogenated and acyclic hydrocarbons such as alkanes and alkenes (Nawar et al., 1977; Le Guen et al., 2000). Table 2 shows the summation of the odor activity values (OAVs) of volatiles within a specific group to reveal similarities and differences between the aroma profiles of microalgae, seaweeds and seafood flavors. Concentrations of each volatile were divided by their individual

Table 2
Summation of the odor activity values (OAV) of volatiles within a specific groups. Aroma profiles of 8 microalgae, 5 commercial seaweed and 8 seafood powders were examined (n=3)

Odor-active compounds	Microalgae species								Seaweeds species					Seafood flavorings						
	Chl	**Dun**	**Tis**	**Nan**	**Pha**	**Rho**	**Ske**	**Tet**	**Pal**	**Lam**	**Sac**	**Ulv**	**Und**	**Cod fish**	**CrabExtr**	**Shrimp Extr**	**Lobster**	**Lobster Extr**	**Mussel**	**Coal fish**
Sulfur-containing compounds																				
Sulfur compounds	47	17	4432	30	7181	117	3602	3149	3.0	1.1	17	671	2.3	212	142	109	229	155	1955	384
Dimethyl sulfide	0	0	4320	6.9	7131	17.5	3523	3089	1.2	0.7	13	666	0	1.0	102	18	3.6	30	1656	0.7
Nitrogen-containing compounds																				
Trimethylamine	0	148	0	0	44	67	0	64	0	0	0	0	0	38	33	2545	532	88	47	137
Pyridines	1.8	14	0.6	0.5	0.8	0.1	5.4	0.3	0	0	0	0	0	0	0	0.3	0.1	0	0.6	0
Pyrazines	4.5	0.5	0.2	0	0	0	0.7	0.9	0	0	0	0	0	0	0.2	1.5	6.3	0.8	4.6	0.5
Fatty acid-derived compounds																				
Linear saturated aldehydes	62	190	67	200	39	35	494	36	43	18	7.7	20	6.4	19	9.5	20	32	5.0	83	13
Unsaturated aldehydes	73	729	224	1421	11	42	810	68	59	0.3	6.7	8.4	22	6.7	6.3	7.5	12	4.9	28	3.0
Unsaturated ketones	245	115	288	484	119	204	2329	173	77	3.7	8.0	1.8	4.3	207	32	4.8	326	14	238	16
Unsaturated alcohols	29	0.1	53	176	16	17	92	32	0	4.0	4.7	3.5	7.0	23	4.5	7.9	39	0.6	35	4.1
Furans	13	93	12	49	9.7	3.8	133	15	4.8	2.5	7.8	0.9	0.2	12	2.1	2.2	17	3.9	8.6	2.7
Saturated ketones	19	39	11	8.3	1.9	1.3	17	4.0	0.6	0.5	2.1	0	0.4	8.9	2.4	13	22	5.2	17	2.3
Carotenoids-derived compounds	193	2466	88	112	20	58	361	67	36	3.5	4.7	1.2	2.2	0.1	0	0	0.2	0	0.3	0
Others																				
Alkyl aldehydes	86	19	223	5.2	55	5.3	318	6.9	5.9	0	4.3	0	0.6	10	5.5	43	22	6.0	20	32
Benzaldehydes	55	35	62	19	11	12	229	12	8.6	1.0	1.1	0.3	0.5	23	6.1	12	23	6.2	28	21
Fatty acid alkyl esters	8.0	0.1	8.5	11	0.6	0	127	0.2	0	0	0	0	0	0.3	0	0.9	1.0	0	0.4	0.8
Diketones	23	11	26	9.2	27	24	15	1.4	0.2	0	0	0.1	0.1	0.2	0.3	0.4	0.6	0.1	0.6	0.1
Monoterpenes	23	9.2	2.0	1.0	0.3	0.4	4.2	0.6	0.8	0.2	0.1	0	0.1	5.0	2.8	47	38	1.3	24	27
Alkyl benzenes	3.2	1.2	0.5	1.2	1.0	0.6	1.4	0.3	0.1	0.1	0	0	0	1.9	0.9	13	11	0.7	7.8	7.7

[a]Odor threshold value, standard deviations and identification methods of the individual compounds can be found in SI.
[b]Chl = *Chlorella vulgaris*; Dun = *Dunaliella salina*; Tis= *Tisochrysis lutea*; Nan = *Nannochloropsis oceanica*; Pha = *Phaeodactylum tricornutum*; Rho = *Rhodomonas salina*; Ske = *Skeletonema costatum*; Tet = *Tetraselmis chui*; Pal = *Palmaria palmata*; Lam = *Laminaria ochroleuca*; Sac = *Saccharina latissima*; Ulv = *Ulva laetevirens/rigida*; Und = *Undaria pinnatifida*

odor threshold value (OTV) determined in water, which can be found in Supplementary Information (SI), to calculate their individual OAVs. Microalgae share important odor-active volatile groups with the investigated seafood flavorings, including fatty acid-derived compounds, sulfuric and nitrogen-containing compounds, alkyl aldehydes and benzaldehydes.

