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Flavor-boosting of *Phaeodactylum tricornutum* by fermentation with edible mushrooms

Marina Rigling[a,1,2], Jiaqi Liang[a,1,3], Isa Entenmann[a], Konstantin Frick[b], Ulrike Schmid-Staiger[b], Can Xiang[a], Lena Kopp[c], Stephan C. Bischoff[c], Yanyan Zhang[a,*,4]

[a] *Institute of Food Science and Biotechnology, Department of Flavor Chemistry, University of Hohenheim, Fruwirthstraße 12, Stuttgart 70599, Germany*
[b] *Fraunhofer Institute for Interfacial Engineering and Biotechnology (IGB), Nobelstrasse 12, Stuttgart 70569, Germany*
[c] *Institute of Clinical Nutrition, University of Hohenhei, Fruwirthstrasse 12, Stuttgart 70599, Germany*

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A B S T R A C T

Microalgae are a promising and sustainable source of nutritious food, especially for use in alternatives to fish and seafood. Among them, *Phaeodactylum tricornutum* (PT) stands out for its potential to revolutionize future diets with its rich nutrient profile and eco-friendly cultivation methods. However, its typically fishy and “brackish water” off-odor has been a significant deterrent. Using 13 basidiomycetes as starter cultures, the dynamic changes in the aroma were studied. To better understand the aroma development during fermentation, odor-active compounds were identified using headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry–olfactometry. By submerged fermentation lasting 39 and 51 hours with *Pleurotus citrinopileatus* (PCI) and *Pleurotus eryngii* (PER), respectively, the unpalatable odor of PT was transformed into savory and seafood-like aromas, while retaining most of the valuable carotenoids (fucoxanthin and β-carotene were retained at 75 % and 90 %) and fatty acids (eicosapentaenoic acid and docosahexaenoic acid were preserved at 80 % of their initial concentrations). Throughout the fermentation process, key odorants responsible for the algae’s initial green, grassy, and unpleasant odor were reduced, while compounds responsible for savory and seafood-like fragrances increased. A series of sulfur compounds, such as dimethyl disulfide, were found to be major contributors to the post-fermentation aroma.

1. Introduction

Current agriculture and food production faces significant challenges due to climate change and continuously expanding global population. The demand to feed nearly 8 billion people while minimizing the impact on the environment has sparked a movement toward innovative and sustainable food sources (Gomez-Zavaglia et al., 2020). Besides intensive research on plant-based protein alternatives, there has also been an increase in research into microalgae. Microalgae possess a series of desirable characteristics, which makes microalgae a promising alternative for future food production. Microalgae are reviewed as promising alternatives to fish and other seafood which currently suffer under high overfishing. Microalgae have been reported to reach more than 70 % protein content and have favorable amino acid composition compared to other plant proteins such as soy or chickpea (Espinosa-Ramírez et al., 2023). The high protein content of microalgae further offers the prospect of processing them into nutritional supplements, functional foods, and even as an alternative protein source (Pereira and Rodrigues, 2021). Besides, microalgae are rich in polyunsaturated fatty acids (PUFAs), especially the ω3 fatty acids docosahexaenoic acid (DHA, 22:6 *n*–3) and eicosapentaenoic acid (EPA, 20:5 *n*–3). These PUFAs are essential constituents of omega-3 fatty acids and are recognized for their beneficial

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* Corresponding author.
E-mail addresses: marina.rigling@uni-hohenheim.de (M. Rigling), jiaqi.liang@uni-hohenheim.de (J. Liang), isa.enm@gmail.com (I. Entenmann), konstantin.frick@igb.fraunhofer.de (K. Frick), ulrike.schmid-staiger@igb.fraunhofer.de (U. Schmid-Staiger), can.xiang@uni-hohenheim.de (C. Xiang), lena.stiefvatter@uni-hohenheim.de (L. Kopp), bischoff.stephan@uni-hohenheim.de (S.C. Bischoff), yanyan.zhang@uni-hohenheim.de (Y. Zhang).

[1] These authors contributed equally to this work

[2] 0000-0002-4318-8758

[3] 0000-0002-8948-5280

[4] 0000-0002-2827-8042

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effects on preventing cardiovascular health and hypertension (Yongmanitchai and Ward, 1991). The high EPA contents also are viewed as promising alternatives for PUFA supplementation of vegans, as in plant-based food available α-linoleic acid is only poorly converted into EPA in the human body (Burdge, 2006). *Phaeodactylum tricornutum* (PT) is a microalgae belonging to the diatom group. It is a unicellular photosynthetic organism of great ecological and biological importance (Basu and Mackey, 2018). PT represents a rich source of nutrient-packed biomass, offering a wide range of potential uses in the food industry. The composition of PT includes proteins (40 % in dry mass), lipids (18 % in dry mass), carbohydrates (26 % in dry mass), vitamins, and minerals, making it a promising ingredient for various products (Rebolloso-Fuentes et al., 2007; Branco-Vieira et al., 2020). Within PT, two important groups of metabolites are of particular interest — polyunsaturated fatty acids (PUFAs) and carotenoids with antioxidant properties (Yongmanitchai and Ward, 1991). PT is highly effective in production of eicosapentaenoic acid (EPA) which plays a critical role in the production of anti-inflammatory eicosanoids and cytokines and the reduction of reactive oxidative species (ROS) in the body. PT is known for its high content in the xanthophyll fucoxanthin, a carotenoid which does not show provitamin A activity. Consumption of fucoxanthin is related with beneficial health effects such as neuroprotective, antioxidant and antiproliferative effects (Mohibbullah et al., 2018). Besides fucoxanthin, PT is also rich in the provitamin A-active β-carotene which is important for the visual sense and also shows antioxidant potential (Chang et al., 2013). However, the aroma of microalgae is highly complex. It is often described as possessing fishy, grassy, and seafood notes, with an unpleasant "brackish water" off-odor, which decreases consumer acceptance. However, cultivation environment and parameters significantly influence the final aroma perception (Wang et al., 2023; Urlass et al., 2023). This emphasizes the importance of improving the odor and flavor of PT for food applications.

For thousands of years, fermentation has played an important part in the processing of food. During fermentation such as by bacterial strains and yeasts, the food can change its flavor, nutritional, and/or structural properties, or new foods can be created through complete transformation (Carballo, 2012). Basidiomycete-mediated fermentation represents a novel and intriguing method in food processing. As members of the higher fungi phylum, basidiomycetes encompass a wide array of over 40,000 species (He et al., 2022). The secretome of basidiomycetes, containing proteases, lipases, carbohydrate-active enzymes, secreted proteins of unknown function, and small-secreted proteins, is a particularly promising source for producing natural flavors through processes such as *de novo* synthesis and biotransformation (Jia et al., 2023). These characteristics have been successfully applied in various research studies. For example, Zhang et al. (Zhang et al., 2014) demonstrated pleasant fruity and plum-like flavors were produced through *Lentinula edodes*-mediated fermentation of wort. In addition, Rigling et al. (Rigling et al., 2021) reported that fermentation of green tea with *Wolfiporia cocos* not only resulted in the desired jasmine tea aroma profile, but also preserved the nutritional quality of the product, especially in terms of its antioxidant properties. However, to the best of our knowledge, no research on the flavor modulation of microalgae by basidiomycete exists. In recent research, microalgae have been fermented using *Bacillus subtilis* and *Lactobacillus*, a process which reduces off-odors and yields creamy flavor compounds, as revealed through SPME analysis (Hung et al., 2023). Nevertheless, it was not possible to maintain or produce a characteristic fishy odor of the microalgae which might be important for future application as alternative fish or seafood applications. Therefore, a basidiomycete-mediated fermentation process can be an effective approach to reduce the unpleasant microalgae flavor while preserving the nutritional values of PT.

