Algal Research 80 (2024) 103538

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

The use of Tetraselmis chuii as seafood flavoring agent in a vegetable broth

Bert Coleman [a,b,*], Christof Van Poucke [c], Bavo De Witte [a], Valentina Casciaro [a], Tanja Moerdijk-Poortvliet [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), Jacobsenstraat 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 Chemistry, Joint Research Centre Zeeland, Groene Woud 1a, 4331, NB, Middelburg, the Netherlands

ARTICLE INFO

Keywords:
Microalgae
Tetraselmis chuii
Seafood alternative
Sensory evaluation
Consumer acceptance
Umami

ABSTRACT

The growing demand of seafood alternatives is driven by concerns on overfishing, marine pollutants and animal welfare in aquaculture and fisheries. Currently, the availability of non-animal-based seafood flavorings on the market is limited, and animal-based seafood flavorings conflict with vegetarian and vegan criteria.

The aim of this study is to explore the use of *Tetraselmis chuii* as a seafood flavoring in a vegetable broth. The flavor of the *T. chuii* broth was compared with a broth containing vegan fish flavoring based on a yeast extract and two broths containing white fish and lobster flavorings. To evaluate the different broths, the study employs a combination of sensory evaluation by a trained panel, chemical flavor analysis for aroma and umami characteristics, and consumer acceptability tests.

Our results indicate that *T. chuii* effectively imparts a fish and shellfish flavor to the broth, which is less intense compared to the white fish and lobster flavorings. Nevertheless, consumers are equally positive of the aroma and flavor of the *T. chuii* broth and the animal-based seafood flavorings broths. The chemical flavor analysis of the *T. chuii* broth identifies volatile organic compounds (VOCs) such as dimethyl sulfide, methanethiol, trimethylamine, and 4-heptenal (Z), which collectively contribute to its distinct seafood aroma.

Consumer preference tests show a preference for the seafood aroma of the *T. chuii* broth over the vegan fish flavoring broth, attributed to the meaty-like off-odor originating from specific VOCs of the yeast extract. In contrast, the vegan fish flavoring broth exhibits a stronger umami taste which is explained by elevated levels of free glutamate and guanosine-5′-monophosphate.

This study highlights the potential of *T. chuii* as innovative seafood flavoring agent to enhance the sensory experience of seafood alternatives, contributing to the ongoing development of sustainable and flavorful non-animal alternatives in the food industry.

1. Introduction

People that are concerned about overfishing, marine pollutants in seafood and animal welfare in aquaculture and fisheries are interested in non-animal-based alternatives for fish and shellfish [1–3]. However, consumers are unwilling to compromise on their sensory experience when consuming these seafood alternatives [4]. Therefore, it is essential for seafood alternatives to deliver flavors that are associated with fish and shellfish [5]. Commercially available seafood flavorings are generated from (parts of) fish or shellfish. Obviously, these seafood flavorings do not comply with vegetarian and vegan criteria.

Understanding the complexity of the seafood flavor is critical for selecting appropriate non-animal-based flavorings for the development of seafood alternatives. The aroma of seafood is determined by a mixture of volatile organic compounds (VOCs) including fatty-acid derived VOCs (e.g. 4-heptenal (Z), 2,6-nonadienal (E,E) and 3,5-octadien-2-one (Z)), sulfur-containing VOCs (e.g. dimethyl sulfide (DMS)) and nitrogen-containing VOCs (e.g. trimethyl amine (TMA)) [6]. In addition, the seafood taste is often associated with the umami taste, which is determined by free amino acids (FAAs) including glutamate (Glu) and

* Corresponding author at: Aquatic Environment and Quality, Cell Blue Biotech and Food Integrity, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Jacobsenstraat 1, 8400 Ostend, Belgium.

https://doi.org/10.1016/j.algal.2024.103538
Received 5 March 2024; Received in revised form 8 May 2024; Accepted 8 May 2024
Available online 13 May 2024

aspartate (Asp) and free 5′-nucleotides including adenosine-5′-monophosphate (AMP), guanosine-5′-monophosphate (GMP) and inosine-5′-monophosphate (IMP) [7,8]. Food scientists and flavor developers employ a combination of flavor enhancers and plant-derived extracts or ingredients to provide these key flavor components to their final food product in order to mimic the unique characteristics of seafood. However, the development of the non-animal-based seafood alternatives is still in its infancy [5].

Currently, only limited commercial non-animal-based flavorings are available that provide the authentic seafood flavor. However, seaweed (extracts) is now being employed to introduce a seafood flavor to these alternatives [4]. Additionally, a vegan fish flavoring (Maxavor Fish W YE), which is based on yeast extract (YE) and contains algal oil derived from heterotrophic microalga *Schizochytrium* sp., was recently launched to mimic the fish flavor [9,10]. Because of the strong umami taste properties of YE, the use of traditional artificial flavor enhancers (in EU assigned by assigned *E*-numbers) such as monosodium glutamate (MSG or E621) and disodium inosinate (E631) can be avoided. This feature of YE is particularly relevant for those who prefer natural or clean-label ingredients in their food products [11]. So far, the entire microalgal biomass has not been utilized as flavoring agents in seafood alternatives, even though certain microalgae exhibit seafood-like characteristics [12]. Some microalgae such as *Tetraselmis chuii* and *Rhodomonas salina* possess strong seafood aroma properties and umami taste features because of the presence of important seafood VOCs (fatty acids-derived VOCs, DMS and TMA) and taste compounds (Glu and AMP), respectively [12]. Coleman et al. (2023) showed that the flavor properties of *T. chuii* biomass depend on the cultivation conditions [13]. Specifically, *T. chuii* grown under nitrogen-sufficient conditions exhibited enhanced seafood flavor attributes, marked by notably diminished off-odors and stronger umami taste, in comparison to *T. chuii* cultivated under nitrogen-limited conditions. These findings suggest that this microalga, when cultivated and processed under favorable conditions, might have potential as innovative flavoring which can be used in the development of seafood alternatives.

The aim of this study is to compare the seafood flavoring capacity of *T. chuii* biomass with two animal-based seafood flavorings and a commercially available vegan fish flavoring based on a YE in a plant-based broth. *T. chuii* was selected for this study because of its interesting seafood flavor properties and because this species is allowed as food ingredient in the EU under the novel food regulation [12–14]. The broths were evaluated by a trained sensory panel and chemical flavor analysis was performed, including the analysis of the VOC profile and chemical markers that contribute to the umami taste. Furthermore, the broths were also evaluated by a consumer panel to reveal the appreciation of *T. chuii* biomass in comparison with the animal-based seafood flavorings and vegan seafood flavoring. Studying the effect of microalgal incorporation on the flavor of the food product is of great importance for new food formulations and will help in the development of seafood alternatives.

2. Materials and methods

2.1. Cultivation of Tetraselmis chuii

Tetraselmis chuii RCC128, acquired from Roscoff Culture Collection (Roscoff, France), was cultivated at ILVO (Ostend, Belgium) as described by Coleman et al. (2023) [13]. Briefly, a 40 L glass bubble column photobioreactor (PBR) (Phyco-Conical PBR Varicon Aqua solutions, United Kingdom) was filled with 36 L of sterilized seawater and inoculated with 2 L of pre-cultured *T. chuii* in the exponential phase. The culture medium was based on F/2 according to Guillard & Ryther (1962) [15] with adjusted concentrations for nitrogen (N) (100 mg NO_3-N/L) and phosphorus (P) (20 mg PO_4-P/L), using $NaNO_3$ and NaH_2PO_4 $2H_2O$, respectively. The PBR was placed in a temperature-controlled room at 20 ± 0.5 °C and continuously illuminated at a photon flux density of 150 μmol photons/m^2 s provided by cool white, fluorescent tubes (white F3 S7, SANlight). The PBR was aerated (5 L/min) and the pH was maintained at 8.0 ± 0.5 by a pH controller using food-grade CO_2. The *T. chuii* culture was harvested after 12 days using a lamella centrifuge (4000 *g*, R. Van Houte). To avoid any flavor deterioration due to storage and processing [16], the microalgal paste was immediately frozen at −20 °C, freeze-dried (Labconco, Kansas City) and stored in airtight glass jars at −80 °C.