3.1.2.1. Fatty acid-derived volatiles. Similar to seafood, a wide variety of aldehydes, ketones and alcohols detected in microalgae arise from the transformation of unsaturated fatty acids by nonselective oxidation (autoxidation) and selective lipoxygenase enzymes (Pohnert and Boland, 2002; Achyuthan et al., 2017). As Table 2 shows, the fatty acid-derived aroma compounds are divided into 6 subgroups: linear saturated aldehydes, unsaturated aldehydes, unsaturated ketones, unsaturated alcohols, furans and saturated ketones. The OAVs of the different chemical subgroups in microalgae *R. salina, P. tricornutum* and *T. chui* are comparable with those of the seafood flavorings, sharing important odor-active compounds such as saturated aldehydes pentanal, hexanal, heptanal, octanal and decanal, unsaturated aldehydes 4-heptenal (Z) and 2,6-nonadienal (E,E), unsaturated alcohol 1-octen-3-ol and unsaturated ketone 3,5-octadien-2-one. The latter contributes to the seafood odors observed in *R. salina, P. tricornutum* and *T. chui* during the sensory evaluation.

In contrast to those three microalgae, the amount of fatty acid-derived compounds observed in the other microalgae, especially in *S. costatum and N. oceanica*, are several factors higher (up to 500 times more) compared to the seafood flavors. The strong grassy, fishy and rancid odor of *N. oceanica* can be explained by the high amounts of grassy smelling (hexanal, heptanal and 2,6-nonadienal), fatty/fishy smelling (2,4-decadienal and 4-heptenal) and fishy/grassy smelling (1-octen-3-ol) volatiles. These volatiles could originate from the high eicosapentaenoic acid (EPA) and arachidonic acid (AA) content in *N. oceanica* (Lindsay, 1990; Huerlimann et al., 2010). Likewise, high EPA content and active lipoxygenase against PUFAs in *S. costatum* (Zhukova and Aizdaicher, 1995; Fontana et al., 2007) could result in the overpowering grassy smelling (heptanal, 1-hexen-3-ol and 2,6-nonadienal) and earthy/mushroom smelling (3,5-octadien-2-one) volatiles, explaining its strong grassy and earthy odor. Notably, fatty/cucumber smelling 2,4-decadienal and 2-nonenal are observed in all microalgae, especially of *N. oceanica, D. salina, S. costatum* and *T. lutea*, however, not observed in the seafood flavorings.

The fatty acid-derived compounds are generally lower in seaweed compared to the microalgae. From all investigated seaweeds, red seaweed *P. palmata* shares the most fatty acid-derived compounds with seafood flavorings. However, compared to microalgae *R. salina, P. tricornutum* and *T. chui,* both seaweeds *P. palmate* and *U.laetevirens/rigid* contain lower amounts of fatty acid-derived compounds important for seafood aroma such as 3,5-octadien-2-one, 1-octen-3-ol, hexanal and heptanal, which could explain the lower seafood odor scores. Furthermore, the lower amount of grassy smelling volatiles (in specific hexanal, heptanal and 1-octen-3-ol) observed in those seaweeds explains the lower observed grassy odor. Finally, all seaweeds possess earthy/musty smelling 1-octen-3-one, which is not detected in seafood and *R. salina, P. tricornutum* and *T. chui.*

3.1.2.2. Sulfur-containing volatiles. The OAVs of the sulfuric volatiles ranges from 109 – 1955 in the seafood flavorings and from 17-7181 in microalgae. From all sulfuric volatile compounds, dimethyl sulfide (DMS), dimethyl disulfide and methanethiol have the highest OAV in microalgae and seafood. These volatiles are characterized by very low odor thresholds and their odor description varies from cabbage/onion to unpleasant rotten/putrid according to their concentration and the product matrix (Rowe et al., 1998). Similar to seafood, sulfuric volatiles in microalgae originate from microbial and thermal degradation of DMSP and sulfur-containing compounds (Fredrickson and Strom, 2009). Additionally for seafood, dimethyl trisulfide is detected, which has similar odor features as the other sulfuric volatiles. Preceding heat treatment (e.g. cooking and high temperature drying) of the seafood could explain this difference, as dimethyl trisulfide can originate from sulfur-containing compounds during thermal processes (Varlet and Fernandez, 2010). DMS is observed in all seafood flavorings, with OAV ranging from 0.7-1 in fish, 3.6-102 in crustaceans and 1656 in mollusks. Some marine organisms, such as mussels, (filter) feed on protein and DMSP rich phytoplankton, accumulating high amounts of sulfuric volatile precursors. As a result, the feeding habit of marine organisms could explain the diversification of sulfuric aroma compounds within the seafood group. According to literature, DMS has an important contribution to the aroma of bivalves (oyster, mussel, scallops), crustaceans (lobster, crab, shrimp), and to a lower extent of fish (Le Guen et al., 2000; Piveteau et al., 2000). Consequently, the presence of DMS in *R. salina, T. chui* and *P. tricornutum* contribute to their observed crab and mussel odor. This result is in line with the study of Van Durme et al (2013) who also linked seafood-like characteristics in microalgae to high levels of sulfuric compounds.