In this study, the fermentation process of a PT algae suspension was screened with thirteen species of basidiomycetes to select a desired aroma profile at first. Subsequently, the aroma compounds of non-fermented and fermented algae suspension were determined. In detail, the odorants were identified and semi-quantified by means of headspace solid-phase microextraction combined with gas chromatography–mass spectrometry–olfactometry (HS-SPME–GC–MS–O). The corresponding odor activity values (OAVs) of each component from both samples were analyzed and compared. To evaluate the nutritional value of the fermented suspension, carotenoid content (fucoxanthin and β-carotene) and fatty acids profile were analyzed.

2. Materials and methods

2.1. Chemicals and materials

Decanal (96 %), dimethyl disulfide (99 %), heptanal (97 %), neral (95 %), nerolidol (97 %), 1-octen-3-ol (98 %), 2-pentanone (99 %), 1-penten-3-one (97 %), 2-pentylfuran (98 %), and sulcatone (98 %) were purchased from Alfa Aesar (Karlsruhe, Germany). *o*-Cymene (analytical standard), geraniol (98 %), 2-nonanol (99 %), 2-nonenal (97 %), octanal (99 %), 3-octanone (98 %), 1-octen-3-one (96 %), and α-pinene (98 %) were purchased from Sigma Aldrich (Steinheim, Germany). Benzaldehyde (99.5 %), β-ionone (100 %), isoamyl alcohol (98.5 %), limonene (95 %), linalool (100 %), and thymol (99 %) were purchased from Carl Roth (Karlsruhe, Germany). Dimethyl trisulfide (98 %) and *trans*-2-octenal (95 %) were purchased from J&K Scientific GmbH (Pforzheim, Germany). 2-Ethyl-1-hexanol (99 %), hexanal (99 %), and 2-undecanone (98 %) were purchased from Merck KaGaA (Darmstadt, Germany). Bornyl acetate (97 %) was purchased from Thermo Fisher (Sindelfingen, Germany), 4-nonanone (98 %) was purchased from TCI (Zwijndrecht, Belgium), and 2-tridecanone (95 %) was purchased from BLD Pharm (Reinbek, Germany). Ethanol (99.9 %) was purchased from VWR Chemicals (Bruchsal, Germany). Basidiomycota strains *Ganoderma lucidum, Laetiporus sulphureus, Pholiota nameko, Pleurotus citrinopileatus, Pleurotus eryngii*, and *Trametes versicolor* were acquired from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig). *Bjerkandera adusta* and *Stereum hirsutum* were obtained from the Institute of Food Chemistry and Biotechnology, Justus-Liebig-University Giessen (LCB, Giessen, Germany). *Lentinula edodes, Mycetinis scorodonius, Polyporus umbellatus*, and *Wolfiporia cocos* were provided by the Centraal Bureau voor Schimmelcultures (CBS, Utrecht, Netherlands) and *Agrocybe aegerita* was obtained from the International Graduate Institute (IHI, Zittau, Germany).

2.2. Preparation of algae suspension

Freeze-dried PT powder (after cell disruption in a cooled ball mill (4 °C)) was provided from Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB (Stuttgart, Germany). To prepare the 10 g/L algae suspension, 1 g of PT powder was added into a 250-mL Erlenmeyer flask. Prior to adding 100 mL of water to the flask, the water was subjected to a heating phase, brought to boiling point for 30 seconds, and then cooled to 80 °C. The flasks were then immediately cooled to prevent any potential heat impairment of the algae. Algae solutions were prepared fresh prior to each fermentation experiment.

2.3. Fermentation procedure

Basidiomycetes (1st generation) were cultivated on agar plates at 24 °C with either 30 g/L malt extract, 3 g/L soy peptone, and 15 g/L agar or 20 g/L malt extract and 15 g/L agar, varying according to the species of basidiomycetes, for 7 days until over 80 % of the agar surface was covered. This growth was then used as the seed culture for the pre-culture medium (30 g/L malt extract and 3 g/L soy peptone, or 20 g/L malt extract, varying based on the species of basidiomycetes). A piece of mycelia (1 cm × 1 cm × 0.5 cm) from the outer edge of the mycelia on the agar plate was transferred to 100 mL of pre-culture medium within 250-mL Erlenmeyer flasks. The mixture was homogenized using an Ultra Turrax T25 homogenizer (IKA, Staufen, Germany) for 10 seconds at

10,000 rpm. Subsequently, the pre-culture was placed in an Innova 42 R incubator shaker at 150 rpm for 7 days in darkness. Ten-millimeter mycelia from the pre-culture were then separated from the media broth by centrifugation (10 min, 2150 *g*, 22 °C) and washed three times with sterile water. The mycelia were resuspended in 10 mL sterile water and transferred into an Erlenmeyer flask containing 100 mL of algae suspension for fermentation. The main culture was incubated in an Innova 42 R incubator shaker (Eppendorf, Hamburg) at 150 rpm and 24 °C in darkness. The sampling of the fermented suspensions was carried out every 2–4 h under sterile conditions until 101 h. To separate the basidiomycota mycelium from the liquid algae suspension culture broth, the suspension was filtered using membrane filters (0.2 µm PES membrane; Merck KaGaA, Darmstadt, Germany) and a VP 220 vacuum pump (VWR, Darmstadt). The supernatant was stored at –20 °C until further analysis.

2.4. Sensory analysis

A total of 24 samples between 0 h and 101 h fermentation time were collected for each basidiomycete. After thawing the stored samples at room temperature for 15 min, they were sensorily described by at least three experienced assessors ($n = 3$, all female, mean age 31) from the University of Hohenheim (Stuttgart, Germany). Panelists underwent sensory training, including odor recognition with standard substances, intensity rating and descriptive vocabulary specialized for evaluation of algal samples for at least one week within the Department of Flavor Chemistry (University of Hohenheim, Stuttgart, Germany). Samples were evaluated in an overall agreement set-up using common descriptors found in the literature (van Durme et al., 2013). Samples with pleasant and potent odors at specific time points were further evaluated by tasting (sip and spit-out). The odor and flavor overall intensity was rated on a unipolar scale of 1–5 (1, very weak; 5, very intense). The promising samples with interesting flavors were re-generated at least three times in the fermentation experiments. According to the odor attribute (savory, seafood-like, fishy, and mild preferred) and odor intensity (intense preferred), the specific fermentation time for each species was defined.

2.5. Headspace solid-phase microextraction (HS-SPME)

Three different samples (non-fermented algae suspension and algae suspension fermented with *Pleurotus citrinopileatus* (PCI) and *Pleurotus eryngii* (PER) were selected for further aroma analysis by headspace solid-phase microextraction combined with gas chromatography–mass spectrometry–olfactometry (HS-SPME–GC–MS–O). For manual HS-SPME a CAR/PDMS (Carboxen™/polydimethylsiloxane, 30/50 µm, 1 cm fiber length) from Supelco (Steinheim, Germany) was utilized. A volume of 7.5 mL of the supernatant sample was added to a 20-mL headspace vial. The sample was incubated for 15 min (250 rpm) at 45 °C, followed by a 30-min extraction at 45 °C. Subsequently, the analytes were desorbed in a thermal desorption unit (TDU). After desorption, the fiber was cleaned at 250 °C for 10 min. Each sample was tested in triplicate (Rigling et al., 2022; Rigling et al., 2023).