2.2. Preparation of the broths

All broth powders were prepared by mixing dried vegetable seasoning, sea salt, maltodextrin and 23 % (*w*/w) of either *T. chuii* biomass, white fish flavoring, lobster flavoring or vegan fish flavoring (Table 1). The seasoning powder contains a mix of different vegetables and herbs, including onion, parsley, carrot, celery, garlic and leek. The two animal-based seafood flavorings were acquired from Flandor Flavors International. The white fish flavoring is a mixture of cod (*Gadus morhua*), saithe (*Pollachius virens*) and haddock (*Melanogrammus aeglefinus*) which were cooked, oven dried and grounded into a powder. The lobster flavoring consists of wild catch lobster (*Homarus americanus*) which was cooked, oven dried and grounded into a powder. The vegan fish flavoring (Maxavor Fish W YE) was acquired from DSM Food Specialties (Delft, the Netherlands). This flavoring is based on yeast extract (YE) and contains algal oil derived from heterotrophic microalga *Schizochytrium* sp. (unknown concentration). All broth powders were stored in approximately 50 g portions in closed food-grade jars. The liquid broths were prepared by homogenizing the broth powders and by diluting into 90 °C mineral water (Cristaline, Jandun, France) to a dosage of 20 g/L. The liquid broths contain a final concentration of 0.46 % (*w*/*v*) of either the *T. chuii* biomass, white fish flavoring, lobster flavoring or vegan fish flavoring.

2.3. Volatile organic compounds analysis

Volatile organic compounds (VOCs) were determined using an automated headspace solid-phase microextraction (HS-SPME) – gas chromatography–mass spectrometry (GC–MS) method according to Coleman et al. (2022) [12]. The broth powders were dissolved to a dosage of 20 g/L in 90 °C mineral water (Cristaline, Jandun, France). The 20 mL amber colored SPME vials were filled with 12 mL solution, spiked with the internal standard mixture (IS) and hermetically sealed. The vials were incubated for 30 min at 40 °C, followed by 30 min extraction at 40 °C using a Gerstel MPS sampler coupled to an Agilent 7890 A GC. The loaded Supelco 50/30 μm DVB/CAR/PDMS fiber was desorbed in splitless mode (250 °C, 3 min) and compounds were separated on a DB-5MS column (30 m × 250 μm × 1 μm; Agilent) using a helium flow rate of 1 mL/min. The oven temperature program was set as follows: start at 35 °C, hold for 3 min, then raised to 220 °C at a rate of 3.5 °C/min. The mass spectra in the electron impact ionization mode

Table 1
Ingredients of the broth powders.

Component (%)	*T. chuii* broth	Lobster broth	White fish broth	Vegan fish broth
Seasoning mixture	36.8	36.8	36.8	36.8
Sea salt[a]	22.8	21.8	22.6	22.2
Maltodextrin	17.4	18.4	17.6	18.0
Tetraselmis chuii powder	23.0	–	–	–
Lobster powder	–	23.0	–	–
Fish powder	–	–	23.0	–
Maxavor Fish YE	–	–	–	23.0
Total	100	100	100	100

[a] The amount of sea salt was adjusted based on the expected Na^+ concentrations to obtain an equal salt concentration in all broths.

were generated at 70 eV and recorded in full scan mode (35–250 *m/z*) utilizing a 5975C inert XL mass spectrometer (Agilent).

To identify the VOCs, Unknown analysis of Masshunter (Agilent) was used. Identification of the VOCs was based on (1) spectral match, compared to the NIST (v2, 2011) and an in-house spectral database, and (2) retention index (RI), compared to the Aroma office 2D (v5.00.00, 2017, Gerstel) and an in-house RI database. RI calibration of the chromatogram was accomplished using a C8-C20 alkane standard solution. VOCs were identified if following criteria were met: (1) peak height is $>10^3$ (counts), (2) signal-to-noise ratio is higher than 3, (3) spectrum match factor is higher than 75 %, and (4) the calculated experimental RI differs <15 from the library RIs. Experimental Kovats RI, library RI and match factor information used for identification can be found in Supplementary Information.

Semi-quantitative determination of the VOCs was performed by spiking 10 μL of the IS mixture to each SPME vial prior to SPME-GC–MS analysis. An IS 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 et al. (2020) [17], the area of the chromatographic peak of each identified VOC was divided by the area corresponding to the IS 2-methyl-3-heptanone. Areas corresponding to identified esters were divided by the area of IS methyl nonanoate. To calculate semi-quantitative concentrations of each VOC, the obtained responses were multiplied with the concentration of IS in the samples (6.8 μg/L for 2-methyl-3-heptanone and 72.9 μg/L for methyl nonanoate), assuming all the response factors were equal. Subsequently, these semi-quantitative concentrations were divided by their individual odor threshold value (OTV) determined in water to calculate the odor activity value (OAV) of each VOC. Since both semi-concentration and the OTV of the VOC are considered, OAVs provide more insight on which VOCs contribute to the flavor. Because of the high OTVs of alkanes and alkenes, we assumed their contribution to the aroma was negligible and were not further considered [18,19]. This approach for calculating semi-quantitative results is comparable to the approach described by Giri et al. (2010) [20] and Van Durme et al. (2013) [21]. In addition, all identified VOCs possess a specific odor descriptions which were retrieved from The Good Scents Company database [22] and the Flavornet database [23]. Three replicate analyses were performed on each liquid broth.

2.4. Free Amino acid analysis

Free amino acids (FAAs) were determined according to Coleman et al. (2022) [12]. 0.2 g of the broth powder was dissolved in 8.5 mL UHPLC-MS water and 0.5 mL of the IS mixture using a vortex for 15 s. The IS mixture contained 134.9 μg/mL methionine-3,3,4,4-d_4; 34.1 μg/mL *N*-methyl-L-valine; 146.3 μg/mL histidine-$^{13}C6^{15}N3$; 282.7 μg/mL L-glutamine $^{13}C5$; 1259.2 μg/mL L-glutamic acid $^{13}C5$; 294.4 μg/mL DL-Lysine-$^{13}C1,2$; 435.7 μg/mL L-aspartic acid-^{15}N-2,3,3-d_3; 634.1 μg/mL L-aspargine-$^{15}N2$; 316.8 μg/mL L-alanine-2,3,3,3-d_4 and 430.9 μg/mL homoarginine dissolved in UHPLC-MS water. After 15 min of extraction at room temperature, 1 mL 35 % sulfosalicylic acid was added to the samples for acid precipitation of proteins and peptides. Afterwards, the samples were centrifuged for 10 min at 4000*g*. Aliquots of 500 μL of the supernatants were diluted with 100 μL acidified (1 % (*v*/v) formic acid) ammonium formate buffer (4 M) and 40 μL ACN. Amino acids were separated according to van 't Land (2019) by HILIC-MS/MS using a LC-MS system [24]. Three μL of aqueous extract was injected onto an Intrada HILIC column (100 mm × 3 mm × 3 μm) 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) acetonitrile and 80/20 (acetonitrile (≥ 99.95 %)/ammonium formate (≥ 99 %)). Amino acids were detected using a Shimadzu triple quadrupole MS (LCMS-8040) equipped with an electrospray ionization source (ESI) source. Nitrogen was used as nebulizer and argon as collision gas. The quantification was performed using 6-point calibration curves for each of the amino acids, in a range comprising the taste threshold. All broth powders were measured in triplicate.