No DMS was found in *D. salina* and *C. vulgaris* which could explain their relatively low seafood scores. Despite the high amount of DMS detected in *T. lutea and S. costatum*, lower mussel and crab odor scores are observed compared to *R. salina, T. chui* and *P. tricornutum*. The latter could be due to the high amounts of fatty acid-derived odor-active compounds (grassy/earthy/rancid odors), alkyl aldehydes (malty/nutty/coffee odors) and benzaldehydes (nutty/almond odors), masking these sulfuric compounds.

Compared to microalgae and seafood, seaweeds contain less sulfuric volatiles, except for green seaweed *U. laetevirens/rigida*. Similar to the study of Lopez-Perez et al., (2017), the DMS found in green seaweed *Ulva* is 2–3 orders higher compared to other seaweeds. The latter can be attributed to degradation of DMSP present in the green algae *Ulva*, while brown algae contain little DMSP (Groene, 1995). The crab and mussel odor scores in *U. laetevirens/rigida* could be attributed to DMS. Likewise, the lower crab scores in *P.palmata* compared to microalgae *R.salina, P.tricornutum* and *T.chui* could be due to the lower amount of sulfuric compounds such as DMS.

3.1.2.3. Nitrogen-containing volatiles. Nitrogen containing volatiles are divided into trimethylamine, pyridines and pyrazines. Trimethylamine (TMA) has a fishy/ammoniacal characteristic odor with a low odor threshold value which is generally present in marine seafood, adding pleasant crustacean-like odors at low concentrations (Cadwallader et al., 1995). However, TMA is also known as an important indicator of seafood spoilage generated by microbial reduction of trimethylamine N-oxide (TMAO) (Seibel and Walsh, 2002). Derived from Dalgaard et al (1993), TMA gives an unpleasant odor when above 0.42g TMA/kg codfish. In our seafood samples, TMA ranges between 0.1-9.6mg TMA/kg, indicating that the seafood samples were not spoiled. The presence of fishy smelling TMA in microalgae *R. salina, T. chui* and *P. tricornutum* could play a role in their observed seafood odors, while in the seaweeds no TMA is observed. Pyridines and pyrazines are present in both seafood and microalgae, but have less impact on the overall aroma of the samples based on the OAVs.

3.1.2.4. Alkyl aldehydes and Benzaldehydes. Finally, both the OAVs of alkyl aldehydes and benzaldehydes in microalgae *R. salina, P. tricornutum* and *T. chui* are comparable with those of the seafood flavorings. Alkyl aldehydes (e.g. 3-methyl-1-butanal and 2-methyl-1-butanal) are microbially formed from amino acids and categorized as spoilage odors in seafood (Aro et al., 2003; Jaffrès et al., 2011). Benzaldehydes are formed by enzymatic and thermal degradation of the amino acid such as phenylalanine (Nierop Groot and De Bont, 1998).

In microalgae *C. vulgaris, T. lutea* and *S. costatum,* higher OAVs of alkyl aldehydes and benzaldehydes are found compared to the other microalgae and seafood, giving malty/nutty/coffee and nutty/almond off-flavors, respectively. In seaweeds, both alkyl aldehydes and benzaldehydes are lower compared to microalgae and seafood flavorings, except

for *P.palmata*. This latter could be an additional reason for the lower observed seafood odors in *U.laetevirens/rigid* compared to microalgae *R. salina, P. tricornutum* and *T. chui.*

3.1.3. Odor-active off-flavors in microalgae and seaweeds

Microalgae contain odor-active groups which do not contribute to the aroma of seafood and therefore could be considered as off-flavors when used in plant-based seafood alternatives. Next to the fatty/cucumber smelling unsaturated aldehydes 2,4-decadienal and 2-nonenal, also breakdown products of carotenoids, diketones and fatty acid alkyl esters are detected in microalgae, which do not exceed the odor threshold in seafood.

3.1.3.1. Carotenoids-derived volatiles. Carotenoids are powerful antioxidants, exclusively synthesized by plants and microorganisms, that help in eliminating reactive oxygen species (ROS) and harvesting light (Di Lena et al., 2019). Due to the unstable conjugated double-bound structure in carotenoids, both xanthophylls and carotenes could easily degrade into a variety of nor-carotenoids (e.g. β-ionone) and methyl ketones (e.g. 6-methyl-5-hepten-2-one) by enzymatic carotenoid cleavage dioxygenases (CCDs) and non-enzymatic reactions stimulated by light, oxygen and temperature (Jüttner, 1995). Nor-carotenoids have low OTVs and are characterized with floral and woody features (Oliveira et al., 2006). The lowest amount of carotenoids-derived are found in the microalgae *P. tricornutum, T. chui* and *R. salina*, which are characterized with the highest seafood odor scores.