2.6. Gas chromatography–mass spectrometry–olfactometry (GC–MS–O)

Gas chromatography (GC) was performed with an Agilent 8890 GC gas chromatograph equipped with thermal desorption unit (TDU) and cooled injection system (CIS) connected to a 5977B mass spectrometry detector (MSD) (Agilent Technologies, Waldbronn, Germany) as well as to an OPD4 olfactory detection port (GERSTEL GmbH & Co KG, Mülheim a. d. Ruhr, Germany). The TDU was equipped with a standard TDU liner (OD 6 mm, ID 4 mm, L 60 mm) (Gerstel, Mühlheim an der Ruhr, Germany). Desorption started at a temperature of 40 °C (1 min) and then ramped at 120 °C/min to 150 °C, and held for 1 min. The cryo-focusing was performed in a CIS (Gerstel) equipped with a glass wool liner (OD 3 mm, ID 2 mm, L 71 mm) with liner-in-liner principle with the TDU liner (Gerstel) in solvent vent mode (40 mL/min). The analytes were released to the gas chromatography system in the CIS with a temperature program starting at 50 °C (1 min), then increased at 720 °C/min to 175 °C, and finally held for 2 min. A J&W polar DB-WAXms column (30 m × 0.25 mm × 0.25 µm film thickness; Agilent Technologies, Waldbronn, Germany) was installed. Helium (5.0) (Westfalen, Münster, Germany) served as carrier gas with a constant flow rate of 1.2 mL/min. The GC oven temperature was initially held at 40 °C (3 min) and then ramped at 5 °C/min to 240 °C (10 min). Split ratio was set to 1:1 between MSD and ODP by a µFlowManager splitter (Gerstel) with a column outlet pressure of 20 kPa. The following parameters were applied for analysis: MS mode, scan; scan range, m/z 33–330; electron ionization energy, 70 eV; source temperature, 230 °C; quadrupole temperature, 150 °C. ODP mixing chamber temperature at 150 °C; N_2 (Westphalia) was used as ODP4 make-up gas. Data collection was accomplished using the software Agilent MassHunter B.07.06 combined with the Gerstel Maestro (Rigling et al., 2022; Rigling et al., 2023).

2.7. Compound identification

The odor-active compounds were identified based on their characteristic odors perceived at the ODP, retention indices (RI) on a polar column, and their mass spectra. These were then compared with those of authentic standards and data available in the MS database (NIST17) and the literature.

2.8. Semi-quantification and calculation of odor activity values (OAVs)

The determined compounds of non-fermented and fermented algae suspension were semi-quantified by internal standard (IS) method. Twenty-five microliter internal standard (thymol, concentration = 6.82 mg/L) were spiked to 7.5 mL of the algae sample. The mixed samples were extracted under the same HS-SPME conditions as *Section 2.5* . The corresponding response factors were determined for each analyte by a mixture of authentic standards in aqueous solutions with ethanolic water. The odor activity values (OAVs) of the compounds were calculated by dividing the calculated concentration in the samples to their odor threshold in water obtained from literature. Each sample was measured in triplicate.

2.9. Analysis of carotenoids and fatty acids

For the analysis of the carotenoid and fatty acid content, the biomass samples were dried and a cell disruption was carried out using a homogenizer (Precellys 24; Bertin Technologies, Montigny-le-Bretonneux, France). The carotenoid content was analyzed using HPLC (1200 Infinity; Agilent, Santa Clara, CA) as described by Derwenskus et al. (Derwenskus et al., 2019) using the method of Gille et al. (Gille et al., 2016).

The fatty acid content was determined using the transesterification method from Lepage and Roy (Lepage and Roy, 1984). The analysis was done using a gas chromatograph (7890 A; Agilent) as previously described (Meiser et al., 2004; Lepage and Roy, 1984).

2.10. Statistical analysis

Statistical analysis was performed using SPSS 27.0 for Windows (SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) and paired t-test were conducted for all analyses, A level of $p < 0.05$ was considered as statistically significant.

3. Results and discussion

3.1. Fermentation screening and sensory evaluation

Thirteen distinct basidiomycetes were screened for fermentation of *Phaeodactylum tricornutum* (PT). Significant aroma changes were noted with most of these fungi. Throughout the fermentation process, both the quality and intensity of the odor and taste varied. Table 1 exhibits the most characteristic and intense odor and taste impressions detected during fermentation. Remarkably, the strong musty, "brackish water"-like, and green characteristic odor of algae suspension diminished greatly or even vanished entirely after submerged fermentation with certain basidiomycetes (e.g., *Pleurotus eryngii, Stereum hirsutum* or *Trametes versicolor*). Simultaneously, a range of appealing and interesting odor impressions were perceived, such as sea-like (e.g., *Polyporus umbellatus, Wolfiporia cocos, Pleurotus eryngii,* and *Agrocybe aegerita*), seafood-like (e.g., *Pleurotus citrinopileatus*), savory (e.g., *Pleurotus citrinopileatus, Wolfiporia cocos*, and *Pleurotus eryngii*), mild (*Pleurotus citrinopileatus*, and *Lentinula edodes*).

Within the basidiomycetes that produced pleasant aromas, *Polyporus umbellatus, Wolfiporia cocos,* and *Agrocybe aegerita* were observed to reduce the intensity of the "brackish water" and green notes in the algae suspension. However, *Polyporus umbellatus* and *Wolfiporia cocos* also produced an unpleasant stinging odor, with *Agrocybe aegerita* failing to diminish the musty odor. The cultures generated a diverse range of odor impressions within the brief fermentation period of up to 4 days. This phenomenon is similar to findings in previous research. Hung et al. (Hung et al., 2023) studied the capability of two microorganisms *(Bacillus subtilis* and *Saccharomyces cerevisiae*) to change odors in green and brown seaweed media. Unpleasant odors (e.g., grassy, fatty, and fishy) were weakened and floral, fruity, and sweet odors were enhanced after an incubation period of 0–3 days. Nevertheless, the seafood-like and savory scents were not generated or preserved. Besides, products derived from the fermentation of microorganisms pose greater safety risks (Ağagündüz et al., 2022). Additionally, the non-fermented algae suspension as blank, did not exhibit a "brackish water" off-odor after 101 hours of fermentation, while a solvent-like off-odor note was observed, highlighting the significant role driven by basidiomycetes. After the sensory evaluation by tasting, the results aligned with Coleman et al., (Coleman et al., 2022), who noted that the non-fermented PT possesses a pronounced umami taste. The fermented algae suspension with *Pleurotus citrinopileatus, Pleurotus eryngii*, and *Trametes versicolor* were viewed as the most pleasant ones, while the nutty and cereal-like odor intensity generated from *Trametes versicolor* was very weak. Compared to the substrate algae, the odor of *Pleurotus citrinopileatus* and *Pleurotus eryngii* culture broth from the typical umami, fishy, "brackish water", and salty (sea-like) odor turned to a pleasant, mild, seafood-like, savory, and no "brackish water" odor. Collectively, *Pleurotus citrinopileatus* (PCI) and *Pleurotus eryngii* (PER) emerge as excellent candidates for creating a new fermentation system. Their appealing odors have garnered significant attention recently. Hence, the algae suspension, modified in odor through fermentation, is expected to have high consumer acceptance and high market potential.

Table 1
Odor impression and flavor impression of PT suspension after fermentation with different basidiomycetes.