2.5. Free 5′-nucleotides analysis

Free 5′-nucleotides were extracted and analyzed according to Moerdijk-Poortvliet et al. (2022) [25]. In brief, 50 mg broth powder and 5 mL Milli-Q were homogenized and extracted for 15 min in a water bath set to 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 HPLC using a DIONEX Ultimate 3000 HPLC system equipped with a SIELC PrimeSep D mixed-mode column (150 mm × 4.6 mm × 5 μm) with a corresponding guard column (10 mm × 4.6 mm × 5 μm). In the mixed-mode column, the retention of the 5′-nucleotides was controlled by ion-exchange interactions and hydrophobic interactions with the stationary phase. 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 of the 5′-nucleotides was 260 nm using an UV (DAD 3000). Quantification of 5′-nucleotides was achieved using a 7-point external calibration curve (5 to 1000 μM). All broth powders were measured in triplicate.

2.6. Equivalent umami concentration

The intensity of the umami taste of a food product can be estimated by measuring the equivalent umami concentration (EUC) expressed in the amount of MSG per 100 g of product. The synergy effect between the umami FAAs and 5′-nucleotides is represented by the equation according to Yamaguchi et al. (1971) [26]:

$$Y = \sum a_i b_i + 1218\left(\sum a_i b_i\right)\left(\sum a_j b_j\right)$$

in which 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 free Glu or Asp; a_j is the concentration (g/100 g) of free 5′-nucleotides IMP, GMP or AMP; b_i is the relative umami 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 free 5′-nucleotides compared to IMP (1 for IMP; 2.3 for GMP and 0.18 for AMP).

2.7. Color analysis

The color of the different broths was determined by measuring the reflectance using a CM-5 reflectance spectrophotometer (Konica Minolta Sensing Americas Inc). The broths were diluted to a concentration of 20 g/L and 2 mL of the sample was placed in a small petri dish. Every liquid broth was measured nine times and the average color values were calculated for each broth. The results were expressed in terms of L*, lightness (from 0 to 100 %); a*, redness to greenness (60 to −60); b*, yellowness to blueness (60 to −60), following the CIELAB system.

2.8. Sensory evaluation by a trained panel

All sensory research performed in this study was in accordance with Ethical Standards of the Commission Flavor and Odor of ILVO (ECSG-ILVO). Prior to the sensory sessions, the *T. chuii* biomass was tested on microbial safety (coagulase positive *Staphylococcus aureus* enumerations, *Escherichia coli* enumerations, *Salmonella* detection, *Listeria* spp. and *Listeria monocytogenes* detection) and trace element analysis (cadmium, lead, mercury, arsenic and iodine). Informed consent was obtained from all participants of the sensory evaluation.

The flavor of the liquid broths was evaluated by a sensory panel which was trained according to the ISO8586:2012 standard. The sensory evaluations were performed by 11 trained panelists which are all highly

experienced (> 3 years). The taste lab is equipped according to the ISO standard 8589:2007 using the Fizz software (Fizz Biosystèmes). A free choice profiling (FCP) session was organized in which the sensory attributes of the different broths were identified. Based on panel discussions, the sensory attributes and reference products were selected for the broths (Table 2). The intensity of the selected sensory attributes was scored using a scale ranging from 0 (absent) to 10 (very strong). The liquid broths for the sensory analysis were prepared in the same way as the VOC analysis. Mineral water (Cristaline, Jandun, France) was boiled using an electric water boiler. Afterwards, the different broths powders were fully dissolved with 90 °C mineral water to a concentration of 20 g/L. The broths were kept warm in a pre-heated oven at 40 °C until the sensory evaluation. Small amounts of the broths (20 mL) were presented in randomly coded and closed 30 mL screw-capped amber colored glass vials. The sensory panel evaluated all broths once. The flavor of the different broths was assessed under red lighting to avoid impact of the color on the sensory evaluation.

2.9. Consumer appreciation test

An appreciation test was organized in which 43 untrained consumers ranked the different broths (*T. chuii* broth, lobster broth, white fish broth and vegan fish broth). The participants, ranging in age from 20 to 60 years, formed a gender-diverse group, with 55 % being men and 45 % women, originating from Flanders, The Netherlands, and the UK. Moreover, the participants were drawn from a wide range of backgrounds, with 45 % representing the public sector (including academia, research and regulation) and 55 % from the private sector (including food and feed industry, retail and hospitality). Additionally, 44 % of participants had no prior experience with algae whatsoever, while 28 % possessed limited knowledge of algae, and another 28 % had prior experience working with algae.

The evaluation criteria focused on aroma, flavor and color appreciation, with the intention of assessing their suitability for use in the preparation of seafood dishes. The most appreciated broth receives a score of 1 and the worst broth receives a score of 4. The sum of the ranks was made in order to evaluate differences between the appreciation features of the broths. Additionally, following questions were asked: "*Does the flavor of the broth remind you of seafood (such as fish, lobster, mussels, …)?*" and "*Do you think the broth contains algae?*". Similar to the sensory evaluation by the trained panel, broths were dissolved to 20 g/L with boiled mineral water (Cristaline, Jandun, France) and kept warm at 40 °C in a pre-heated oven. Small amounts of the broths (20 mL) were presented in randomly coded and closed 30 mL screw-capped amber colored glass vials. Initially, the aroma and flavor appreciation were evaluated and the two questions were answered under red light. Afterwards, the color appreciation was ranked under day light.

Table 2
Sensory attributes associated to the different broths, their descriptions and used reference products.

Sensory attributes	Description	Reference product
Grassy odor	The odor associated with sweet, freshly cut grass	0.2 g/L 1-hexen-3-ol solution
Fishy odor/ flavor	The odor/flavor of fish	10 g/L white fish mix powder
Shellfish odor/ flavor	The odor/flavor of lobster, shellfish	10 g/L lobster powder
Chicken-like odor	The odor associated with chicken (cooked), meat	No reference
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 MSG	0.595 g/L MSG
Sweet	The taste on the tongue associated with sucrose	0.195 g/L sucrose
Sour	The taste associated with citric acid	0.43 g/L citric acid
Herbal flavor	The flavor associated with herbals, spicy, broth	No reference

2.10. Statistical analysis

Using R (version 4.0.5), sensory scores proved to fit a normal distribution, based on the evaluation of the Quantile Quantile plot and a histogram of the residuals. A linear mixed-effect model was conducted for each sensory attribute (lmer function, "lsmeans" package). The effect of the assessors was considered random. To determine significant differences between the sensory attributes of the different broths, ANOVA (Type III) was used at an α risk of 5 %. This method is commonly applied to data from descriptive analysis [27]. Non-parametric Friedman analysis was applied to evaluate significant differences between the sum of the ranks data obtained from the consumer appreciation test of the broths, using an α risk of 5 %.

3. Results and discussion

3.1. Sensory evaluation of the broths by the trained panel

Fig. 1 summarizes all average odor, taste and flavor scores which are obtained from the sensory evaluation of the four different broths. Interestingly, the sensory evaluation showed that the broth containing *T. chuii* biomass possessed fishy and shellfish aroma features. However, its fishy and shellfish odor intensities were significantly lower compared to the broths containing the white fish and lobster flavorings, respectively (both $p < 0.05$). In contrast, the *T. chuii* broth was characterized with a higher grassy odor compared to the other broths (both $p < 0.05$) which might be an off-flavor when using this microalga in seafood alternatives. The sensory results also indicated that the *T. chuii* broth had stronger seafood aroma features compared to the broth containing the vegan fish flavoring ($p < 0.05$). This vegan fish broth was characterized by chicken and meaty-like odor ($p < 0.05$), which could originate from the YE present in the flavor solution. Currently, YEs are being explored to replace the flavor of certain animal-based products because of its meaty-like flavors [11].

