JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Evaluation of the Volatile Composition and Sensory Properties of Five Species of Microalgae

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ABSTRACT: Due to their high content of polyunsaturated fatty acids, antioxidants, and proteins, microalgae hold a lot of potential for nutritional applications. When microalgae are integrated into foodstuffs, the aroma is an important aspect to consider. In this study the aroma properties of microalgae were studied by correlating data on the volatile composition with sensory evaluations. Four species of marine microalgae (*Botryococcus braunii,*, *Rhodomonas*, *Tetraselmis* species, and *Nannochloropsis oculata*) and one fresh water microalga (*Chlorella vulgaris*) were investigated. Multivariate data processing revealed that microalgal samples having a seafood-like odor character contain high levels of sulfuric compounds (dimethyl disulfide, dimethyl trisulfide, and methional), diketones, α-ionone, and β-ionone. Fresh green, fruity flavors were linked with typical aldehydes such as 2,4-alkadienals and 2,4,6-alkatrienals. The presence of these compounds in fresh microalga pastes is explained by aroma formation mechanisms such as enzymatic lipid oxidation, enzymatic and chemical degradation of dimethylsulfoniopropionate (generating dimethyl sulfide), phenylalanine (generating benzaldehyde), and carotenoids (generating ionones).

KEYWORDS: *microalgae, volatile organic compound analysis, sensory analysis, principal component analysis, partial least-squares regression*

INTRODUCTION

Because of interesting nutritional properties, microalgae hold a lot of potential for nutritional applications. Microalgae or extracts of microalgae may be consumed as such or may be integrated in a variety of foodstuffs.[1,2] When integrating microalgae in foodstuffs, the aroma properties are an important parameter to take into account. These aroma properties may be unwanted in some applications or highly desirable in others, yet very little information is available about the flavor of microalgae.

Microalgae produce a biomass that is poor in lignin, yet rich in lipids, proteins, and/or carbohydrates.[3] Some microalgae (e.g., *Nannochloropsis*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Haematococcus*) are rich in polyunsaturated fatty acids (PUFA) and are, therefore, attractive as a replacement for fish oil.[4,5] Other species such as *Arthrospira* are rich in proteins and could in the future replace eggs, milk, and soybeans in food and feed applications.[6] Microalgae are also a rich source of antioxidants, and some species are currently produced commercially for use as carotenoid antioxidants.[1]

It is known from ecophysiological studies that microalgae produce a wide array of volatile compounds that may influence the flavor or aroma of the biomass; these compound classes include unsaturated aldehydes, dimethyl sulfide, and organohalogens.[7] Such volatiles may positively or negatively affect food materials enriched with microalgae. For example, some freshwater microalgae are known to produce earthy off-flavors in the form of geosmin and 2-methylisoborneol. When these compounds are present in drinking water reservoirs, they affect the flavor of drinking water.[8] It has been shown that geosmin produced by microalgae may negatively affect the flavor of cultivated fish such as carp.[9] There is also evidence that some microalgae possess marine or seafood aromas. For instance, norisoprenoids derived from microalgal carotenoids impart a positive green flavor in natural sea salts.[10] Carotenoid degradation products, together with long-chain aldehydes derived from microalgal unsaturated fatty acids, have a positive influence on the flavor of oysters.[11] Chee et al.[2] reported fishy or marine off-flavors in yogurt that was enriched with omega-3-rich oils from heterotrophic microalgae. Although fishy aromas may be unwanted in some applications, they can also be appreciated when microalgae are used as a fish or fish oil replacement.

In contrast to microalgae, much more information is known about flavors of macroalgae (i.e., seaweeds). Seaweeds have been used as a source of food for many centuries, particularly in East Asia.[12] Important flavor compounds in seaweeds are organohalogens, aldehydes derived from unsaturated fatty acids, and dimethyl sulfide.[13,14] Unfortunately, it is not known whether microalgae possess similar flavors as macroalgae.

The goal of this study is to study the volatile fraction and aroma properties of microalgae using HS-SPME-GC-MS analysis coupled with sensory evaluation. SPME is a powerful extraction

Received: July 19, 2013
Revised: October 18, 2013
Accepted: October 19, 2013
Published: October 19, 2013

dx.doi.org/10.1021/jf403112k | J. Agric. Food Chem. 2013, 61, 10881−10890

Table 1. Fatty Acid Composition and Dry Matter of Selected Microalga Pastes

			% of total fatty acids			
	total lipid (g/100 g)	dry matter (%)	C18 MUFA[a]	C18 PUFA[a]	EPA[a]	DHA[a]
Chlorella[15]	12	26.5	17	28		0.3
Rhodomonas[19]	19	24.5	11	35	2.4	3.0
Tetraselmis[19]	13	27.5	12	30	2.4	0.1
Nannochloropsis[16,18]	8−15	28.5	2.5	5.5	22	
Botryococcus[17]	20	19.9	13	22		

[a]MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentanoic acid; DHA, docosahexanoic acid.

technique that has been routinely used in combination with GC and GC-MS and successfully applied to a wide variety of compounds, especially for the extraction of volatile and semivolatile organic compounds from environmental, biological, and food samples.[11] In this study, a detailed analysis was carried out on biomass derived from five different species of microalgae. The aim of this paper is to focus on the volatile fraction to investigate to what degree the addition of microalgae in food products might influence the occurrence of wanted and/or unwanted aroma and flavor attributes.