Extremely high amounts of nor-carotenoids (e.g. β-ionone, isophorone and β-cyclocitral) are found in *D. salina* (OAV of 2466) compared to the other microalgae, explaining its significantly higher floral sensory score. This observation is possibly linked to the high β-carotene content (up to 10% DW) in *D. salina*, which provides this microalgae its orange color (Xu and Harvey, 2019). Microalgae are one of the richest producers of carotenoids (Di Lena et al., 2019), explaining the higher OAVs (ranging from 20-2466) for carotenoids-derived aroma compounds compared to seaweeds (ranging from 1.2-36).

3.1.3.2. Diketones and Esters. Spoilage bacteria are responsible for the production of odor-active diketones (e.g. 2,3-butanedione and 2,3-heptanedione) in shrimps (Jaffrès et al., 2011). The higher amount of diketones in microalgae compared to seaweeds and seafood is attributed to the presence of buttery smelling 2,3-butanedione (Prost et al., 2004; Senger-Emonnot et al., 2006).

Fatty acid alkyl esters can be formed by esterification of carboxylic acids and lipid-derived alcohols or microbial fermentation, giving fruity odors (Peterson and Chang, 1982; Jüttner, 1995). In microalgae *P. tricornutum, T. chui* and *R. salina* and seaweeds this group does not exceed the OTVs, while for the other microalgae higher amounts are found.

3.2. Seafood taste in algae

3.2.1. Taste evaluation of microalgae and seaweed

Additionally to the odor evaluation, Fig. 2A and 2B reveal the taste scores of the eight different microalgae evaluated by the expert panel.

Interestingly, the highest umami score is observed in *P. tricornutum* (5.3) followed by *T. chui* (4.7) and *R. salina* (4.2), while compared to these microalgae, significantly lower umami taste is found in *D. salina* (0.9), *T. lutea* (2.2) and *N. oceanica* (2.5) (p value < 0.05). Similar to the umami score, the highest salty score is observed for *R. salina* (3.6), *T. chui* (3.4) and *P. tricornutum* (3.1). High bitterness is generally not appreciated in food and could be an off-flavor in plant-based seafood. Microalgae *S. costatum* (5.7) has a significant higher bitter score compared to the others (p value < 0.05), except for *T. lutea* (4.4). The bitter score is the lowest in *N. oceanica* (1.3) and *T. chui* (1.4), which could give these microalgae an advantage for food-applications. The sweet score ranges from 0.8-2.5, with *S. costatum* (0.8) having the lowest sweet score, possibly due to its high bitterness.

In line with the odor evaluation, a fishy taste is found in several microalgae such as *T. chui* (2.9), *N. oceanica* (2.8), *R. salina* (2.2) and *P. tricornutum* (2.2), while almost nonexistent in *D. salina* (0.9) and *S. costatum* (0.7). Furthermore, notes of crab and mussel taste are found in *P. tricornutum, T. chui* and *R. salina*. Within the seaweed group, see SI, the highest umami taste is observed in *U. laetevirens/rigida.* The observed crab taste in *U. laetevirens/rigida* is significantly higher than the other seaweeds (p value < 0.05) and *P. palmata* has scored the highest on fishy and mussel taste.

Figure 2C compares the taste of microalgae *R. salina, P. tricornutum* and *T. chui* and seaweeds *U. laetevirens/rigida* and *P. palmata*. The umami taste of the microalgae is significantly higher compared to *P. palmata* (p value < 0.05). In addition, both *T. chui* and *P. tricornutum* score significantly higher on umami taste compared to *U. laetevirens/rigida*. Microalgae *P. tricornutum* is significantly more bitter compared to both seaweeds *P. palmata*, while microalgae *R. salina* is also significantly more bitter than *P. palmata* (p value < 0.05). Microalgae *T. chui* and *P. tricornutum* have significantly higher mussel taste compared to both seaweeds (p value < 0.05), while the mussel taste in *R. salina* is significantly higher than *U. laetevirens/rigida*. Significantly higher crab taste is observed in *T. chui* compared to *P. palmata* (p value < 0.05). No significant differences in fishy taste are found between the evaluated seaweeds and microalgae.

3.2.2. Comparison of seafood taste markers in microalgae and seaweed

Table 3 shows the taste activity values (TAVs) of the free amino acids (FAAs) important for seafood, 5'ribonucleotides and salts (K^+ and Na^+) as well as the calculated equivalent umami concentration (EUC) in all microalgae, commercial seaweeds and seafood flavorings. Complete FAA profiles can be found in SI.