No difference in umami taste was observed between the broth containing *T. chuii* biomass and the broths containing the animal-based seafood flavorings. In contrast, the broth containing the vegan fish flavoring was characterized with the strongest umami taste ($p < 0.05$). Additionally, this vegan fish broth possessed a higher taste intensity, salty taste and herbal flavor compared to the other broths which might be explained by the flavor enhancing properties of umami [11]. No difference in shellfish flavor was observed between the *T. chuii* broth and the lobster broth. However, the *T. chuii* broth possessed a significantly lower fishy flavor compared to the white fish broth ($p < 0.05$). In contrast, the broth containing the vegan fish flavoring possessed a significantly lower shellfish flavor compared to the broth containing the lobster flavoring ($p < 0.05$), whereas its fishy flavor did not significantly differ from the broth containing the white fish flavoring.

This sensory evaluation indicated that the *T. chuii* broth had lower seafood odor intensities than broths with animal-based seafood flavorings. Yet, in comparison to the vegan fish flavoring broth, the seafood odor of *T. chuii* broth was more similar to animal-based seafood broths. The umami intensity in the vegan fish flavoring was significant stronger than the other broths, while the umami taste intensity of the *T. chuii* biomass broth was comparable to the broths with animal-based seafood flavorings. Interestingly, this sensory evaluation also revealed that *T. chuii* biomass was more effective in substituting for shellfish flavor, while the vegan fish flavoring effectively mimicked the fish flavor.

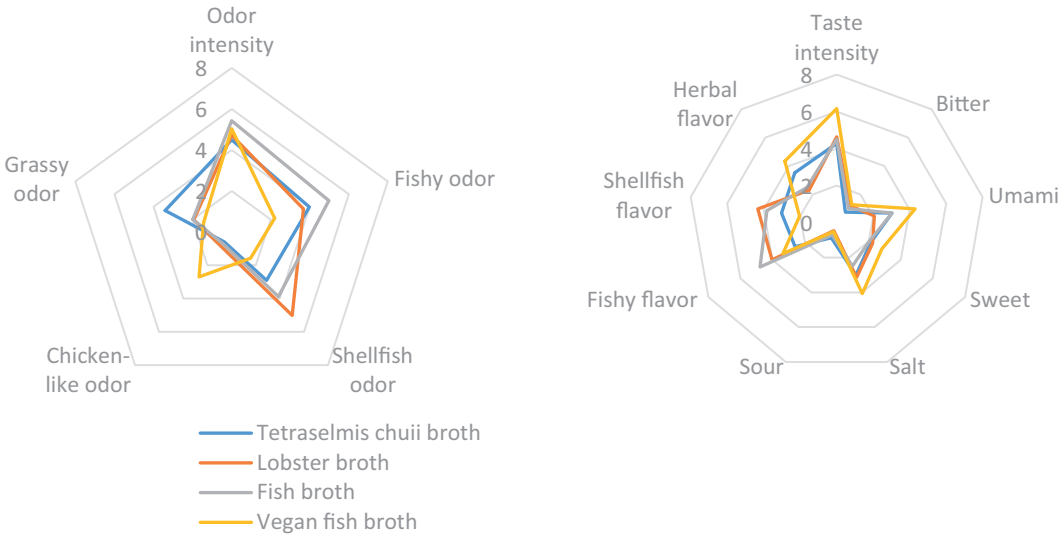

Fig. 1. The sensory odor (left) and taste/flavor (right) scores of the broths visualized in a radar chart. Average scores of the 11 trained members scores (on a scale from 0 to 10) are shown after quantitative descriptive sensory evaluation ($n = 1$).

3.2. Ranking appreciation test of the broths by a consumer panel

All 43 untrained assessors of the consumer panel ranked the different broths from the best (rank 1) to the worst (rank 4) broth for aroma, flavor and color appreciation meant to be used as seafood flavoring. Additionally, two questions were asked: "*Does the flavor of the broth remind you of seafood (such as fish, lobster, mussels, …)?*" and "*Do you think the broth contains algae?*"

Table 3 shows the sum of the rank scores of consumers, in which the lowest sum of rank represents the highest appreciation score. The consumer appreciation test revealed that the aroma of the broth containing *T. chuii* biomass was equally appreciated as the aroma of the broths containing the animal-based seafood flavorings. Notably, the higher grassy odor of the *T. chuii* broth (Fig. 1), observed by the trained panel, did not negatively affect its aroma appreciation. In contrast, the aroma of the vegan fish broth was less appreciated for the seafood flavoring application compared to the other broths ($p < 0.05$). This could be explained by its chicken and meaty-like odor features which were observed in the sensory evaluation by the trained panel (Fig. 1). No significant differences were found between the flavor appreciation of the *T. chuii* broth and the other broths.

The visual consumer acceptance of the broth was negatively affected by the addition of the microalga ($p < 0.05$). The color measurements showed lower L* and a* values in the *T. chuii* broth compared to the other broths (Table 4), explaining its dark green color which is associated with the high chlorophyll content in *T. chuii* biomass [12].

This study revealed that *T. chuii* did not negatively affect the flavor appreciation of the broth. Furthermore, 70 % of the consumers indicated that the flavor of the *T. chuii* broth reminded them of animal-based, and only 58 % of the consumers thought that the *T. chuii* broth contained microalgae (Table 3). Remarkable, the lobster broth reminded consumers more of microalgae than the broth containing the microalgae *T. chuii.* As a result, *T. chuii* can be a suitable substitute for the animal-based seafood flavorings. However, the color could repel some consumers.

While the number of participants (43) in the study was sufficient for comparing the different broths and detecting differences between them, the sample size was not adequate for conducting comparisons across different consumer segments. However, this is an interesting direction for further research and investigation.

Table 3
Ranking appreciation test of the different broth for seafood application by a consumer panel ($n = 43$). The most appreciated broth receives a score of 1 and the worst broth receives a score of 4. Sum of ranks is presented in the table. Different letters correspond to significant differences. The results of the two questions indicate the percentage of the answer "yes".

	T. chuii broth	Lobster broth	White fish broth	Vegan fish broth	*p*-value
Aroma appreciation suitability for use in the seafood dishes*	97 ^{a}	99 ^{a}	99 ^{a}	135 ^{b}	<0.05
Flavor appreciation suitability for use in the seafood dishes*	107 ^{ab}	127 ^{b}	102 ^{ab}	94 ^{a}	<0.05
Visual appreciation suitability for use in the seafood dishes	126 ^{b}	100 ^{ab}	111 ^{ab}	93 ^{a}	<0.05
Does the flavor of the broth remind you of seafood? (Yes %)*	70 %	65 %	88 %	56 %	
Do you think the broth contains algae? (Yes %)*	58 %	63 %	49 %	44 %	

* Evaluated under red light.

3.3. Comparison of the volatile profile of the different broths

A total of 48 different VOCs were identified in the different broths (Table 5). Several sulfur-containing VOCs (e.g. dipropyl (di/tri)sulfides, diallyl (di)sulfides) and other VOCs (o-cymene, 3-carene, caryophyllene) detected in the broths originate from the seasoning mixture [28–30]. This was verified by performing a VOC analysis on the

Table 4
Colorimetric values of the different broths ($n = 9$).

	T. chuii broth	Lobster broth	White fish broth	Vegan fish broth
Colorimetric values				
L*: Lightness (0–100 %)	10.7 ± 0.4	49.9 ± 0.8	44.9 ± 0.3	46.0 ± 0.2
a*: Greenness (−60) to Redness (+60)	−7.0 ± 0.1	8.3 ± 0.1	0.9 ± 0.0	1.6 ± 0.0
b*: Blueness (−60) to Yellowness (+60)	9.6 ± 0.4	14.6 ± 0.1	17.4 ± 0.1	20.1 ± 0.5

Table 5
The mean odor activity values (OAVs) of all identified VOCs in the different broths ($n = 3$). Library retention index, experimental Kovats retention index and spectral match factor information used for compound identification can be found in Supplementary Information. ND = not detected.