No.	Species	Fermentation time (h)	Odor impression	Flavor impression
1	Blank	0	musty, brackish water, fishy, green (I = 4)[a]	umami, fishy, brackish water, salty (I = 3)
2	*Bjerkandera adusta*	27	savory, sea-like, fruity (I = 2)	umami, fishy, sourish (I = 2)
3	*Polyporus umbellatus*	27	sea-like, stinging (I = 4)	salty, sour, umami (I = 3)
4	*Pleurotus citrinopileatus*	39	seafood-like, savory, mild (I = 4)	umami, seafood-like, mild (I = 4)
5	*Wolfiporia cocos*	39	savory, sea-like, stinging (I = 4)	sour, umami (I = 5)
6	*Stereum hirsutum*	44	mild, fishy, sweetish (I = 1)	mild, fishy, slightly bitter (I = 1)
7	*Ganoderma lucidum*	46	fishy, sea-like, citrus-like (I = 2)	green, sour, savory (I = 4)
8	*Pholiota nameko*	48	savory, broth-like, fishy (I =2)	savory, fishy, bitter (I = 3)
9	*Pleurotus eryngii*	51	savory, sea-like, fishy (I = 4)	pleasant, savory, fishy (I = 4)
10	*Trametes versicolor*	64	nutty, cereal-like, alcoholic (I = 1)	nutty, cereal-like (I = 1)
11	*Laetiporus sulphureus*	67	fresh, sea-like (I = 3)	algae, fishy, bitter (I = 2)
12	*Mycetinis scorodonius*	68	mild, cereal-like, fish feed-like (I = 1)	stale, savory, fishy (I = 4)
13	*Agrocybe aegerita*	76	musty, sea-like, fishy (I = 4)	musty, fishy, stale (I = 2)
14	*Lentinula edodes*	95	mild, cereal-like, soy sauce (I = 3)	savory, slightly bitter (I = 3)
15	Blank	101	algae, fishy, musty, solvent-like (I = 3)	umami, fishy, musty, salty (I = 2)

[a] I = intensity; 0 odorless/tasteless;1 very weak; 2 weak; 3 moderate; 4 intense; 5 very intense.

3.2. Qualitative investigation of aroma profile

Aroma-active compounds extracted from non-fermented and fermented microalgae samples by HS-SPME were analyzed using GC–MS–O. As shown in Table 2, a total of 88 odor-active regions were perceived by two trained assessors, with 54 of these being identified based on retention indices (RI), odor characteristics, and comparison with authentic standards where available. The aroma-active compounds comprise six major chemical groups including aldehydes, ketones, alcohols, terpenes, sulfur compounds, and aromatic hydrocarbons. Unfortunately, 34 aroma-active regions from the non-fermented or fermented samples could not be identified due to too weak or unclear MS signals so far. Further investigations should be conducted, to help identify the remaining aroma compounds to complete the aroma profiles of the different samples.

Odor impressions within the non-fermented algae samples were predominantly green or grassy volatiles, such as hexanal and α-pinene. A considerable presence of sweet or even floral volatiles was also detected, including geraniol and β-ionone. Furthermore, the fermented samples contained fishy, sharp, and garlic-like sulfide compounds, such as dimethyl trisulfide and trimethylamine. In detail, 41, 36, and 50 odor-active compounds were detected in the blank, PCI-, and PER-fermented samples, respectively. During fermentation with PCI, the compounds transitioned from grassy and green to savory and wheat-like odor impressions, with certain compounds (e.g., α-pinene and limonene) being undetectable after fermentation, with newly perceived aromas (e.g., dimethyl trisulfide, safranal, and zingiberene) post-fermentation. After the fermentation with PER, the dominant odor impression of algae suspension was sweetish, fruity, and savory, thereby strikingly different from that of non-fermented product. The absence of the sweetish and fruity odors in sensory evaluation is likely related to overlay or masking by the dominant savory and fishy odors. Furthermore, 14 and 22 specific odorants were detected in algae fermented by PCI and PER, respectively, with only 6 components common to both after fermentation, namely acetone (sharp and wheat-like odor impression), 2-butanone (grainy and cereal-like odor impression), 6-methyl-2-heptanone (fruity and flowery odor impression), 1-octen-3-

Table 2
Aroma-active compounds detected in non-fermented, PCI-, and PER-fermented PT microalgae samples.

	RI[a]	RI standard	Compound	Odor impression	Blank	PCI	PER	Identifier
1	< 800	< 800	*Unknown*	savory				O
2	< 800	< 800	Trimethylamine	fishy, fermented			x[b]	O, MS, RI
3	< 800	< 800	*Unknown*	savory, sweetish	x			O
4	802	802	Acetone	sharp, wheat-like		x	x	O, MS, RI
5	897	897	2-Butanone	grainy, cereal-like, grassy, sharp		x	x	O, MS, RI
6	937	937	Ethanol	sharp, grassy	x		x	O, MS, RI
7	980	980	3-Pentanone	rotten, sweetish	x	x	x	O, MS, RI
8	1007	1007	Benzene	musty, chemical, sweetish	x		x	O, MS, RI
9	1018	1019	3-Methyl−2-pentanone	grassy, stinging, chemical			x	O, MS, RI
10	1022	1022	*α*-Pinene	grassy, green	x			O, MS, RI
11	1022	1022	1-Penten−3-one	stinging, sharp, fishy			x	O, MS, RI
12	1056	1056	3-Hexanone	sharp, unknown			x	O, MS, RI
13	1074	1074	Dimethyl disulfide	savory, sharp			x	O, MS, RI
14	1082	1082	Hexanal	chemical, grassy, green	x	x	x	O, MS, RI
15	1089	n.d.	2-Methyl−2-butenal	stinging, chemical, rotten	x	x	x	O
16	1096	1093	Isopropyl vinyl ketone	chemical, playdough-like	x		x	O, MS, RI
17	1129	1127	2,3-Hexanedione	sweetish			x	O, MS, RI
18	1134	1134	*trans*−2-Pentenal	chemical, sweetish			x	O, MS, RI
19	1145	n.d.	*Unknown*	chemical, herbal			x	O
20	1173	1173	1-Penten−3-ol	sweetish		x		O, MS, RI
21	1187	1187	Heptanal	grassy	x	x		O, MS, RI
22	1196	1196	Limonene	sweetish, grassy	x			O, MS, RI
23	1219	1219	Isoamyl alcohol	tard, musty	x	x		O, MS, RI
24	1226	1226	2-Pentylfuran	green, beany		x		O, MS, RI
25	1241	1237	6-Methyl−2-heptanone	tutti frutti, flowery, playdough-like		x	x	O, MS, RI
26	1258	1258	3-Octanone	savory, acidic, sharp		x		O, MS, RI
27	1260	1260	Styrene	sweetish	x			O, MS, RI
28	1273	1272	*o*-Cymene	sweetish, savory	x			O, MS, RI
29	1291	1291	Octanal	green, savory			x	O, MS, RI
30	1295	n.d.	*Unknown*	fishy, algae-like			x	O
31	1298	n.d.	*Unknown*	fishy, algae-like	x			O
32	1303	1303	1-Octen−3-one	savory, mushroom-like, mussel-like		x	x	O, MS, RI
33	1325	1326	2-Methyl−3-octanone	sweetish, wheat-like			x	O, MS, RI
34	1331	1322	4-Nonanone	savory			x	O, MS, RI
35	1342	1342	Sulcatone	baked rice-like	x	x	x	O, MS, RI
36	1394	n.d.	*Unknown*	green, savory	x			O
37	1375	n.d.	*Unknown*	savory	x		x	O
38	1380	n.d.	Ectocarpene	green, fishy, sulphurous	x	x		O
39	1386	1386	Dimethyl trisulfide	garlic-like, fishy, fermented		x	x	O, MS, RI
40	1404	n.d.	*Unknown*	sweetish			x	O
41	1418	n.d.	*Unknown*	chemical		x		O
42	1422	n.d.	*Unknown*	wheat-like	x			O
43	1437	1437	*trans*−2-Octenal	straw-like, hay-like, grassy	x	x	x	O, MS, RI
44	1455	1455	1-Octen−3-ol	hay-like, earthy, mushroom-like		x	x	O, MS, RI
45	1469	n.d.	Sirenin	vegetables, sea-like	x	x	x	O
46	1488	1488	2-Nonanol	musty	x			O, MS, RI
47	1494	1494	2-Ethyl−1-hexanol	sweetish			x	O, MS, RI
48	1501	1502	Decanal	green, grassy, savory	x	x		O, MS, RI
49	1520	1520	Linalool	sweetish, wheat-like	x			O, MS, RI
50	1531	1531	Benzaldehyde	cake-like, cookies-like	x	x	x	O, MS, RI
51	1543	1543	2-Nonenal	stinging, green			x	O, MS RI
52	1546	n.d.	*Unknown*	sourish, fruity			x	O
53	1570	n.d.	*Unknown*	savory, bread crust-like, sweetish			x	O
54	1589	n.d.	*Unknown*	cake-like, bread-like, sweetish	x			O
55	1591	1591	Bornyl acetate	herbal, playdough-like	x	x	x	O, MS, RI
56	1603	1603	2-Undecanone	sweetish, fruity		x		O, MS, RI
57	1614	n.d.	*Unknown*	sweetish, fruity, melon-like			x	O
58	1633	1633	β-Cyclocitral	sweetish, herbal			x	O, MS, RI
59	1657	1648	Safranal	savory, hay-like		x		O, MS, RI
60	1657	1659	Cryptone	grain-like, green	x			O, MS, RI
61	1672	1673	Neral	grassy, sharp	x	x	x	O, MS, RI
62	1701	n.d.	*Unknown*	savory, wheat-like		x		O
63	1726	1738	Zingiberene	wheat-like, woody		x		O, MS, RI
64	1740	n.d.	*Unknown*	chemical, playdough-like		x		O
65	1796	1796	Myrtenol	herbal, wheat-like, grassy	x		x	O, MS, RI
66	1815	1815	2-Tridecanone	sweetish, stinging	x	x	x	O, MS, RI
67	1850	1850	Geraniol	musty, perfume, sweetish	x	x	x	O, MS, RI
68	1879	n.d.	*Unknown*	musty			x	O
69	1952	1952	β-Ionone	perfume, cotton candy-like	x	x	x	O, MS, RI
70	1960	n.d.	*Unknown*	ashy	x			O
71	1963	n.d.	*Unknown*	ashy			x	O
72	1972	n.d.	*Unknown*	sweetish, perfume	x			O
73	2005	2007	β-Ionone epoxide	sweetish		x		O, MS, RI
74	2044	2044	Nerolidol	sweetish, perfume, cotton candy-like	x		x	O, MS, RI