In line with the studies of Konosu et al. (1978) and Finne (1992), lower taste contribution of FAAs is observed in the examined fish species (coal and codfish) in comparison with the shellfish species (crab, shrimp, lobster and mussel). Within the investigated microalgae, the taste contribution of the FAAs in both *D. salina* and *N. oceanica* is low. In contrast to those two microalgae, the FAA profiles of the other investigated microalgae show similarities with those of the shellfish as their profiles are both dominated by glutamic acid (Glu), alanine (Ala) and arginine (Arg). However, glycine (Gly), which is important for the sweet taste of shellfish, is absent in all microalgae. Konosu et al. (1978) demonstrated that Glu, Gly, Arg and Ala constitute the core of the taste of boiled crab with the taste synergism between 5′-ribonucleotides (AMP and GMP) elevating overall preference. For mussel, it was found that Glu and Asp had the highest impact on taste followed by Arg, Gly and Ala (Cha et al., 1998).

Compared to shellfish and microalgae (except for *D. salina* and *N. oceanica*), generally lower amounts of FAAs are detected in seaweeds, as only a few FAAs exceed their taste threshold. Green seaweed *U. laetevirens/rigida* is dominated by Glu and serine (Ser), while missing important seafood FAAs such as Gly, Arg and Ala. Red seaweed *P. palmata* possess a high amount of Arg, Glu and isoleucine (Ile), however, containing lower amounts of Ala and Gly. Remarkably, lower amounts of umami tasting Glu are found in seaweeds (ranging from 0.02-1.39 mg Glu/g DW) compared to microalgae such as *P. tricornutum* (11.4 mg Glu/g DW), *S. costatum* (7.18 mg Glu/g DW) and *T. chui* (5.66 mg Glu/g DW). Similar Glu concentrations in seaweeds were found in the study of Mouritsen et al. (2019), where low amounts of Glu were observed in *S. latissima* (0.06-0.43 mg Glu/g DW), *U. pinnatifida* (0.0075-1.85 mg Glu/g DW) and various *Laminaria*-species (0.155-0.75 mg Glu/g DW). In contrast, Mouritsen et al. (2019) measured high amounts of free Glu in *Saccharina japonica* (2.15-15.5 mg Glu/g DW), indicating that the total FAA composition of seaweeds is very species-specific. As shown in Table 3, most microalgae, except for *D. salina* and *N. oceanica*, contain AMP and/or GMP exceeding their taste threshold. In contrast, no taste threshold of 5'ribonucleotides was exceeded in seaweeds. Besides the seafood aroma of *R. salina, P. tricornutum* and *T. chui*, the similarity and higher amount of seafood taste markers (FAAs, nucleotides) in

Table 3
Taste activity values of the free amino acids important for seafood, 5'ribonucleotides and salts measured in all microalgae, commercial seaweeds and seafood flavorings (n=3) as well as the equivalent umami concentration (EUC) values calculated according the equation by Yamaguchi et al. (1971).

Taste compounds	Taste attribute	Taste threshold (mg/ml)	Microalgae species								Seaweed species					Seafood flavorings						
Free amino acids			**Chl**	**Dun**	**Tis**	**Nan**	**Pha**	**Rho**	**Ske**	**Tet**	**Pal**	**Lam**	**Sac**	**Ulv**	**Und**	**Cod fish**	**Crab Extr**	**Shrimp Extr**	**Lobster**	**Lobster Extr**	**Mussel**	**Coal fish**
Arginine	Bitter/sweet (+)	0.5	1.1		15.1		7.5	2.6	7.4	25.2	6.5						9.2	20.8	6.6	5.6	6.1	0.5
Alanine	Sweet (+)	0.6	5.8		13.3		14.6	2.3	12.7	3.5	0.9		6.9	0.6	4.2	1.3	2.7	3.3	3.5	2.5	3.4	1.3
Glycine	Sweet (+)	1.3			0.7		0.7		0.5							0.6	1.2	22.4	1.7	1.2	22.7	
Glutamic acid	Umami (+)	0.3	8.1	0.1	8.6	1.6	38.1	3.0	23.9	18.9	1.8	0.1	4.6	3.4	4.1	1.6	1.6	3.0	2.5	2.8	7.0	1.2
Aspartic acid	Umami (+)	1	0.7		2.8		0.9	0.5	1.2	1.1	0.1		0.2	0.1	0.2	0.2	0.2	0.3	0.3	0.3	1.7	0.1
Nucleotides																						
GMP	Umami (+)	0.125	2.8	0.1	5.4	0.1	0.5	4.7	3.8	3.3		0.1			0.6	0.7	2.0	6.4	0.7	0.2	3.0	1.0
IMP	Umami (+)	0.25		0.1	0.5	0.1	2.4		2.5				0.3	0.2	0.2	0.1	0.4		0.2	0.2	0.1	0.3
AMP	Sweet/umami (+)	0.50		0.2	0.8		4.8		5.3			0.6	0.3	0.5		0.1	0.4	10.5			1.4	24.2
Equivalent Umami Concentration																						
g MSG/100g DW			8.0	0.0	30	0.4	285	5.0	219	21	0.1	0.0	3.3	1.9	3.0	0.9	2.9	21	1.8	1.4	14	15
Salts																						
Na+	Salt (+)	1.8	4	7	16	21	11	26	12	27	14	13	18	15	39	6	62	66	19	70	12	4
K+	Salt (+)	1.3	9	1	11	5	26	9	22	15	44	47	27	26	31	8	3	3	5	2	8	11
Sum salts			13	8	27	26	37	35	34	42	58	60	45	41	70	14	65	69	24	72	20	15