MATERIALS AND METHODS

Microalgae. A frozen paste of the marine microalgae *Botryococcus braunii* (Chlorophyta), *Rhodomonas* species (Cryptophyta), *Tetraselmis* species (Chlorophyta), and *Nannochloropsis oculata* (Eustigmatophyta) was provided by Necton S.A. (Olhão, Portugal). Samples (1 kg) arrived in vacuum-sealed plastic-lined aluminum foil bags to avoid photodegradation and minimize oxidation. Samples were stored frozen at −20 °C until analyzed (within 3 months of arrival). In addition, biomass of the freshwater microalga *Chlorella vulgaris* SAG211-11B was produced in-house in a pilot-scale airlift photobioreactor. *C. vulgaris* was cultured in Wright's Cryptophyte (WC) medium, illuminated in 12:12 light−dark cycle (125 μmol of photons m^{-2} s^{-1}, Philips Cool White fluorescent tubes), and mixed with filter-sterilized air. Biomass was harvested at the end of the logarithmic phase by centrifugation and immediately frozen and stored at −20 °C until analyzed (within 3 months).

These species are already commercially produced on a medium to large scale, and biomass is therefore available for use in nutritional applications. This selection includes representatives from three different groups of microalgae (Chlorophyta, Cryptophyta, and Eustigmatophyta) and includes both marine and freshwater species. They should give a view of the variety of aroma properties that can be encountered in microalgae. The protein content of each microalga was in the range of 25−30 wt %. In Table 1 the fatty acid composition and dry matter content are summarized for the selected microalga species.[15−19]

Chemicals. Several analytical standards were purchased at Sigma-Aldrich (Diegem, Belgium): ethanol (99%), methional (98%), dimethyl disulfide (99%), 1-penten-3-ol (99%), 3-hydroxy-2-butanone (96%), 1-octen-3-ol (98%), 2,3-butanedione (97%), 3-methylbutanal (99%), pentanal (97%), hexanal, *cis*-4-heptenal (98%), heptanal (92%), nonanal (95%), 4-ethylbenzaldehyde (97%), *tr*,*tr*-2,4-decadienal (85%), and 3-methylbutanol (99%). For each of these reference compounds, a stock solution was prepared in methanol at 0.1 g/L. From this stock solution, 1 μL was transferred to a closed 20 mL headspace vial and analyzed using the GC-MS method described below.

Isolation of Volatile Organic Compounds. Volatiles were extracted with an MPS-2 XYZ autosampler equipped with a headspace solid phase microextraction unit (multi-PurposeSampler or MPS, Gerstel, Mülheim an der Ruhr, Germany). For extraction, 150 mg of the wet microalgal paste was hermetically sealed in 20 mL vials and incubated for 30 min at 40 °C using agitation. Different SPME fibers were compared (CAR/PDMS, PDMS, CAR/DVB/PDMS) for extraction at 40 °C and 15 min of extraction time. The most effective fiber type proved to be CAR/DVB/PDMS. Using this fiber type, measurements were done with extraction temperatures of 40, 60, and 80 °C and a extraction time of 15 min. Next, also the extraction time was optimized using the selected fiber type (CAR/DVB/PDMS) for, respectively, 15, 30, and 45 min. On the basis of these tests it was observed that a 30 min extraction time was optimal, when preceded by incubation of the sample for 30 min at 40 °C.

Gas Chromatography−Mass Spectrometry: HS-SPME GC-MS. For compound identification, mass spectra were acquired using a Hewlett-Packard gas chromatograph 6890 series (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent mass selective detector 5973 series (Agilent Technologies). After thermal desorption of the loaded SPME fiber (250 °C, 3 min), volatiles were separated using a cross-linked methyl silicone column (HP-PONA, 50 m × 0.20 mm, 0.5 μm film thickness; Agilent Technologies). The injected compounds were separated using helium as the carrier gas (flow rate = 1 mL min^{-1}). The temperature gradient was from 40 °C (5 min) to 250 °C at 5 °C min^{-1}, held for 5 min. The injector and transfer lines were maintained isothermally at 250 and 280 °C, respectively. Mass spectra in the electron ionization (MS-EI) mode were generated at 70 eV, and recording was performed in the mass range from 40 to 250 amu (scan mode) and an analysis time of 60 min. Instrument control and data collection were performed using the GC-MSD Chemstation (G2070AA, Agilent Technologies).

All samples were spiked with an internal standard for semiquantitative analysis, and all data were normalized to this reference compound, 2,4,6-trimethylpyridine. A stock solution of the internal standard was prepared at 5.58 μg/mL in methanol, and samples were spiked with 3 μL (or 16.74 ng) prior to HS-SPME-GC-MS. This approach is comparable to the approach described by Giri et al.[20] Five replicate analyses were performed on each sample.

When standards were not available, compounds were tentatively identified by matching the obtained mass spectra using the Wiley 275 mass spectral library containing EI mass spectral data (John Wiley and Sons, Hewlett-Packard, Hoboken, NJ, USA) and by comparing the retention index value to values available in the literature. It was ascertained that authentic reference compounds were used for the determination of these retention indices for the same stationary phase when referred to the literature.[21−35] Experimental retention indices were calculated for all detected volatiles, which were based on the retention times obtained after injection of an in-house prepared *n*-alkane standard mixture (C7−C14) analyzed using the configuration described above. The Kovats indices were calculated according to the method of Van den Dool and Kratz[36] and compared with peer-reviewed Kovats values available in the NIST library (distributed by Agilent Technologies).