(*continued on next page*)

Table 2 (*continued*)

	RI[a]	RI standard	Compound	Odor impression	Blank	PCI	PER	Identifier
75	2063	n.d.	*Unknown*	tart, woody			x	O
76	2073	n.d.	*Unknown*	green, sea-like			x	O
77	2089	2088	Elemol	fruity, pineapple-like	x			O, MS, RI
78	2104	n.d.	*Unknown*	sweetish, pie-like, fresh		x		O
79	2109	n.d.	*Unknown*	fresh, light sweetish	x			O
80	2132	n.d.	*Unknown*	fishy			x	O
81	2155	n.d.	*Unknown*	herbal, green		x		O
82	2157	n.d.	*Unknown*	tart	x			O
83	2190	n.d.	*Unknown*	fishy			x	O
84	2216	n.d.	*Unknown*	tart	x			O
85	2233	n.d.	*Unknown*	herbal, green		x		O
86	2234	n.d.	*Unknown*	playdough-like, chemical, fruity			x	O
87	2297	n.d.	*Unknown*	herbal, flowery		x		O
88	2300	n.d.	*Unknown*	tart, alcoholic	x			O

[a] retention indices;
[b] "×" perceivable; identifier: O olfactometry, MS mass spectra authentic standard or literature; RI retention index.

one (savory and mushroom-like odor impression), dimethyl trisulfide (garlic-like and fishy odor impression), and 1-octen-3-ol (hay-like and earthy odor impression), reflecting the specificity of basidiomycetes. Compared to 41 components detected in the blank sample, Prestegard et al., (Prestegard et al., 2015) and Uzlasir et al., (Uzlasir et al., 2023) identified 61 and 34 aroma compounds in different strains of PT, respectively. This inconsistency could be due to the specificity of the samples and the differences in cultivation and pre-treatment methods. Besides, key seafood-like aroma compounds, including those derived from fatty acids and trimethylamine, as well as the typical green and grassy odorant decanal, were also identified here (Coleman et al., 2022; Nunes et al., 2023). However, it is worth mentioning that trimethylamine was only detected in samples fermented with PER, the extraction method utilized might not be suitable for its identification due to the rapid elution and volatility, which resulted in the inability to verify it using RI and authentic standards (Bekhit et al., 2021). Moreover, dimethyl sulfide, a critical aroma compound in microalgae, was not detected in this study, the reason for this is that the high volatility and low RI of dimethyl sulfide, which provided a challenge for the mass spectrometry (MS) detector used. Additionally, some of the typical microalgae volatiles detected in the samples, such as ectocarpene and sirenin, for which authentic standard and RI data were unavailable, making their final identification difficult.

3.3. Quantitative investigation of aroma profile

To better understand the odorants variation and the savory as well as pleasant odor generated by PCI and PER, the relative changes in concentrations of forty-nine aroma compounds were semi-quantified before and after fermentation. Subsequently, the odor activity value (OAV) was determined for each compound using its odor threshold (OT) in water, as reported in the literature (Table 3). All quantified compounds have been categorized into different chemical groups.

As shown in Table 3, the concentration of the grain-like odorant, 2-butanone, was highest in both blank and PER-fermented samples. Besides, in the PCI-fermented sample, safranal, characterized by an herbal and savory odor, exhibited the highest concentration. Regarding the concentration of the different chemical groups in the non-fermented algae suspension, the aldehydes including hexanal, 2-methyl-2-butenal, and *trans*-2-pentenal, are worth mentioning, as they exhibited high values. Particularly noticeable ketones were 2-butanone, 3-pentanone, 3-methyl-2-pentanone, 1-penten-3-one, 3-hexanone, 2,3-hexanedione, 6-methyl-2-heptanone, and 2-methyl-3-octanone, as well as cryptone, *β*-ionone, and *β*-ionone epoxide. In addition, isoamyl alcohol and 1-octen-3-ol displayed relatively high concentrations in alcohols. The overall profile also contained high concentrations of terpenes, including *α*-pinene, ectocarpene, sirenin, safranal, elemol, and zingiberene. Benzene was also present in noteworthy amounts in hydrocarbons. In terms of the variation in aroma compounds resulting from fermentation with the two fungi, nineteen aroma compounds demonstrated a significant increase, whereas the concentration of seventeen odorants significantly decreased after fermentation with PCI. Similarly, fermentation with PER resulted in a significant increase in twenty-one different aroma compounds, while another twenty-one odorants saw a significant decrease in concentration. Among them, sixteen compounds (3-octanone, dimethyl trisulfide, benzaldehyde, 2-nonanol, dimethyl disulfide, 1-octen-3-one, 2-tridecanone, 3-hexanone, 2-methyl-3-octanone, sirenin, *trans*-2-pentenal, 2-undecanone, 2-ethyl-1-hexanol, nerodidol, styrene, and zingiberene) exhibited a common increase, while fifteen compounds (heptanal, hexanal, 2-pentylfuran, *o*-cymene, 1-penten-3-one, 2-butanone, benzene, 2,3-hexanedione, 6-methyl-2-heptanone, ectocarpene, 2-methyl-2-butenal, cryptone, *α*-pinene, safranal, and *β*-ionone epoxide) demonstrated a concurrent decrease. Within the three distinct samples subjected to assessment, concentration analysis plays a fundamental role in the identification of key aroma compounds and offers valuable insights into the aroma composition of the analyzed samples. However, the calculation of OAVs further enhances these insights. OAVs serve as a critical tool for discerning the most influential odorants within a sample, achieved by juxtaposing the concentration of each compound against its odor threshold (OT) (Cadwallader, 2007). Odorants with elevated OAVs emerge as dominant contributors to the overall aroma profile. As can be observed, the compound with the highest concentration, 2-butanone, displayed very low OAVs (< 1) due to its very high OT, indicating its grainy odor contributes minimally to the overall aroma of the algae samples. The results also revealed that *β*-ionone (OAV 27741, 29621, and 27383 in three samples, respectively), 1-octen-3-one (OAV 869, 1943, and 2015 in three samples, respectively), and dimethyl trisulfide (OAV 995 in PER-fermented sample) were the most important contributors to the overall odor impression of the algae suspensions. One unexpected finding was the extent to which *β*-ionone (perfume-like odor impression) consistently emerged as the compound with the highest OAV across all samples. A possible explanation could be that *β*-ionone is produced through the degradation of both α-carotene and β-carotene; consequently the amount of β-ionone is substantial within the samples as the algae PT is rich in carotenoids (Francezon et al., 2021). Intriguingly, the OAVs of β-ionone did not change much after fermentation, although it has been suggested that β-ionone is a preferred product of carotenoid degradation by basidiomycete enzymes (Paparella et al., 2021). This implies that the basidiomycetes may have preferred to degrade other compounds (e.g., β-cyclocitral) instead of β-ionone within the samples, which is also identified as degradation product (Rigling et al., 2021). 1-Octen-3-ol is one of the main contributors to the typical mushroom-like odor (Heuger et al., 2015). In the overall sensory evaluation of the fermented samples, no clear mushroom-like smell could be detected even though concentration and OAV increased by the factor 2. However, the increased levels of 1-octen-3-ol might have some