	OTV (ppb)	*T. chuii* broth	Lobster broth	White fish broth	Vegan fish broth
Sulfur-containing VOCs					
Methanethiol (MeSH)	0.02 [c]	7.47 ± 1.33	ND	5.21 ± 1.08	6.81 ± 0.59
1-Propanethiol	3.1 [a]	1.25 ± 0.12	0.08 ± 0.02	0.21 ± 0.01	0.18 ± 0.02
Dimethyl sulfide (DMS)	0.12 [a]	201.1 ± 16.7	1.85 ± 0.04	1.39 ± 0.16	1.51 ± 0.12
Dimethyl disulfide (DMDS)	0.29 [a]	0.49 ± 0.04	0.67 ± 0.04	1.03 ± 0.09	0.65 ± 0.06
Dimethyl trisulfide (DMTS)	0.01 [a]	4.73 ± 0.92	ND	32.72 ± 1.25	28.82 ± 2.48
Methyl 2-propenyl disulfide	0.14 [a]	10.47 ± 1.01	6.54 ± 1.62	9.22 ± 1.64	5.71 ± 0.54
Methyl propyl disulfide	0.29 [a]	3.40 ± 0.31	2.44 ± 0.41	2.36 ± 0.13	2.03 ± 0.16
Dipropyl disulfide	0.22 [a]	46.05 ± 10.05	70.17 ± 2.87	48.35 ± 8.12	22.35 ± 1.51
Dipropyl trisulfide	0.22 [a]	40.17 ± 2.16	27.86 ± 2.77	57.51 ± 1.91	23.09 ± 3.47
Diallyl sulfide	0.22 [a]	2.58 ± 0.31	2.58 ± 0.32	3.14 ± 0.13	2.69 ± 0.27
Diallyl disulfide	0.22 [a]	6.35 ± 0.51	3.80 ± 0.50	4.87 ± 1.39	4.48 ± 0.31
Thiophene, 3,4-dimethyl-	0.56 [a]	0.97 ± 0.09	1.27 ± 0.04	1.15 ± 0.06	1.05 ± 0.11
Nitrogen-containing VOCs					
Trimethylamine (TMA)	0.37 [a]	2.51 ± 0.30	7.37 ± 0	0.87 ± 0.02	ND
Alkyl aldehydes					
2-Methylpropanal	0.1 [b]	ND	ND	ND	1.00 ± 0.18
3-Methylbutanal	1.1 [d]	0.12 ± 0.01	0.07 ± 0.00	0.09 ± 0.01	0.78 ± 0.11
2-Methylbutanal	0.2 [e]	ND	ND	0.47 ± 0.03	4.05 ± 0.49
Furans					
2-Methylfuran	2.3 [d]	0.11 ± 0.00	0.03 ± 0.00	0.09 ± 0.00	0.10 ± 0.01
2-Ethylfuran	2.3 [d]	0.32 ± 0.02	0.15 ± 0.01	0.45 ± 0.02	0.14 ± 0.01
2-Pentylfuran	5.8 [d]	0.17 ± 0.02	0.10 ± 0.00	0.07 ± 0.01	0.05 ± 0.00
Saturated aldehydes					
Hexanal	2.4 [e]	0.72 ± 0.09	0.48 ± 0.04	0.24 ± 0.02	0.10 ± 0.01
Heptanal	0.8 [b]	0.23 ± 0.05	0.37 ± 0.02	0.34 ± 0.04	0.13 ± 0.04
Octanal	0.587[d]	0.14 ± 0.02	0.19 ± 0.00	0.30 ± 0.01	0.13 ± 0.02
Nonanal	1 [b]	0.22 ± 0.04	0.33 ± 0.01	0.46 ± 0.02	0.33 ± 0.08
Unsaturated aldehydes					
4-Heptenal, (*Z*)-	0.8 [b]	0.07 ± 0.01	0.13 ± 0	0.19 ± 0.01	ND
Benzaldehydes					
Benzaldehyde	1.5 [a]	0.22 ± 0.02	0.31 ± 0.07	0.54 ± 0.05	0.22 ± 0.01
Benzeneacetaldehyde	4 [b]	ND	ND	ND	0.04 ± 0
Carotenoids-derived VOCs					
5-Hepten-2-one, 6-methyl-	50 [b]	0.01 ± 0.00	ND	ND	ND
Cyclohexanone, 2,2,6-trimethyl-	100 [b]	< 0.05	ND	ND	ND
Isophorone	0.3 [b]	0.67 ± 0.10	ND	ND	ND
α-Cyclocitral	5 [b]	0.02 ± 0.00	ND	ND	ND
β-Cyclocitral	5 [b]	0.05 ± 0.01	ND	ND	ND
α-Ionone	2.6 [b]	0.28 ± 0.02	ND	ND	ND
β-Ionone	3.5 [e]	0.24 ± 0.04	ND	ND	ND
Ketones					
2,3-Octanedione	9.8 [a]	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	< 0.05
2-Octanone	50 [d]	< 0.05	< 0.05	< 0.05	< 0.05
2-Decanone	5.5 [a]	ND	0.03 ± 0.00	0.03 ± 0.00	ND
2-Undecanone	5.5 [a]	0.07 ± 0.01	0.15 ± 0.00	0.11 ± 0.01	0.04 ± 0.01
Aromatic hydrocarbons					
Benzene	470 [b]	ND	ND	< 0.05	< 0.05
Toluene	21 [b]	ND	0.01 ± 0.01	0.03 ± 0.00	0.12 ± 0.01
Benzene, pentyl-	177 [b]	< 0.05	< 0.05	< 0.05	< 0.05
Benzene, 1,3-dimethyl-	41 [b]	< 0.05	< 0.05	< 0.05	< 0.05
Styrene	35 [b]	< 0.05	< 0.05	< 0.05	< 0.05
o-Cymene	5.01 [b]	0.05 ± 0.01	0.51 ± 0.09	0.17 ± 0.05	0.04 ± 0.00
3-Carene	6 [b]	0.02 ± 0.00	0.18 ± 0.05	0.18 ± 0.11	0.02 ± 0.00
D-Limonene	1.8 [b]	0.60 ± 0.07	2.14 ± 0.29	0.91 ± 0.26	0.46 ± 0.04
γ-Terpinene	6 [b]	ND	0.07 ± 0.01	0.03 ± 0.01	ND
α-Cubebene	64 [b]	< 0.05	< 0.05	0.01 ± 0.00	< 0.05
Caryophyllene	64 [b]	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00

vegetable broth without any *T. chuii* biomass, seafood flavoring or vegan fish flavoring (Supplementary Information).

Typical VOCs that originate from the white fish and lobster flavoring were detected in the animal-based broths including fatty acid-derived VOCs (e.g. 4-heptenal (Z), hexanal, heptanal, octanal) and nitrogen-containing trimethylamine (TMA). Additionally, the white fish broth was distinguished form the lobster broth by the presence of sulfur-containing VOCs methanethiol (MeSH) and dimethyl trisulfide (DMTS). Sulfur-containing VOCs DMS and DMDS were detected in the seafood broths which can originate from the lobster and white fish flavorings as well as from the onion and garlic in the seasoning mixture [28,30].

The broth containing *T. chuii* biomass was characterized by a higher amount of DMS compared to the other broths. DMS is a key aroma compounds for shellfish such as clams, oyster and mussel [6,28]. Similar to the white fish broth, the sulfur-containing VOCs MeSH and DMTS were also observed in the *T. chuii* broth. In addition, the *T. chuii* broth contained also nitrogen-containing TMA and fatty acid-derived 4-heptenal (Z) which have characteristic fishy odor features [28]. Earlier studies have showed that DMS, MeSH, TMA and 4-heptenal (Z) are linked to the seafood aroma of microalgal biomass, which could explain the observed seafood aroma in the *T. chuii* broth [12,13,21].