Sensory Evaluation of Headspace Using Quantitative Descriptive Analysis. Descriptive sensory analyses on the microalga headspaces were performed using an internally available experienced and trained panel of 12 assessors (5 males, 7 females, aged between 24 and 42 years). The sensory laboratory used in this study was equipped according to ISO8589:2007 standards. Training of the selected panelists was performed according to ISO8586, by which they were familiarized with five relevant aroma attributes by presenting food products having the following characteristic odors: (1) grassy, vegetable, cucumber; (2) fruity (pineapple, citrus); (3) cooked shrimp, cooked seafood; (4) fresh marine, fishy; and (5) rancid, fatty odor. These selected attributes were based on the sensory vocabulary found in the literature and after internal discussion. Small amounts of the microalga samples (200 mg) were presented in randomly coded and closed 20 mL screw-capped glass vials

Table 2. Mean Semiquantitative Results (Nanograms per Gram with Standard Deviation in Parentheses; $n = 5$) of Headspace Concentrations of Microalga Pastes by HS-SPME-GC-MS (Electron Ionization) Analyses, Experimental and Literature Retention Indices, and Identification Methods Used

compound	OTV[a20,38,69]	Tetra[a]	Rhodo[a]	Nannochl[a]	Botryo[a]	Chlor[a]	KI_{exptl}[a]	$KI_{lit.}$[a]	Idm[b]
linear aldehydes									
pentanal	12−42		60 (29)				690	690	A
cis-2-pentenal	1500	10 (2)	90 (33)	180 (35)	320 (76)	4100 (470)	734	731[26]	B
hexanal	2.4	5 (1)	210 (62)	96 (39)	690 (190)	1900 (210)	779	772[27]	A
trans-2-hexenal	110			68 (28)	740 (210)	1200 (240)	825	847[27]	B
cis-4-heptenal	0.8−10		45 (10)		390 (110)		872	871	A
heptanal	0.01		35 (7)		230 (70)		877	875	A
c,tr-2,4-heptadienal	95		690 (210)		730 (240)		965	989	B
tr,tr-2,4-heptadienal	15		1700 (480)		4300 (1200)		979	996	B
2-octenal	3			30 (0)	490 (110)		1030	1056[26]	B
nonanal	1		82 (14)	21 (1)	410 (97)	170 (6)	1082	1083[26]	A
c,tr,tr-2,4,6-nonatrienal	0.03				21000 (2900)		1247		C
tr,tr,tr-2,4,6-nonatrienal	0.03				32000 (6600)		1260		C
c,tr-2,4-decadienal	0.027				3900 (980)		1275	1267[28]	B
tr,tr-2,4-decadienal	0.027				3600 (1100)		1297	1297	A
total		15	3000	400	69000	7400			
branched and aromatic aldehydes									
3-methylbutanal	0.2	43 (3)	130 (24)	89 (23)		39 (10)	661	661	A
furfural	3000		47 (11)				801	802[29]	B
benzaldehyde	350	4800 (440)	30900 (8500)	590 (200)	2200 (310)	3300 (730)	930	926[27]	B
phenylacetaldehyde	4			33 (7)		98 (27)	1015	1017[30]	B
4-ethylbenzaldehyde	123				2100 (430)		1122	1122	A
total		4800	31000	720	2300	3500			
alcohols									
ethanol	100000	13 (3)		1500 (490)		100 (37)	440	440[22]	B
1-penten-3-ol	400	13 (1)	120 (30)	580 (140)	250 (31)	16000 (1800)	685	665[28]	A
3-methylbutanol	4	10 (3)		590 (200)		550 (55)	727	725[31]	B
2-methylbutanol	16	6 (2)		120 (45)		210 (22)	731	729[22]	B
1-pentanol	4000	9 (1)	140 (44)	86 (30)	55 (15)	2000 (140)	755	751[26]	B
cis-2-penten-1-ol	400	44 (10)	120 (32)	420 (90)	360 (77)	7000 (550)	757	746[32]	B
3-hexen-1-ol	3.9					6100 (910)	831	834[27]	B
1-hexanol	2500	2 (0)		330 (75)		4400 (940)	849	850[30]	B
1-octen-3-ol	1		420 (94)	67 (11)			959	968[26]	A
total		97	790	3700	660	36000			
ketones									
2,3-butanedione	1	5 (1)	67 (17)	160 (19)		210 (60)	622	619[31]	A
1-penten-3-one	1−1.3	4 (1)	39 (11)	26 (5)	300 (61)	4800 (260)	682	658[33]	B
2,3-pentanedione	5506	4 (1)	38 (10)			390 (110)	688	668[29]	B
3-pentanone	70000			81 (11)	140 (32)	4600 (700)	691	701[24]	B
3-hydroxy-2-butanone	800		580 (96)	2200 (1100)		4400 (1500)	694	674[28]	A
2,3-octanedione			92 (32)				956	959[24]	B
6-methyl-5-hepten-2-one[b]	50	130 (30)					960	967[26]	B
c,tr-3,5-octadien-2-one	0.0012		440 (140)				1040	1040	C
tr,tr-3,5-octadien-2-one	0.0012		430 (160)				1063	1040	C
total		140	1700	2500	440	14000			
terpenes/norisoprenoids									
β-cyclocitral	5	27 (4)	170 (40)	23 (5)	360 (120)	890 (70)	1206	1196[22]	B
α-ionone	3	30 (7)	1400 (290)		220 (62)	300 (24)	1426	1424[22]	B
β-ionone	3.5	66 (14)	140 (30)			85 (11)	1488	1466[22]	B
total		120	1900	23	590	1300			
esters									
ethyl acetate	5−5000			21 (8)			639	600[31]	B
methyl hexanoate	70−84			42 (11)		1200 (120)	909	911[21]	C
methyl phenylacetate	650			34 (6)			1146	1154[34]	B