[a]Taste thresholds (mg/ml) and taste of free amino acids in water according to Nishimura & Kato (1988) and Shallenberger (1993)
[b]Taste thresholds of nucleotides (mg/ml) in water according to Fuke and Ueda (1996) and Yamaguchi et al. (1971)
[c]Taste thresholds of salts (mg/ml) in water according to Rotzoll et al. (2006)

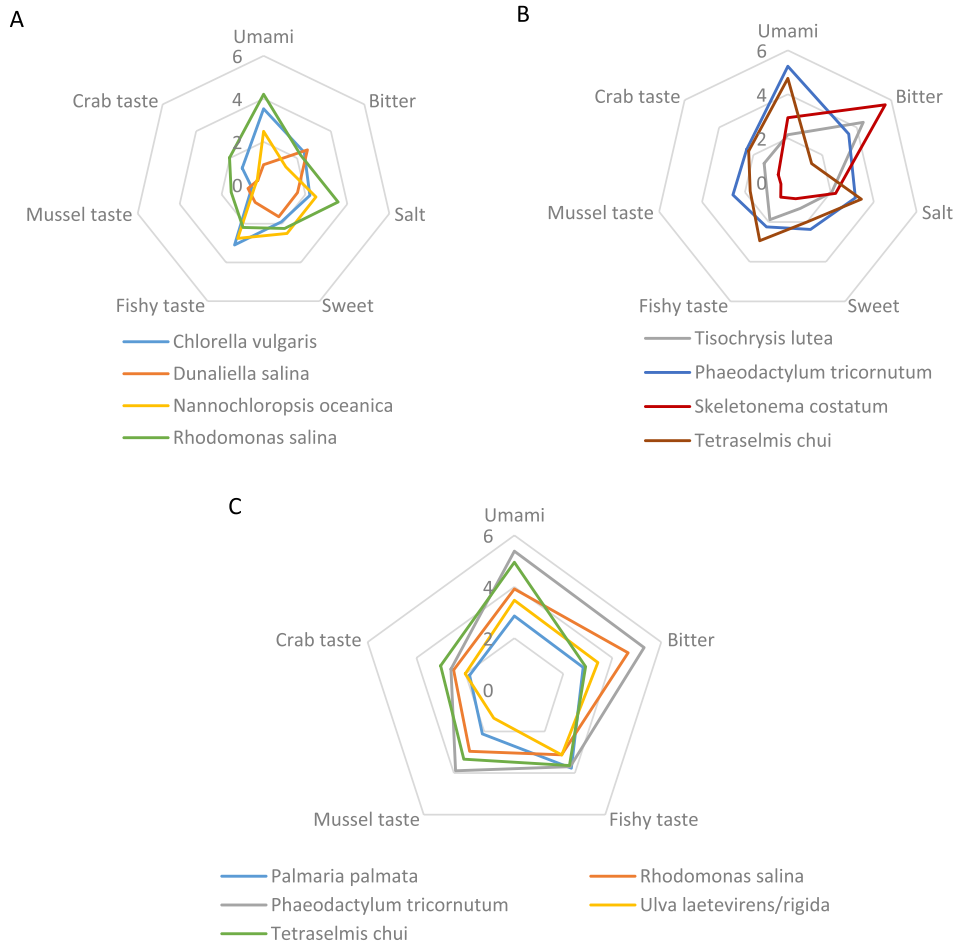

Figure 2. Spider diagrams of taste evaluation of 8 microalgae (A & B). Taste evaluation of microalgae *T. chui*, *P. tricornutum* and *R. salina* compared to seaweeds *P. palmata* and *U. laetevirens/rigida* (C). Average scores are shown after quantitative descriptive sensory evaluation.

those three microalgae compared to seaweeds *P. palmata* and *U. laetevirens/rigida* could explain the higher observed mussel and crab taste in those microalgae.