Table 3

Quantitative distribution of aroma-active compounds detected in non-fermented, PCI-, and PER-fermented PT microalgae samples.

			Blank		PCI		PER	
	Compound	**OT (ppb)**	**c (μg/L)**	**OAV**	**c (μg/L)**	**OAV**	**c (μg/L)**	**OAV**
	Aldehydes:							
1	Hexanal	5^b	642.32±97.31	128	494.33±32.91^m	99	208.35±19.09^{m,n}	42
2	2-Methyl−2-butenal^s	500^d	359.50±18.81	<1	296.78±21.94^m	<1	26.76±2.60^{m,n}	<1
3	*trans*−2-Pentenal^s	1.5^j	261.11±23.85	174	343.16±51.03^m	229	516.80±34.95^{m,n}	345
4	Heptanal	3^b	43.98±1.96	15	16.59±0.79^m	6	5.14±0.76^{m,n}	2
5	Octanal	0.7^b	4.61±0.06	7	5.91±0.11	8	3.28±0.38	5
6	*trans*−2-Octenal	3^b	1.79±0.02	<1	4.23±0.05^m	1	2.82±0.03	1
7	Decanal	2^b	3.37±0.05	2	4.05±0.05	2	2.97±0.03	1
8	Benzaldehyde	350^b	15.89±0.58	<1	507.92±47.02^m	1	386.06±56.20^{m,n}	1
9	2-Nonenal	58^d	0.11±0.01	<1	0.23±0.01	<1	0.78±0.01^m	<1
10	Neral	30^h	1.18±0.03	<1	0.63±0.01	<1	6.71±0.41^m	<1
	Ketones:							
11	2-Butanone^s	50000^a	6800.43±1144.25	<1	2036.11±22.76^m	<1	3588.45±620.81^{m,n}	<1
12	3-Pentanone	70000^a	738.12±89.81	<1	642.17±78.08	<1	325.46±40.25^{m,n}	<1
13	3-Methyl−2-pentanone^s	n.a.	263.65±23.06	n.a.	106.32±8.39^m	n.a.	977.63±94.64^{m,n}	n.a.
14	1-Penten−3-one	1.3^d	410.60±30.34	316	312.15±30.01^m	240	245.26±39.23^{m,n}	189
15	3-Hexanone^s	41^d	173.16±24.74	4	479.41±50.97^m	12	520.60±46.56^m	13
16	2,3-Hexanedione^s	n.a.	1190.09±92.13	n.a.	455.00±79.56^m	n.a.	639.09±87.59^{m,n}	n.a.
17	6-Methyl−2-heptanone^s	n.a.	656.00±99.15	n.a.	379.59±58.08^m	n.a.	224.37±22.42^{m,n}	n.a.
18	3-Octanone	21^d	1.88±0.35	<1	15.46±4.62^m	1	7.67±1.46^{m,n}	<1
19	1-Octen−3-one	0.005^e	4.35±0.40	869	9.71±1.62^m	1943	10.07±1.44^m	2015
20	2-Methyl−3-octanone^s	n.a.	394.48±44.61	n.a.	546.26±43.16^m	n.a.	562.33±52.98^m	n.a.
21	4-Nonanone	8.2^d	0.22±0.01	<1	0.44±0.01^m	<1	0.25±0.01	<1
22	Sulcatone	50^h	14.35±1.70	<1	15.08±0.98	<1	14.17±0.82	<1
23	2-Undecanone	7^b	0.33±0.01	<1	1.75±0.11^m	<1	2.01±0.25^m	<1
24	2-Tridecanone	n.a.	0.03±0.01	n.a.	0.25±0.01^m	n.a.	0.81±0.01^{m,n}	n.a.
25	Cryptone^s	n.a.	182.22±17.36	n.a.	119.51±8.89^m	n.a.	89.03±3.53^{m,n}	n.a.
26	*β*-Ionone	0.007^c	194.18±38.35	27741	351.66±44.68^m	29621	191.68±6.32^n	27383
27	*β*-Ionone epoxide^s	n.a.	351.86±30.00	n.a.	197.69±30.38^m	n.a.	173.01±18.68^m	n.a.
	Alcohols:							
28	Isoamyl alcohol	300^b	3585.93±644.86	12	3863.58±330.51	13	483.96±50.87^{m,n}	2
29	1-Octen−3-ol	1^b	161.78±23.77	162	177.93±19.10	178	190.57±9.25^m	191
30	2-Nonanol	58^d	0.11±0.01	<1	0.23±0.01^m	<1	0.78±0.02^{m,n}	<1
31	2-Ethyl−1-hexanol	270000^a	53.28±8.73	<1	102.23±9.12^m	<1	81.40±15.58^{m,n}	<1
32	Linalool	6^c	2.17±0.03	<1	2.88±0.05	<1	1.69±0.01	<1
33	Geraniol	40^f	3.97±0.23	<1	4.29±0.68	<1	4.61±0.23	<1
34	Nerolidol	10^d	0.73±0.01	<1	4.47±0.08^m	<1	2.11±0.05^{m,n}	<1
	Terpenes:							
35	α-Pinene	6^a	2841.07±382.23	474	2169.97±239.66^m	362	1598.15±301.16^{m,n}	266
36	Limonene	10^c	5.91±0.78	<1	5.22±0.81	<1	3.79±0.52^{m,n}	<1
37	*o*-Cymene	n.a.	0.59±0.01	n.a.	0.34±0.01^m	n.a.	0.15±0.01^{m,n}	n.a.
38	Ectocarpene^s	n.a.	4398.14±415.76	n.a.	971.42±53.28^m	n.a.	382.02±46.65^{m,n}	n.a.
39	Sirenin^s	n.a.	186.78±26.52	n.a.	314.76±41.37^m	n.a.	311.12±37.30^m	n.a.
40	Safranal^s	n.a.	6004.61±748.50	n.a.	4755.59±554.87^m	n.a.	3175.25±496.99^{m,n}	n.a.
41	Elemol^s	n.a.	635.86±75.00	n.a.	201.87±32.42^m	n.a.	841.80±40.45^{m,n}	n.a.
42	Myrtenol^s	n.a.	57.82±7.42	n.a.	51.21±6.09	n.a.	18.37±0.88^{m,n}	n.a.
43	Zingiberene^s	n.a.	537.22±52.48	n.a.	831.53±90.29^m	n.a.	644.30±39.63^{m,n}	n.a.
	Sulfur compounds:							
44	Dimethyl disulfide	1.2^d	7.53±0.59	6	132.10±23.01^m	110	443.16±80.91^{m,n}	369
45	Dimethyl trisulfide	0.01^e	0.13±0.01	13	1.11±0.01^m	111	9.95±0.01^{m,n}	995
	Hydrocarbons:							
46	Benzene^s	1500^d	182.62±8.69	<1	141.80±19.82^m	<1	133.34±15.27^m	<1
47	Styrene^s	3.6^d	11.33±1.45	3	76.42±5.14^m	21	52.45±9.84^{m,n}	15
	Others:							
48	2-Pentylfuran	6^g	8.23±0.64	1	5.95±1.15^m	<1	2.28±0.38^{m,n}	<1
49	Bornyl acetate	75^a	0.17±0.01	<1	0.16±0.01	<1	0.03±0.01^{m,n}	<1