dx.doi.org/10.1021/jf403112k | J. Agric. Food Chem. 2013, 61, 10881−10890

Table 2. continued

compound	OTV[a20,38,69]	Tetra[a]	Rhodo[a]	Nannochl[a]	Botryo[a]	Chlor[a]	KI_{exptl}[a]	$KI_{lit.}$[a]	Idm[b]
methyl octanoate	200		320 (100)	92 (21)	1000 (220)	1600 (110)	1102	1109[35]	B
methyl decanoate	0.006			67 (20)			1305	1307[21]	B
total			320	310	1200	3900			
furans									
2-ethylfuran					75 (44)		702	698[25]	B
2-pentylfuran	6				110 (30)	870 (86)	976	975[30]	B
total					190	870			
sulfuric compounds									
dimethyl sulfide	0.84	39 (4)					501	500[28]	B
dimethyl disulfide	0.16−12	21 (1)	34 (5)				733	733	A
methional	0.2		32 (6)				864	864	A
dimethyl trisulfide	0.005	32 (8)	180 (51)				948	950[28]	B
total		92	240						
acids									
acetic acid	20		130 (24)	72 (5)	150 (22)	190 (54)	639		C

[a]OTV, odor threshold value; Tetra, *Tetraselmis*; Rhodo, *Rhodomonas*; Nannochl, *Nannochloropsis*; Botryo, *Botryococcus*; Chlor, *Chlorella*; KI, Kovats index. [b]Idm, identification methods: A, identification based on MS database, retention index values from the literature when available (ascertained from authentic reference compounds), and spiking with authentic reference compound; B, tentative identification based on the MS database and retention index values from the literature (ascertained from authentic reference compounds); C, when only MS or retention index values were available (ascertained from authentic reference compound), it must be considered as a tentative identification.

that were wrapped with aluminum foil. These samples were incubated for 1 h at 40 °C. The five microalga samples were evaluated and compared in one session, which is in agreement with the recommendation that in descriptive tests the number of samples should not be more than six (ISO6658:2005). The panelists had to randomly select one sample, remove the glass stopper, and immediately smell the headspace. Each panelist assessed the intensity of the five attributes using a scale ranging from 0 (aroma absent) to 5 (very strong aroma). Mean scores for each attribute were calculated. In this paper the obtained sensory data for each microalga were visualized by a spider diagram.

Statistical Analysis. Exploratory data analysis of the chemical information was performed by means of principal component analysis (PCA) (Unscrambler 9.7, Camo, Oslo, Norway). Using the same software, partial least-squares regression (PLS2) was applied to examine possible interrelationships between sensory descriptions and the aroma compounds detected by GC-MS. All data were weighted with 1/SD (standardization), and a leverage correction was used to validate the models.

On the sensory data, paired comparison tests (*t* test) were performed using SPSS Statistics 21 software to evaluate if differences in terms of sensory properties existed between the selected microalgae. Significances for the differences were established at an α risk of 5%.

RESULTS

Semiquantitative HS-SPME-GC-MS Analyses on Microalgae. Identification and semiquantitative information of the volatile components from the measured microalgae are shown in Table 2. Despite the selective character of HS-SPME, a total of 58 volatile compounds were identified in the investigated samples, with aldehydes, alcohols, and ketones as the most abundant groups.

Despite literature information that freshwater microalgae produce off-flavors in the form of geosmin and 2-methylisoborneol,[7,8] these compounds were not detected in this study.

Generally, for each microalga species, aldehydes proved to be the most prevalent (Table 2) and, due to their low odor threshold values, might be important headspace volatiles compounds contributing to desirable aromas as well as rancid odors and flavors. Saturated aldehydes have a green-like, hay-like, paper-like odor, whereas unsaturated aldehydes have a fatty, oily, frying odor. From Table 2 it can be seen that the aldehydes were divided into two subgroups, more specifically, the linear and the branched/aromatic aldehydes. Whereas the shorter chain linear aldehydes are often derived from chemical lipid oxidation, branched and aromatic aldehydes are typically formed due to enzymatic lipid and protein oxidation. Within the marine microalga samples, *Botryococcus* was shown to have the highest total aldehyde content (71.2 μg/g) followed by *Rhodomonas* (33.9 μg/g), both species with a relatively high content in C18-polyunsaturated fatty acids (PUFA) and total lipids.[19,37] *Tetraselmis* (4.8 μg/g) and *Nannochloropsis* (1.1 μg/g) contained only very small quantities of aldehydesn both species having a relatively low total lipid content.[18,19]

In *Botryococcus* the most important aldehydes were unsaturated *trans,trans,trans*-2,4,6-nonatrienal (32.2 μg/g) (tentative), *cis,trans,trans*-2,4,6-nonatrienal (20.8 μg/g) (tentative), *cis,trans*-2,4-heptadienal (4.3 μg/g), and *cis,trans*-2,4-decadienal (3.9 μg/g).