Besides VOCs that are essential for the seafood aroma, the *T. chuii* broth also contained the highest amount of hexanal which possesses a grassy odor feature [28]. In addition, the carotenoid-derived VOCs (e.g. isophorone, α-cyclocitral, β-cyclocitral, α-ionone and β-ionone) were characteristic for the *T. chuii* broth. These VOCs have floral, violet, woody, fruity-like features and are key odorants of some flowers (e.g. crocus, violet, tulip and rose) and some berry species (e.g. blackberry, raspberry and cranberry) [28]. However, no floral notes were observed

in the broth containing *T. chuii* biomass by the trained sensory panel. Possibly, the combination of the carotenoid-derived VOCs and fatty acid-derived hexanal in the *T. chuii* broth could have contributed to its higher observed grassy odor during the sensory evaluation by the trained panel. However, the consumer evaluation showed that this grassy odor did not affect its aroma acceptance for seafood applications.

Both TMA and 4-heptenal (Z) were not detected in the broth containing the vegan fish flavoring. Although the vegan fish broth contains *Schizochytrium*-derived algal oil, no specific fatty acid-derived VOCs were observed. Similar to the white fish broth and the *T. chuii* broth, the vegan fish broth possessed sulfur-containing VOCs MeSH and DMTS. Importantly, the vegan fish broth contained several key VOCs that are typically associated with yeast including Strecker aldehydes (e.g. 2-methylpropanal, 3-methylbutanal, 2-methylbutanal and benzeneacetaldehyde) and sulfur-containing DMTS [11,28,31]. Strecker aldehydes possess musty, cocoa, coffee, nutty and malty-like odor features and can be formed from amino acids (e.g. leucine and isoleucine) either via the Strecker degradation during thermal processing of the yeast or via microbial activity during the cultivation of the yeast [11,32]. The amount of Strecker aldehydes in the vegan fish broth was several times higher compared to the other broths. DMTS can provide cooked and meaty-like odor features and can be generated from the thermal degradation of sulfur-containing compounds such as methionine or thiamin [11,31]. Furanthiols and pyrazines have been reported to be essential VOCs of YE [11]. Furanthiols (e.g. 2-methyl-3-furanthiol) possess chicken-like and meaty-like flavors, whereas pyrazines generally possess roasted odor features [31]. However, both furanthiols and pyrazines were not detected in this study which could be attributed to the limited amount of vegan fish flavoring present in the broth (0.46 % (*w/v*)). Nonetheless, these VOCs could still influence the aroma of the vegan fish broth even at very low concentrations (under the detection limit) because of their low OTVs [11]. The presence of high concentrations of common yeast VOCs and the absence of VOCs such as TMA and 4-heptenal (Z) could explain the lower observed fishy and shellfish odor and the higher observed chicken-like odor in the vegan fish broth by the trained panel. This chicken-like odor feature of the vegan fish broth negatively affected its aroma appreciation for seafood applications in the consumer evaluation.

Odor detection threshold values (OTV) determined in water (in ppb): [a]Murnane et al. (2013) [33]; [b]Leffingwell (1991) [34]; [c]The Good Scents Company (2022) [22]; [d] Giri et al. (2010) [20]; [e]Czerny et al. (2008) [35].

3.4. Comparison of the free amino acids and 5′-nucleotides profile of the broth powders

The FAAs Glu and Asp and free 5′-nucleotides AMP, GMP and IMP of the different broth powders are shown in Table 6. The complete FAAs and free 5′-nucleotides profiles can be found in Supplementary Information. The FAAs content in the broth powder containing *T. chuii* biomass was dominated by free Glu (2.29 mg Glu/g DW) which was several orders higher compared to the amount of free Glu in the broth powders containing the white fish (0.10 mg Glu/g DW) and lobster (0.16 mg Glu/g DW) flavorings. Remarkably, the free Glu content of the broth powder containing the vegan fish flavoring (5.81 mg Glu/g DW) was more than twice the amount of free Glu content in the *T. chuii* broth powder. The free 5′-nucleotides GMP and IMP were absent in the *T. chuii* and lobster broth powders and found in only trace amounts in the white fish broth powder. In contrast, the vegan fish broth powder possessed very high amounts of the flavor enhancers GMP (8.76 mg GMP/g DW) and IMP (10.59 mg IMP/g DW). These extremely high FAAs and free 5′-nucleotides content in the broth powder containing the vegan fish flavoring can be explained by the presence of YE in the flavoring. Yeasts are produced by fermentation which involves the breakdown of complex molecules, such as proteins, DNA and RNA, into simpler compounds like amino acids and nucleotides. Additionally, the yeasts are heated to rupture the cells which enables the inherent digestive enzymes to hydrolyze sugars, proteins and nucleic acids, producing reducing sugars, amino acids, peptides and nucleotides [36]. The heating of reducing sugars and amino acids generates Strecker aldehydes (e.g. 2-methylpropanal, 3-methylbutanal, 2-methylbutanal and benzeneacetaldehyde) which are highly present in the VOC profile of the broth containing the vegan fish flavoring (Table 5).

The high amounts of free Glu in combination with the high amounts of GMP and IMP resulted in an extremely high EUC value for the vegan fish broth powder (2197.9 g MSG/100 g DW) compared to the *T. chuii* (1.17 g MSG/100 g DW), white fish (0.08 g MSG/100 g DW) and lobster (0.10 g MSG/100 g DW) broth powders (Table 6). These EUC values explain the stronger umami taste observed by the sensory panel in the vegan fish broth compared to the other broths. Despite the correction of the sea salt concentration in the broths, the Na^+ and K^+ salts were higher in the vegan fish broth compared to the other broths. This might be due to the variable salt concentrations of the YE [37]. The higher EUC value and salt content of the vegan fish broth were responsible for the higher observed salty taste and taste intensity of this broth compared to the other broths.

Because of the strong umami taste of YE, it is often utilized as a healthy salt replacer, a masker for sour and bitter tastes and a flavor enhancer, replacing traditional flavor enhancers such as MSG (E621) and disodium inosinate (E631) [36]. Although a slightly higher EUC value was calculated for the *T. chuii* broth powder compared to the animal-based seafood broth powders, no difference in umami taste was observed by the sensory panel. Possibly, the difference between the EUC

Table 6
Free amino acids, 5′-nucleotide and salt concentrations in the dried broths ($n = 3$) expressed in mg/g DW.

Compound		Taste threshold (mg/mL)[a,b,c]	*T. chuii* broth powder	Lobster broth powder	White fish broth powder	Vegan fish broth powder
Free amino acids						
Glutamic acid	Glu	0.3	2.29 ± 0.28	0.16 ± 0.00	0.10 ± 0.00	5.81 ± 0.31
Aspartic acid	Asp	1	0.24 ± 0.03	0.08 ± 0.00	0.05 ± 0.00	0.69 ± 0.04
Free 5′-nucleotides						
Adenosine monophosphate	AMP	0.5	0.18 ± 0.05	0.24 ± 0.03	0.12 ± 0.01	0.15 ± 0.02
Guanosine monophosphate	GMP	0.125	ND	ND	ND	8.76 ± 1.18
Inosine monophosphate	IMP	0.25	ND	ND	0.04 ± 0.00	10.59 ± 1.35
Equivalent umami concentration (g MSG/100 g)	EUC		1.17	0.10	0.08	2197.9
Salts						
Sodium	Na^+	1.8	110.0	110.0	110.0	160.0
Potassium	K^+	1.3	4.1	1.1	1.5	17.0

[a] Taste thresholds of free amino acids (mg/mL) in water according to Kato & Nishimura (1989) [38] and Shallenberger (1993) [39].
[b] Taste thresholds of free 5′-nucleotides (mg/mL) in water according to Yamaguchi et al. (1971) [26] and Fuke and Ueda (1996) [40].
[c] Taste thresholds of salts (mg/mL) in water according to Rotzoll et al. (2006) [41].

values of the *T. chuii* broth powder and the animal-based seafood broth powders were too low to notice a significant umami taste distinction, even by trained assessors. Possibly, a higher dosage of the *T. chuii* biomass would be necessary to profit from its umami taste properties.