Finally, both potassium (K^+) and sodium (Na^+) play a role in the perception of salty and umami taste. A positive correlation was found between salt score and umami score (r: +0.86; p-value <0.01), showing the importance of salt in the umami perception and vice versa. Most seafood, except codfish and coalfish, contains higher Na^+ compared to K^+, whereas in seaweeds the TAV of K^+ is higher than Na^+. This latter is in line with the data of Mouritsen et al. (2019), where K^+ salts outbalance Na^+ salts often with a factor 2–3 in concentration in seaweeds. The total taste contribution of the salts is similar for microalgae *R. salina, P. tricornutum* and *T. chui* and seaweeds *P. palmata* and *U. laetevirens/rigida.*

3.2.3. *Umami taste of microalgae and seaweed*

The equivalent umami concentration (EUC) was determined using the amount of FAAs Glu and Asp in combination with 5'ribonucleotides (IMP, GMP and AMP). The EUC value in seafood varies from 0.9 to 21 g MSG/100g DW. Low EUC value were calculated for *D. salina* (0 g MSG/100g DW) and *N. oceanica* (0.4 g MSG/100g DW), while the highest EUC values were attributed to *P. tricornutum* (285 g MSG/100g DW), *S. costatum* (219 g MSG/100g DW), followed by *T. lutea* (30 g MSG/100g DW) and *T. chui* (21 g MSG/100g DW). Figure 3 reveals the relation between the perceived umami intensity obtained from sensory evaluation and the calculated EUC values in microalgae. No significant positive correlation was found (r = +0.57; p-value = 0.14 > 0.05), as bitter tasting microalgae such as *T. lutea* and *S. costatum* have high EUC values, but lower umami scores, indicating that strong bitterness could mask the umami taste. This masking effect might be explained by the particularly complex relationship between bitter and umami, as bitter-tasting chiral isomers of sweet compounds can bind to the umami receptor, eliciting the bitter sensation (Temussi, 2009). In contrast, it has been suggested that umami substances (Glu and AMP) and sodium salts could suppress the tastes of various bitter compounds by binding to the bitter-taste receptor (Shim et al., 2015; Kemp and Beauchamp, 1994; Keast and Breslin, 2003). EUC value is a quantitative and objective measurement for the umami potential of food, which has shown a good correlation with umami intensity in several food such mushrooms (Phat et al., 2016) meat, seafood, dairy and vegetable products (Zhu et al., 2022). However, EUC does not take into account the contribution of salts and the possible taste suppression by other taste-active compounds (e.g. bitter tasting compounds) in the umami perception of complex matrixes such as microalgae.

Bitter tasting microalgae *S. costatum* and *T. lutea* score low on seafood taste attributes regardless of their overlap in taste markers with shellfish. Bitterness itself could not be explained by bitter tasting FAAs (e.g. phenylalanine, tyrosine, isoleucine and histidine) in microalgae, as no significant correlation was found (r = +0.47; p-value = 0.24 > 0.05). Possibly other compounds such as oxidized free fatty acids (Gläser et al., 2021), which were not measured in this study, contribute to the overwhelming bitter taste in some microalgae (e.g. *T. lutea* and *S. costatum*). Whereas umami taste receptors respond only to several chemical substances (Glu, Asp, IMP,…), multiple chemical substances for the sweet receptors and an enormous variety of compounds with diverse chemical structures can elicit the bitter taste receptors (Roper and Chaud-

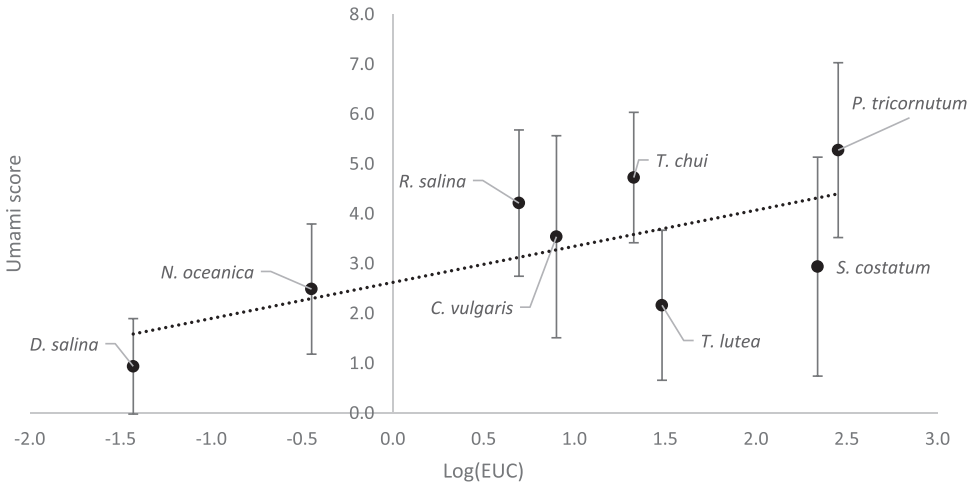

Figure 3. The relation between the perceived umami intensity and the calculated logarithmic EUC values in microalgae

hari, 2017), which makes it hard to chemically quantify bitterness and sweetness in complex food matrixes.