Table 2 also shows that several ketones and alcohols were present in the microalga samples. A number of these ketones seemed to be prevalent throughout the microalga samples, such as 1-penten-3-one, 3-pentanone, and 3-hydroxy-2-butanone. Similarly, a number of alcohols were measured in important quantities in all selected microalgae. 1-Penten-3-ol, 1-pentanol, and *cis*-2-penten-1-ol were identified in all samples. In particular, for *Chlorella*, high semiquantitative concentrations of 1-penten-3-ol (16000 ± 1800 ng/g), *cis*-2-penten-1-ol (7000 ± 550 ng/g), 3-hexen-1-ol (6100 ± 900 ng/g), 1-penten-3-one (4800 ± 260 ng/g), and 3-pentanone (4600 ± 700 ng/g) were measured.

Sulfur compounds, such as dimethyl trisulfide, dimethyl disulfide, and dimethyl sulfide, were detected only in *Tetraselmis* and *Rhodomonas* species. Low levels of methional (32 ± 6 ng/g) were detected in *Rhodomonas*.

Some terpenes/norisoprenoids, such as β-cyclocitral (tentative), α-ionone, and β-ionone (tentative), were measured in most

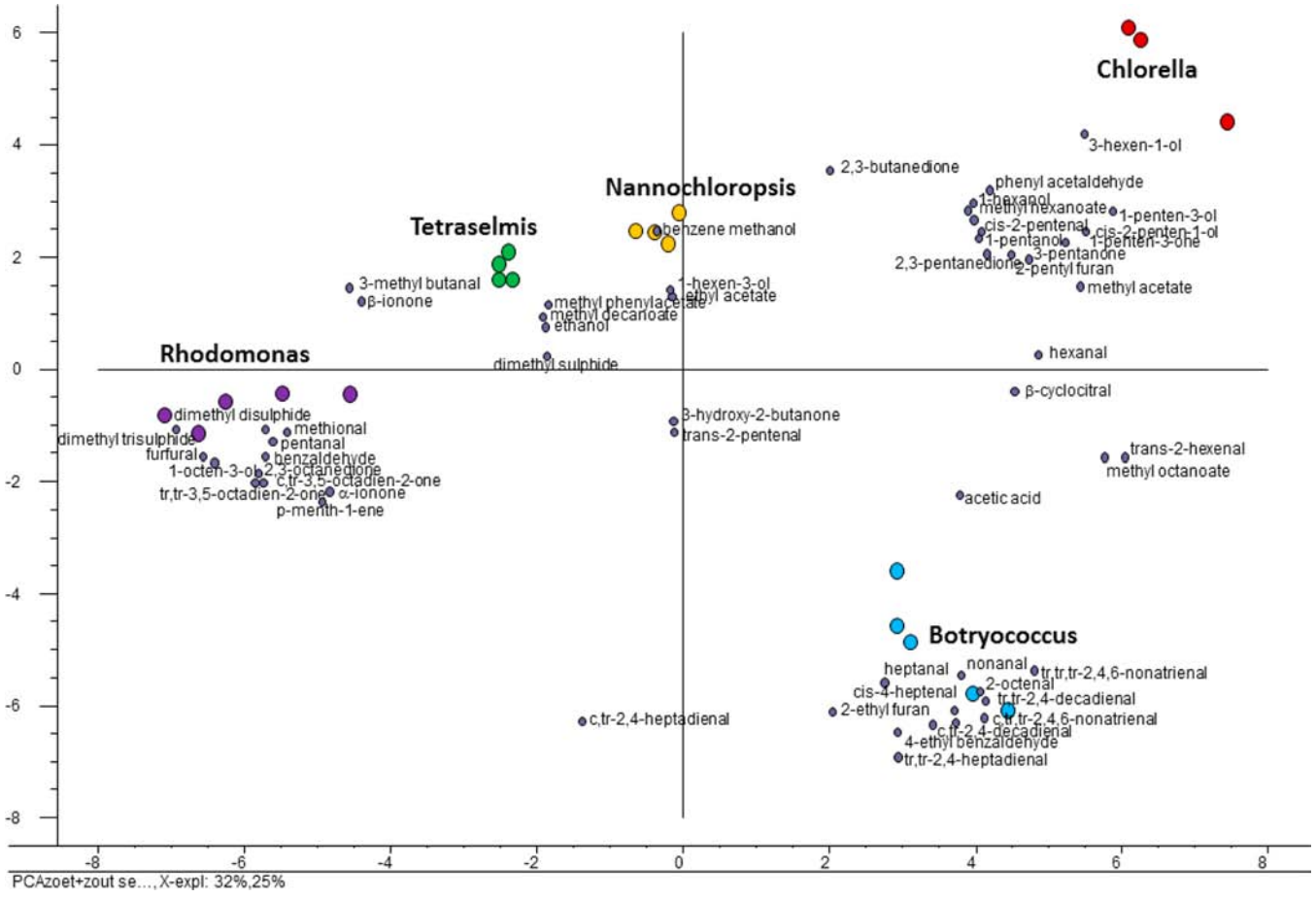

Figure 1. 2D principal component analysis (PCA) biplot of the semiquantitative HS-SPME-GC-MS (electron ionization) data of all microalga samples (n = 5).

microalga samples, with the exception of *Nannochloropsis*. These compounds are characterized by low odor threshold values, making them important aroma compounds. *Rhodomonas* contained a relatively high concentration of α-ionone (1400 ± 290 ng/g); this compound was also detected in *Chlorella* (300 ± 24 ng/g), *Botryococcus* (220 ± 62 ng/g), and *Tetraselmis* (30 ± 7 ng/g).