^{s}Labeled compounds were measured *via* a standard reference of a compound of the same class; ^{a} (Fazzalari, 1978); ^{b} (Guadagni et al., 1963); ^{c} (Buttery et al., 1971); ^{d} (Burdock, 2016); ^{e} (Buttery et al., 1990); ^{f} (Takeoka et al., 1990); ^{g} (Fors, 1983); ^{h} (Buttery et al., 1987); $^{m,\ n}$ $p < 0.05$.

synergistic effects on other aroma compounds helping to form the overall odor impression perceived from the fermented microalgae. Sulfur compounds are generally known to contribute to a series of savory odors, e.g., meaty or fishy flavors. Microalgae are a good source of sulfur-containing volatile and non-volatile compounds while basidiomycetes are known to be able to synthesize sulfur aroma compounds by bioconversion or *de novo* synthesis (Stöppelmann et al., 2024). Additionally, to visually observe the changes of OAVs, heatmaps are presented in Fig. 1A (OAV < 21) and Fig. 1B (OAV > 21). Based on Fig. 1B, the results of hexanal, 1-penten-3-one, and α-pinene decreased their OAVs, whereas the OAVs of *trans*-2-pentenal, 1-octen-3-one, 1-octen-3-ol, dimethyl disulfide, and dimethyl trisulfide increased after both fermentations. Specifically, the olfactory impression of hexanal and α-pinene is typically characterized as grassy and green (Sharma et al., 2010; Zhu et al., 2021). The reduction of this aldehyde and terpene, which is a distinctive feature of algae, and thus their green and grassy odor, is advantageous in the context of the desired aroma modifications (Nedele et al., 2021). 1-Penten-3-one, characterized by a stinging, sharp, and fishy odor, was the only ketone to decrease in concentration while having an OAV greater than 1, making it crucial for the aroma profile of PT. It has been reported as a useful marker for assessing fishy and metallic off-odors and flavors in fish oil products, demonstrating the

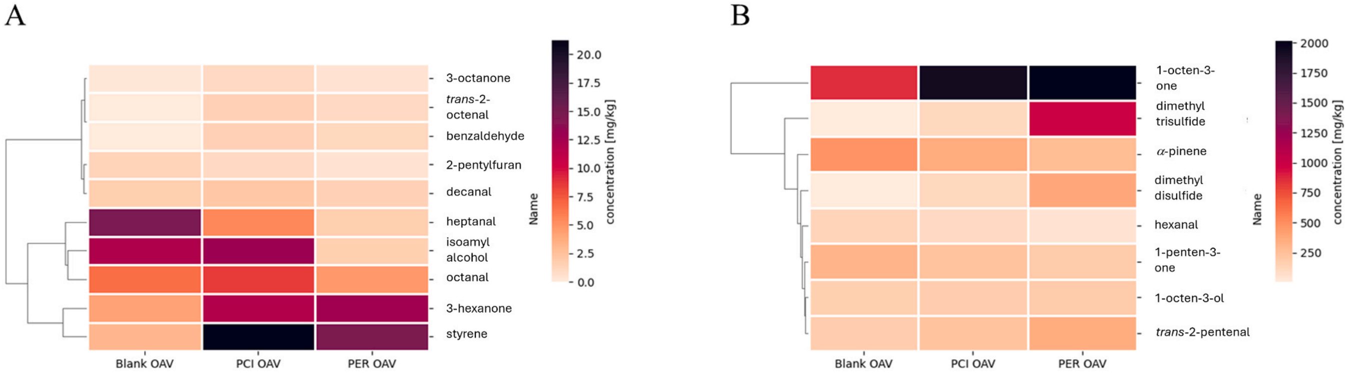

Fig. 1. Heatmaps odor activity values (OAVs) of Blank, PCI-, and PER-fermented PT microalgae samples; for OAV < 21 **(A)** and OAV > 21 **(B)** (excluding β-ionone due to its excessively high OAVs).

importance of the fermentation (Venkateshwarlu et al., 2004). In terms of the rise in odorant, 1-octen-3-one (savory and mushroom-like odor impression), exhibited the highest OAVs among the increased ketones, significantly influencing the overall odor impression with a savory effect. This volatile compound formed through the degradation of unsaturated fatty acids such as linoleic acid present in the algae by a chemical autoxidation process (Darriet et al., 2002). Another two sulfur-containing garlic-like, savory, and fishy compounds dimethyl disulfide and dimethyl trisulfide, increased significantly during the fermentation induced by the basidiomycetes PCI and PER. Van Durme et al. (2013) reported that seafood flavors can be mainly attributed to the presence of lower concentrations of sulphuric compounds, including dimethyl disulfide, dimethyl trisulfide, and methional. Dimethyl trisulfide is a major contributor to the aroma of cooked shrimp and seafood, emphasizing the critical role of these compounds and their influence on the overall savory and seafood-like odor profile. These sulfur-containing compounds typically originate from the Strecker degradation of amino acids (Tressl et al., 1994). Referring to Fig. 1A, there was a decrease in the OAVs of heptanal and 2-pentyl-furan, while an increase was observed in trans-2-octenal, benzaldehyde, 3-hexanone, and styrene. The concentration and OAVs of 2-pentyl-furan were found to decrease significantly after fermentation, with the OAVs falling below 1. It is reported that 2-pentyl-furan may contribute to green and grassy aroma in various algae species (Urlass et al., 2023; Bao et al., 2018). Comparable effects were found in a study by Du et al., (Du et al., 2021) where microbial fermentation of algae was found to greatly reduce 2-pentyl-furan, among other compounds, resulting in an improvement in odor. Moreover, two increased aldehydes compounds, trans-2-octenal and benzaldehyde, in the non-fermented blanks did not achieve an OAV ≥ 1, while the ones in the fermented samples exceeded this threshold. While benzaldehyde produced a cake-like and spicy aroma, trans-2-octenal evoked hay-like notes, both of which contributed to the aroma complexity and richness found in the fermented samples. They potentially interact with other compounds within the sample to create a fishy savory aroma. Ammari & Schroen, (Ammari and Schroen, 2018) identified the presence of both compounds in the aroma profile of cooked shrimp, characterized by a fishy odor. Additionally, it is noteworthy that trans-2-octenal only achieved an OAV of ≥ 1 in the case of PCI fermentation. This could account for the more pronounced savory odor detected in the samples fermented with PCI compared to the samples fermented with PER. Overall, it could be shown, that algae odor is a complex mixture defined by a series of different aroma compounds from several chemical classes. Even though, so far not all aroma compounds are identified and not all OAVs could be calculated, this study can give some distinct evidences on the key aroma compounds of PT. Further, it could be shown that an odor modulation by a basidiomycete-mediated fermentation can improve the odor of the microalgae making in more attractive for potential food applications.