3.5. Economic considerations for integrating microalgal biomass as flavoring agents in food

The additional cost to incorporate microalgal biomass in food should be reasonable. By utilizing microalgae as flavoring agents, only low incorporation levels (< 1 %) are required. Currently (year 2024), a large amount of freeze-dried *T. chuii* biomass can be purchased from a European industrial microalgal cultivator at a retail price ranging from €143.5/kg DW (produced outdoor in Portugal) to €220/kg DW (produced indoor in Belgium). This price is still higher compared to the current retail prices of white fish flavorings (€20–40/kg), lobster flavorings (€45–60/kg) and vegan fish flavoring (€30–50/kg). However, the production cost of microalgal biomass is predicted to drop by increasing the production scale and productivity of the microalgal cultivation [42]. Furthermore, dewatering of microalgae using centrifugation and freeze-drying are energy and cost-intensive processes. The production cost of microalgae could be reduced by combining ultrafiltration and spray drying [42].

4. Conclusion

The sensory assessment reveals that *T. chuii* biomass can serve as a seafood flavoring agent, effectively imparting a seafood flavor to the vegetable broth without compromising consumer appreciation of the product's aroma and flavor. The chemical flavor analysis indicates that *T. chuii* provide essential VOCs including DMS, MeSH, TMA and 4-heptenal (Z) to the broth, creating a distinct seafood aroma. Compared to animal-based seafood flavorings (white fish and lobster flavorings), the seafood flavor in the broth containing *T. chuii* biomass is milder. Although, the addition of the *T. chuii* biomass in the broth elevates the free Glu and Asp content compared to the addition of seafood flavorings, the sensory evaluation conducted by the trained panel revealed a comparable umami taste intensity in the broths. To benefit from the umami properties higher concentrations of microalga should be incorporated into the final food product. However, this will intensify the overall flavor intensity of the food product.

The consumer appreciation test shows a preference for the seafood aroma characteristics of *T. chuii* broth over the broth containing vegan fish flavoring. This preference is linked to the chicken-like and meaty-like off-odors detected by the trained sensory panel in the vegan fish flavoring, which originates from particular VOCs found in the yeast extract (YE). In comparison to the vegan fish flavoring, the umami taste characteristics of *T. chuii* biomass are less pronounced. This can be attributed to the elevated levels of free Glu and Asp along with the high amounts of free 5′-nucleotide IMP and GMP present in the vegan fish flavoring. The sensory evaluation reveals that *T. chuii* biomass is more effective in substituting for shellfish-like flavor, while the vegan fish flavoring effectively mimics the fish flavor.

This study shows that *T. chuii* proves to provide fish and shellfish flavor to plant-based broths. Further research is needed to investigate the behavior of the microalgal flavor in food products during food processing and storage. This would provide important information about the shelf-life of the algal-containing food products and further help in the development of seafood alternatives.

Statement of informed consent, human/animal rights

No conflicts or animal rights applicable. The sensory research procedures in this study followed the guidelines that constrain the use of human subjects from the Nuremberg code of ethics in medical research and the declaration of Helsinki (as revised in 2013).

Funding

This work was supported by the ValgOrize project, and is subsidized by the Interreg 2 seas programme 2014–2020 and co-financed by the European Regional Development Fund under subsidy contract Nr. 2S05-17.

CRediT authorship contribution statement

Bert Coleman: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Christof Van Poucke:** Writing – review & editing, Visualization, Validation, Supervision, Software, Methodology, Investigation, Data curation, Conceptualization. **Bavo De Witte:** Writing – review & editing, Visualization, Validation, Supervision, Software, Methodology, Investigation, Data curation, Conceptualization. **Valentina Casciaro:** Writing – original draft, Formal analysis. **Tanja Moerdijk-Poortvliet:** Writing – review & editing, Formal analysis. **Koenraad Muylaert:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Conceptualization. **Johan Robbens:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT 3.5 (OpenAI) in order to improve the writing process. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank the technicians of the marine analytical lab (MAL) of the Aquatic Environment and Quality department (ILVO) for their assistance in free amino acid analysis. Thanks to the lab technicians of the Department of Technology, Water and Environment (HZ) for their assistance in free nucleotide analysis. The authors would also like to thank Bart Ampe to help with statistical analyses and Jurgen De Meester of Flandor Flavors International for preparing the different broth powders.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2024.103538.

References

[1] B. Franks, C. Ewell, J. Jacquet, Animal welfare risks of global aquaculture. *Science*, Advances 7 (14) (2021), https://doi.org/10.1126/sciadv.abg0677.
[2] B. De Witte, B. Coleman, K. Bekaert, S. Boitsov, M.J. Botelho, J. Castro-Jiménez, A. D. Turner, Threshold values on environmental chemical contaminants in seafood in the European economic area, Food Control 138 (2022) 108978, https://doi.org/10.1016/j.foodcont.2022.108978.
[3] FAO, The State of World Fisheries and Aquaculture 2022: Towards Blue Transformation, 2022, https://doi.org/10.4060/cc0461en.

[4] M. Kazir, Y.D. Livney, Plant-based seafood analogs, Molecules 26 (6) (2021), https://doi.org/10.3390/molecules26061559.

[5] Good Food Institute. (2021). Industry update: alternative seafood. 2021, Retrieved from: Alternative Seafood Industry Update.docx (gfi.org).

[6] F. Shahidi, J.R. Botta, Seafoods: chemistry, processing technology and quality, in: Seafoods: Chemistry, Processing Technology and Quality, 1994, https://doi.org/10.1007/978-1-4615-2181-5.

[7] W. Chen, De, M. Zhang, Non-volatile taste active compounds in the meat of Chinese mitten crab (*Eriocheir sinensis*), Food Chem. 104 (3) (2007) 1200–1205, https://doi.org/10.1016/j.foodchem.2007.01.042.

[8] A. Fuentes, I. Fernández-Segovia, I. Escriche, J.A. Serra, Comparison of physico-chemical parameters and composition of mussels (*Mytilus galloprovincialis Lmk.*) from different Spanish origins, Food Chem. 112 (2) (2009) 295–302, https://doi.org/10.1016/j.foodchem.2008.05.064.

[9] DSM, Royal DSM Integrated Annual Report 2021, 2021. Retrieved from Maxavor® Fish YE wins Food Ingredients Europe 2021: Plant-based innovation category (dsm.com).

[10] Commission implementing regulation (EU) 2022/1365. Amending Implementing Regulation (EU) 2017/2470 as regards the conditions of use of the novel food *Schizochytrium* sp. oil rich in DHA and EPA. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022R1365.

[11] Diez Simon, C. (2021). Volatile characterization: optimizing GC-MS methods to reveal the chemistry and aroma of savoury food ingredients. [internal PhD, WU, Wageningen University]. Wageningen University. doi:10.18174/550790.

[12] B. Coleman, C. Van Poucke, B. De Witte, A. Ruttens, T. Moerdijk-Poortvliet, C. Latsos, K. De Reu, L. Blommaert, B. Duquenne, K. Timmermans, J. van Houcke, K. Muylaert, J. Robbens, Potential of microalgae as flavoring agents for plant-based seafood alternatives, J. Future Foods 5 (2022) 100139, https://doi.org/10.1016/j.fufo.2022.100139.

[13] B. Coleman, C. Van Poucke, B. De Witte, V. Casciaro, T. Moerdijk-poortvliet, K. Muylaert, J. Robbens, Fine-tuning the flavor of Tetraselmis chuii by modifying nitrogen supply, Algal Res. 74 (2023) 103208, https://doi.org/10.1016/j.algal.2023.103208.