PCA of the Semiquantitative HS-SPME-GC-MS Data. A simultaneous representation of volatile compounds from the different microalgae on the first two principal components of the PCA plot is presented in Figure 1. PCA was used to evaluate the semiquantitative HS-SPME-GC-MS results and to identify the most representative volatile compounds for each microalga species on which they could be classified (Figure 1).

The PCA plot as presented in Figure 1 explains 57% of the total variance. The first and second principal components each explain a variance of 32% (PC1) and 25% (PC2), respectively. Generally, Figure 1 shows well clustered replicates for each microalga species, indicating that each is clearly differentiated from one another on the basis of the volatile composition in the headspace. PCA clearly shows that the freshwater microalga *Chlorella* is well differentiated from the marine microalgae. As will be discussed later, this is in agreement with sensory evaluations (Figure 2).

Generally it can be concluded that alkenals, alkedienals, and alkatrienals are typical for *Botryococcus*, whereas *Rhodomonas* and also *Tetraselmis* are classified by a number of sulfuric compounds as well by some ionones. The volatile chemical composition of *Nannochloropsis* seems to be between *Tetraselmis* and *Chlorella*. The latter is characterized by some typical short-chain alcohols, ketones, and aldehydes, which often have a characteristic green odor.

Sensory Analysis on Microalga Pastes. Descriptive sensory analyses on the headspace for the five aroma attributes were performed on the selected marine microalga samples (*Tetraselmis*, *Botryococcus*, *Nannochloropsis*, *Rhodomonas*) and the freshwater microalga (*Chlorella*). Figure 2 illustrates the average sensory scores perceived in every microalga paste.

The freshwater microalga *Chlorella* (Figure 2E) had relatively low scores compared to the marine microalgae (Figure 2A–D), resulting in a rather bland flavor profile that was dominated by 'grassy, vegetable, cucumber' aromas. *Botryococcus braunii* (2D) and *Nannochloropsis oculata* (2C) were characterized by flavor profiles with higher scores for 'grassy, vegetable, cucumber' and 'fruity' aromas compared with the other marine microalgae (p value < 0.05 in all cases), whereas no significant differences were observed with *Chlorella* (p values > 0.05). *Tetraselmis* (2A) and *Rhodomonas* (2B) both had significantly more pronounced 'fresh marine, fishy' and 'cooked shrimp/cooked seafood' aromas when compared with other microalga pastes (p value < 0.05 in all cases). Between *Tetraselmis* (2A) and *Rhodomonas* (2B) no significant differences were observed for the attribute 'fresh marine, fishy' (p value = 0.37 > 0.05), whereas *Rhodomonas* scored higher than *Tetraselmis* for the sensory attribute 'cooked shrimp/cooked seafood' (p value = 0.044 < 0.05).