3.4. Fatty acid and carotenoid profile

Fucoxanthin, a member of the xanthophylls synthesized by photosynthetic organisms, serves roles in photo protection and light harvesting (Mikami and Hosokawa, 2013; Bertrand, 2010). Notably abundant in brown (micro)algae and seaweeds, fucoxanthin has shown accumulation in the liver and adipose tissue of mice, along with safe metabolism and intake in humans. Its antioxidant, anti-inflammatory, and anti-obesity properties have spurred investigations into its potential health benefits. While microalgae typically serve as primary producers of several polyunsaturated fatty acid, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), PT primarily yields EPA where variations in biomass composition can occur based on species and environmental factors (Stiefvatter et al., 2022; Gao et al., 2017). EPA and DHA play crucial roles in human nutrition due to insufficient conversion of the essential fatty acid α-linolenic acid into these compounds (Burdge, 2006). Recommended daily intake of 250–300 mg EPA+DHA or regular fish consumption aids in maintaining immune balance towards anti-inflammation (EFSA Panel on Dietetic Products, Nutrition, and Allergies, 2010). As fish obtain *n*–3 PUFAs by consuming microalgae, direct consumption of microalgae offers a sustainable alternative. Therefore, besides a pleasant aroma impression obtained from the algae, also the nutritional values before and after fermentation were studied (Table 4).

In regard of the carotenoids, it could be shown that especially a fermentation of the microalgae with PCI led to a decreased content for fucoxanthin and β-carotene by 10 % and 23 %, respectively. The degradation of carotenoids by basidiomycetes is widely studied so far. It is known that carotenoids can be transferred into a series of norisoprenoic-structured aroma compounds by the basidiomycetes. Fungal peroxidases for cleavage of β−carotene towards e.g., β-ionone have been reported in the basidiomycetes *Mycetinis scorodonius* and *Lepista irina* so far (Rigling et al., 2022; Zorn et al., 2003). However, so far there has been no evidence that basidiomycetes are also able to metabolize fucoxanthin. As increased levels of β-ionone (351.66 ± 44.68 μg/L), a typical β-carotene metabolite, were found in the fermented samples with PCI, this finding is in accordance to the decreased carotenoid levels. Interestingly, on the other hand after fermentation with PER the levels of both carotenoids were increased by 3 % and 10 %, respectively. The levels of β-ionone after fermentation with PER were comparable to the non-fermented sample (194.18 ± 38.35 μg/L (non-fermented) and 191.68 ± 6.32 μg/L (fermented PER) respectively) which might be explained by a poor metabolism of carotenoids by PER. However, also a potential release of bound carotenoids from the algae cells during the fermentation can be a potential explanation for the slightly increased carotenoid levels.

Interestingly, it was be shown that the characteristic polyunsaturated fatty acids (PUFAs), especially EPA and DHA could be well preserved

Table 4
Carotenoids and fatty acid content of the non-fermented, PCI-, and PER-fermented PT microalgae samples.

	Non-fermented PT	PT fermented with PCI	PT fermented with PER
Carotenoids (% biomass)			
Fucoxanthin	0.85	0.78	0.94
β-Carotene	0.26	0.20	0.27
Fatty acid (% biomass)			
C14:0 (myristic acid)	0.62	0.25	0.29
C14:1 (myristoleic acid)	0.12	0.11	0.25
C16:0 (palmitic acid)	1.13	0.77	0.58
C16:1*n*–7 (palmitoleic acid)	1.88	0.73	0.75
C18:1*n*–9 t (oleic acid)	0.03	0.02	0.01
C18:2*n*–6c (linoleic acid)	0.25	0.10	0.08
C18:3*n*–6 (*γ*-linolenic acid)	0.03	0.02	0.01
C18:3*n*–3 (*α*-linolenic acid)	0.06	0.02	0.01
C20:3*n*–3 (eicosatrienoic acid)	0.24	0.19	0.19
C20:5*n*–3 (eicosapentaenoic acid, EPA)	2.82	2.24	2.21
C20:4*n*–6 (arachnidonic acid)	0.06	0.05	0.05
C22:6*n*–3 (docosahexaenoic acid, DHA)	0.06	0.05	0.07
C24:0 (lignoceric acid)	0.11	0.11	0.15
Sum fatty acids	7.41	4.65	4.54

during the fermentation process in contrast to the shorter and more saturated fatty acids which were degraded to up to 40 %. Free fatty acids and lipids are important odor and flavor precursors and fungal lipases are very efficient tools for fatty acid cleavage into a series of aliphatic aldehydes and alcohols (Wagner et al., 2024). The preservation of EPA and DHA is a crucial finding in regard of the potential health benefits related to the PUFAs. In addition, basidiomycetes are known to produce a series of unusual unsaturated and branched fatty acids, which makes the combination of microalgae with basidiomycetes a unique approach for supply of important and healthy fatty acids (Řezanka and Mareš, 1987). However, the production of new and unusual fatty acids derived from the basidiomycetes were not analyzed in this study.

4. Conclusion

In summary, the proposed hypothesis which posits that the "brackish water" off-odor in PT can be diminished while retaining most of its nutritional composition through a novel fermentation method *via* PCI and PER has been confirmed. Within short fermentation times from up to 2 days fermentation time, the aroma profile of the PT changed from "brackish water"-like, green, and musty to savory and seafood-like by sensory evaluation, which optimized its inherent fishy aroma. The instrumental analysis results revealed that the odorants hexanal, α-pinene, and 2-pentylfuran which contributed green and grassy notes decreased, while the key savory and seafood-like aroma compounds dimethyl disulfide and dimethyl trisulfide increased. However, the ultimate impact of the algae pheromones ectocarpene and sirenin on the aroma profile remains uncertain due to the lack of authentic standards and odor threshold data. The degradation in fatty acid concentration, reduced by around 40 % of the original content, contrasts with the stability observed in EPA and DHA levels. Additionally, the carotenoid levels before and after fermentation remain comparable. Considering the final consumption of PT microalgae in terms of thermal stability, product application and consumer acceptance, these properties should be analyzed in further studies to open new application possibilities for novel, innovative and nutritious foods.

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Ethical statement

Ethical approval to conduct sensory evaluation involving human objects is not a requirement of the University of Hohenheim. All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Participants gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for sensory evaluation.

CRediT authorship contribution statement

Lena Kopp: Project administration, Conceptualization. **Can Xiang:** Investigation, Data curation. **Yanyan Zhang:** Writing – review & editing, Supervision, Project administration. **Stephan C. Bischoff:** Supervision, Project administration. **Isa Entenmann:** Investigation, Data curation. **Jiaqi Liang:** Writing – original draft, Investigation, Data curation, Conceptualization. **Ulrike Schmid-Staiger:** Writing – review & editing, Supervision, Project administration. **Konstantin Frick:** Writing – original draft, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Marina Rigling:** Writing – original draft, Project administration, Methodology, Investigation, Data curation, Conceptualization.

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.

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