[14] AECOSAN, Report of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) on a Request for Initial Assessment for Marketing of the Dried Marine Microalgae *Tetraselmis Chuii* in Food Supplements under Regulation (EC), 2017.

[15] R.R. Guillard, J.H. Ryther, Studies of marine planktonic diatoms. *I. Cyclotella nana Hustedt*, and *Detonula confervacea* (*cleve*) *gran*, Can. J. Microbiol. 8 (1962) 229–239, https://doi.org/10.1139/m62-029.

[16] B. Coleman, C. Van Poucke, B. De Witte, V. Casciaro, T. Moerdijk-Poortvliet, K. Muylaert, J. Robbens, The effect of drying, cell disruption and storage on the sensory properties of Nannochloropsis sp, Algal Research 71 (2023) 103092, https://doi.org/10.1016/j.algal.2023.103092.

[17] Isleten Hosoglu, Karagul-Yuceer Muge, Y., & Guneser, O., Aroma characterization of heterotrophic microalgae *Crypthecodinium cohnii* using solid-phase microextraction and gas chromatography–mass spectrometry/olfactometry during different growth phases, Algal Research 49 (2020) 101928, https://doi.org/10.1016/j.algal.2020.101928.

[18] Matthäus, Oxidation of edible oils, Management in Different Industry Sectors, Woodhead Publishing Series in Food Science, Technology and Nutrition 2010 (2010) 183–238, https://doi.org/10.1533/9780857090331.2.183.

[19] L. Moran, G. Bou, N. Aldai, M. Ciardi, A.M. España, A.S. Zurano, T. Lafarga, Characterisation of the volatile profile of microalgae and cyanobacteria using solid - phase microextraction followed by gas chromatography coupled to mass spectrometry, Sci. Rep. 12 (2022) 3661, https://doi.org/10.1038/s41598-022-07677-4.

[20] A. Giri, K. Osako, T. Ohshima, Identification and characterisation of headspace volatiles of fish miso , a Japanese fish meat based fermented paste , with special emphasis on effect of fish species and meat washing, Food Chem. 120 (2) (2010) 621–631, https://doi.org/10.1016/j.foodchem.2009.10.036.

[21] J. Van Durme, K. Goiris, A. De Winne, L. De Cooman, K. Muylaert, Evaluation of the volatile composition and sensory properties of five species of microalgae, J. Agric. Food Chem. 61 (46) (2013) 10881–10890, https://doi.org/10.1021/jf403112k.

[22] The Good Scents Company, TGSC Information System.website, 2023. http://www.thegoodscentscompany.com/misc/about.html.

[23] Flavornet (2004) website: http://www.flavornet.org.

[24] van 't Land, M., Fish Silage as Protein Ingredient in Animal Feeds : The Pioneering of Fishery Byproduct Utilisation in Belgium, Ghent University Faculty of Bioscience Engineering, 2019.

[25] T.C.W. Moerdijk-Poortvliet, D.L.C. de Jong, R. Fremouw, S. de Reu, J.M. de Winter, K. Timmermans, G.C.H. Derksen, Extraction and analysis of free amino acids and 5′-nucleotides, the key contributors to the umami taste of seaweed, Food Chem. 370 (2022) 131352, https://doi.org/10.1016/j.foodchem.2021.131352.

[26] S. Yamaguchi, T. Yoshikawa, S. Ikeda, T. Ninomiya, Measurement of the relative taste intensity of some L-α- amino acids and 5′-nucleotides, J. Food Sci. 36 (6) (1971) 846–849, https://doi.org/10.1111/j.1365-2621.1971.tb15541.x.

[27] O.G. Mouritsen, L. Duelund, M.A. Petersen, A.L. Hartmann, M.B. Frøst, Umami taste, free amino acid composition, and volatile compounds of brown seaweeds, J. Appl. Phycol. 31 (2) (2019) 1213–1232, https://doi.org/10.1007/s10811-018-1632-x.

[28] H. McGee, Nose Dive: A Field Guide to the world's Smells, Penguin Press, 2020.

[29] Y. Kurobayashi, E. Kouno, A. Fujita, Y. Morimitsu, K. Kubota, Potent odorants characterize the aroma quality of leaves and stalks in raw and boiled celery, Biosci. Biotechnol. Biochem. 70 (4) (2006) 958–965, https://doi.org/10.1271/bbb.70.958.

[30] M. Iranshahi, A review of volatile sulfur-containing compounds from terrestrial plants: biosynthesis, distribution and analytical methods, J. Essent. Oil Res. 24 (4) (2012) 393–434, https://doi.org/10.1080/10412905.2012.692918.

[31] K. Mahadevan, L. Farmer, Key odor impact compounds in three yeast extract pastes, J. Agric. Food Chem. 54 (19) (2006) 7242–7250, https://doi.org/10.1021/jf061102x.

[32] B.A. Smit, W.J.M. Engels, G. Smit, Branched chain aldehydes: production and breakdown pathways and relevance for flavour in foods, Appl. Microbiol. Biotechnol. 81 (6) (2009) 987–999, https://doi.org/10.1007/s00253-008-1758-x.

[33] Sharon S. Murnane, Alex H. Lehocky, P.D. Owens, Odor Thresholds for Chemicals with Established Occupational Health Standards, 2nd edition, American Industrial Hygiene Association (AIHA), 2013. Retrieved from, https://swesiaq.se/onewebmedia/Dokument/ODORTHRESHOLDS.pdf.

[34] J.C. Leffingwell, D. Leffingwell, GRAS flavor chemicals-detection thresholds, Perfumer & Flavorist 16 (1991) 1–19. Retrieved from, http://www.leffingwell.com/odorthre.htm.

[35] M. Czerny, M. Christlbauer, M. Christlbauer, A. Fischer, M. Granvogl, M. Hammer, Schieberle, P., Re-investigation on odor thresholds of key food aroma compounds and development of an aroma language based on odor qualities of defined aqueous odorant solutions, Eur. Food Res. Technol. 228 (2008) 265–273, https://doi.org/10.1007/s00217-008-0931-x.

[36] D. Tomé, Yeast extracts: nutritional and flavoring food ingredients, ACS Food Science \& Technology 1 (4) (2021) 487–494, https://doi.org/10.1021/acsfoodscitech.0c00131.

[37] M. Mohammadzadeh, E. Berizi, S.S. Shekarforoush, Influence of limited replacement of NaCl with KCl and yeast extract on microbiological, chemical, sensory, and textural properties of emulsion-type chicken sausages, Food Sci. Nutr. 9 (4) (2021) 2308–2315, https://doi.org/10.1002/fsn3.2216.

[38] H. Kato, M.R. Rhue, T. Nishimura, Role of free amino acids and peptides in food taste, Flavo Chemistry 13 (1989) 154–174, https://doi.org/10.1021/bk-1989-0388.ch013.

[39] R.S. Shallenberger, Taste of amino acids, in: *Taste Chemistry*, *London*, Blackie Academic and Professional, London,. UK, 1993, pp. 226–233.

[40] S. Fuke, Y. Ueda, Interactions between umami and other flavor characteristics, Trends Food Sci. Technol. 7 (12) (1996) 407–411, https://doi.org/10.1016/S0924-2244(96)10042-X.

[41] N. Rotzoll, A. Dunkel, T. Hofmann, Quantitative studies, taste reconstitution, and omission experiments on the key taste compounds in morel mushrooms (*Morchella deliciosa* Fr.), J. Agric. Food Chem. 54 (7) (2006) 2705–2711, https://doi.org/10.1021/jf053131y.

[42] B. Vázquez-Romero, J.A. Perales, H. Pereira, M. Barbosa, J. Ruiz, Techno-economic assessment of microalgae production, harvesting and drying for food, feed, cosmetics, and agriculture, Sci. Total Environ. 837 (2022) 155742, https://doi.org/10.1016/j.scitotenv.2022.155742.