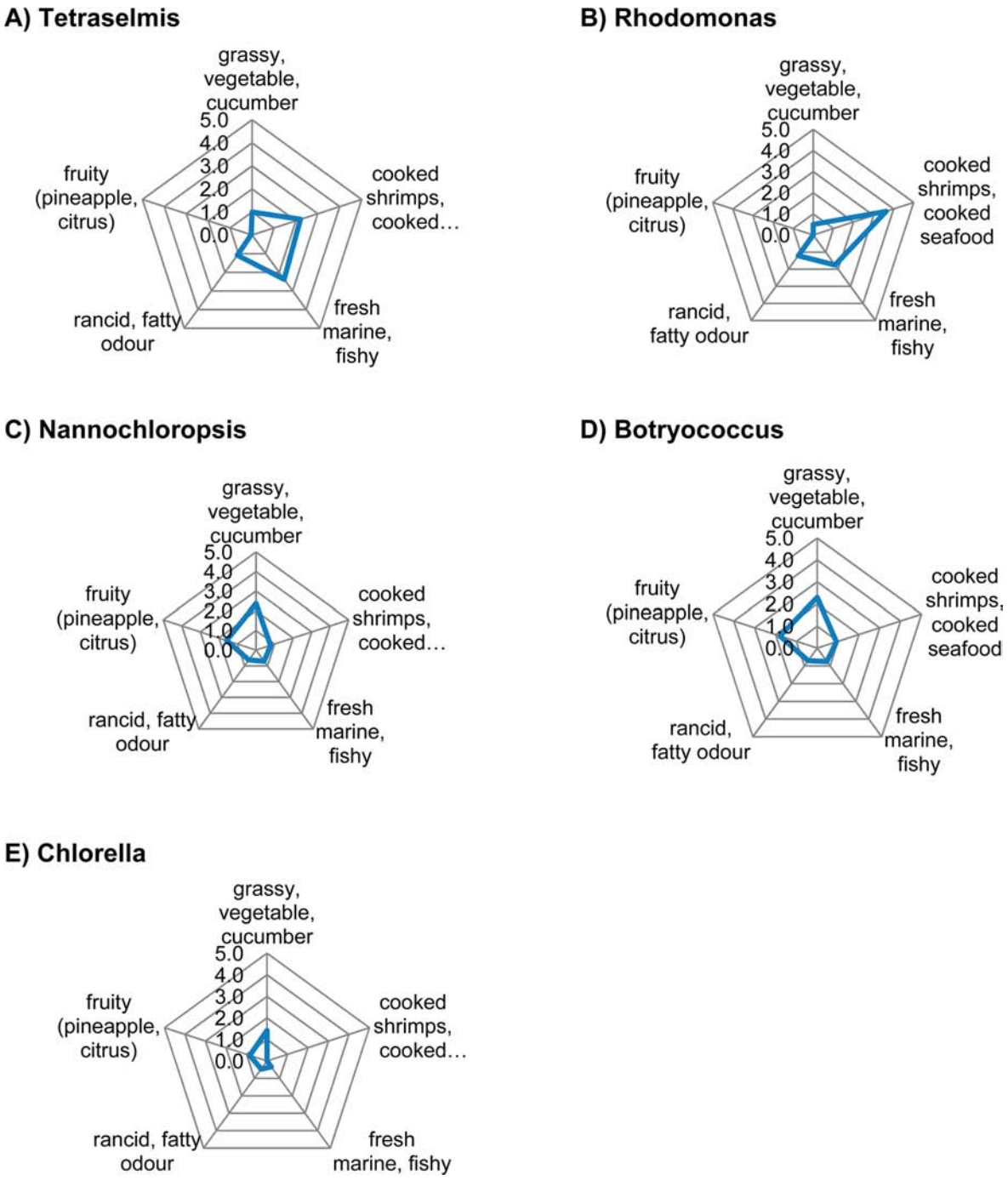

Figure 2. Spider diagram of sensory evaluation of four marine (*Tetraselmis, Botryococcus, Nannochloropsis, Rhodomonas*) and one type of freshwater microalga (*Chlorella*). Average scores are shown after quantitative descriptive sensory evaluation by 12 experienced panelists.

Correlation between Sensory and Chemical−Analytical Analysis. PLS2 was performed on the total data set for the selected microalgal species to determine the relationships between desired sensory attributes and the identified headspace volatiles. Previous studies correlated the concentration of volatile compounds in the matrix to their odor threshold value as a useful tool for the estimation of the potential odor contribution of each individual compound.[38] This ratio of concentration to odor threshold has been assigned as aroma value or odor activity value (OAV). Because the odor detection threshold is a crucial criterion for the impact of single aroma compound to the perceived overall aroma, it is these OAVs that were correlated with sensory data using the PLS2 approach. The *X*-axis contained OAVs for each identified volatile (= semiquantitative concentration/odor threshold), and the *Y*-axis contained the sensory scores for the earlier described attributes. Figure 3 shows the correlation between the *X*- and *Y*-variables.

PLS data interpretation indicated that both the attributes 'cooked shrimp/cooked seafood' and 'fresh marine, fishy' aromas correlated with sulfuric compounds (dimethyl disulfide, dimethyl trisulfide, and methional). It was previously described that these sulfur compounds have low odor threshold values and can have a great impact on the aroma properties of microalgae as also observed in other seafoods.[7] Additionally, it was also seen that