In addition to the higher amount of seafood taste markers (FAAs, nucleotides) in microalgae *R .salina, P. tricornutum* and *T. chui* compared to seaweeds *P. palmata* and *U. laetevirens/rigida,* the higher observed shellfish taste in the microalgae could also be explained by their higher umami taste, as umami is important for the perception of seafood taste. Calculated EUC values in *P. palmata* (0.1 g MSG/100g DW) and *U. laetevirens/rigida* (1.9 g MSG/100g DW) are lower compared to *R. salina* (5.0 g MSG/100g DW), *P. tricornutum* (285 g MSG/100g DW) and *T. chui* (21 g MSG/100g DW), explaining the higher umami taste in the microalgae. Latsos et al. (2021) reported higher EUC values (23.4 g MSG/100 g DW) in *R. salina* at a salinity of 40 and pH 8.5 indicative of the fact that EUC can be influenced by cultivation conditions and processing method (Latsos et al., unpublished results). Interestingly, positive correlations were found between mussel taste and umami score ($r = +0.77$; p-value <0.05) and crab taste and umami score ($r = +0.88$; p-value <0.05) observed by the expert panel, indicating the importance of umami in the perception of seafood taste.

4. Conclusion

Based on the taste and odor evaluation, the most promising microalgae from the examined species for plant-based seafood alternatives are *R. salina, T. chui* and *P. tricornutum.* These microalgae share important odor-active compounds and taste-active compounds with the seafood flavorings. More importantly, essential seafood aroma compounds such as dimethyl sulfide (DMS), trimethylamine (TMA) and lipid-derived poly-unsaturated aldehydes, alcohols and ketones are present in the same range as in the seafood flavorings. In the other investigated microalgae these seafood compounds are absent or high amount of lipid-derived compounds are present, resulting in earthy, rancid and grassy odors. However, all microalgae contain typical aroma compounds which are not present in seafood, such as nor-carotenoids, diketone 2,3-butanedione and fatty acid-derived compounds 2,4-decadienal and 2-nonenal (E) which can be seen as off-flavors when used in plant-based seafood alternatives. These compounds give some microalgae their characteristic aroma such as floral notes of β-ionone in *D. salina*. Positively, the microalgae *R. salina*, and *P. tricornutum* contain low concentrations of these off-flavors. Furthermore, the taste-active compounds in *R. salina, T. chui* and *P. tricornutum* show similarities with the FAAs and 5′-ribonucleotides profile of shellfish, however, the sweet tasting glycine is missing in all microalgae. As important aroma and taste compounds are formed via oxidation and microbial degradation of biological molecules such as fatty acid and carotenoids, the cultivation and pre-processing such as drying and storage conditions of the commercially acquired microalgae could be important for their flavor.

Microalgae *R. salina, T. chui* and *P. tricornutum* possess stronger seafood odor and taste features compared to the evaluated seaweeds. *R. salina* and *T. chui* have higher crab and fishy odor compared to seaweed which can be linked to higher amounts of odor-active seafood compounds such as sulfur containing compounds, fatty acid-derived compounds and TMA. Furthermore, seaweeds contain also off-flavors for plant-based seafood such as nor-carotenoids and fatty acids-derived 1-octen-3-one and 2-nonenal. Additional to the seafood aromas, higher seafood taste compounds such as 5’ ribonucleotides and FAAs are detected in microalgae compared to the seaweeds, which could enhance the higher observed mussel and crab taste in microalgae. However, higher bitterness of *R. salina* and especially in *P. tricornutum* could be unwanted in plant-based seafood alternatives. Interestingly, based on the EUC values and taste evaluation, microalgae *T. chui* and *P. tricornutum* have higher umami potential than the evaluated commercial seaweeds, making these microalgae an useful umami source next to their seafood flavoring capacity.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Bert Coleman: Conceptualization, Formal analysis, Investigation, Software, Methodology, Visualization, Data curation, Writing – original draft. **Christof Van Poucke:** Conceptualization, Investigation, Methodology, Data curation, Validation, Visualization, Software, Supervision, Writing – review & editing. **Bavo Dewitte:** Conceptualization, Investigation, Methodology, Data curation, Software, Supervision, Validation, Visualization, Writing – review & editing. **Ann Ruttens:** Formal analysis, Writing – review & editing. **Tanja Moerdijk-Poortvliet:** Formal analysis, Writing – review & editing. **Christos Latsos:** Conceptualization, Writing – review & editing. **Koen De Reu:** Formal analysis, Writing – review & editing. **Lander Blommaert:** Conceptualization, Writing –

review & editing. **Barbara Duquenne:** Methodology, Software, Writing – review & editing. **Klaas Timmermans:** Funding acquisition, Writing – review & editing. **Jasper van Houcke:** Conceptualization, Writing – review & editing. **Koenraad Muylaert:** Conceptualization, Investigation, Methodology, Supervision, Visualization, Writing – review & editing. **Johan Robbens:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2022.100139.

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