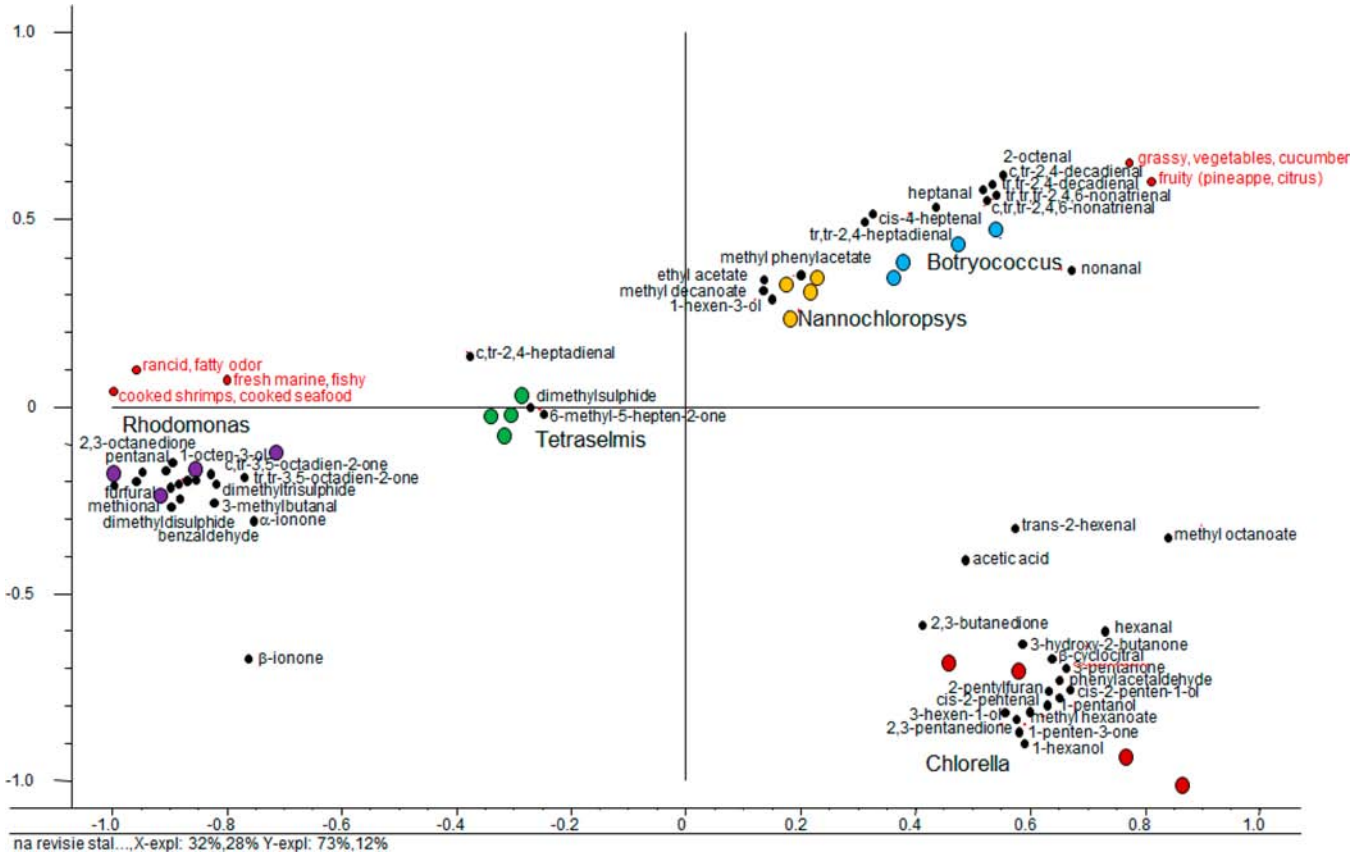

Figure 3. Partial least-squares regression (PLS2) correlation loadings plot (*X*-variables, odor activity values; *Y*-variables, sensory scores) of microalgae (*Nanochloropsis*, *Botryococcus*, *Rhodomonas*, *Tetraselmis*, *Chlorella*).

the diketones (e.g., *c,tr*-3,5-octadien-2-one; *tr,tr*-3,5-octadien-2-one; 2,3-octadione) correlated well with typical seafood attributes, which is in agreement with literature data.[39] Although the samples with highly perceived seafood flavors contained high levels of ionones, their contribution to this flavor attribute is unlikely as ionones are well-known for their floral sensory properties.

The grassy and fruity flavors observed in both *Nannochloropsis* and *Botryococcus* seemed to be well correlated with the reported 2,4-alkadienals (e.g., *tr,tr*-2,4-decadienal, *c,tr*-2,4-decadienal; *tr,tr*-2,4-heptadienal) and 2,4,6-trialkadienals (*c,tr,tr*-2,4,6-nonatrienal; *tr,tr,tr*-2,4,6-nonatrienal). Additionally, some typical shorter chain aldehydes (1-hexen-3-ol, heptanal, 2-octenal, nonanal) were also correlated to the reported fruity, vegetable flavors. Many of these aldehydes can provide several notes to food matrices (fatty, green, woody, fatty, nutty, floral, citrus, waxy, and sweet) depending on the number of carbon atoms and the degree of saturation.[40] The presence of some typical esters, such as methyl hexanoate, methyl octanoate, and methyl decanoate, also seemed to be correlated with the sensory findings detected in *Nannochloropsis*, *Botryococcus*, and *Chlorella*.

The rather bland sensory properties of *Chlorella* also seem correlated with some other short-chain aldehydes, ketones, and aldehydes. It is observed that C5 compounds (e.g., 3-pentanone, 1-penten-3-one, *cis*-2-penten-1-ol, 2,3-pentadione, *cis*-2-pentenal) are typical compounds for *Chlorella*.

DISCUSSION

On the basis of measurements of the headspace volatiles (Table 2) and after multivariate data processing (Figure 3), patterns were visualized, and selected microalgae were classified on the basis of their volatile headspace composition. This analysis indicated that a number of volatiles were correlated with the observed sensory characteristics. Several of these volatiles were measured in concentration ranges exceeding their odor threshold value (OTV) (Table 2), making them important odorants that influence the total aroma and flavor properties of microalgae. These classes include linear aldehydes, terpenes/isoprenoids, and sulfuric compounds. The OTV of hexanal (4.5−5 ng/g), for example, was exceeded in every tested microalga with an order of magnitude up to 200 (*Chlorella*). A high diversity of alkadienals and alkatrienals was detected in *Botryococcus*, and again, many exceeded the OTV with several orders of magnitude. Terpenes/isoprenoids were particularly important in *Rhodomonas*, *Chlorella*, and *Botryococcus*. Sulfuric compounds have a very low OTV and were detected in *Rhodomonas* and to a lesser extent in *Tetraselmis* at high concentrations relative to their OTV. Apart from these three classes of compounds, some isolated volatiles were equally important (Figure 2), such as benzaldehyde and 1-octen-3-ol being present well above their threshold values.

The occurrence of most of the identified volatiles can be ascribed to the particular composition of microalgal biomass. Microalgae are relatively rich in polyunsaturated fatty acids. Marine microalgae contain mostly very long-chain PUFA such as eicosapentanoic acid and docosahexanoic acid, whereas, for example, *Chlorella* mainly contains shorter PUFA such as α-linolenic acid. Species having low PUFA concentrations will contain significantly fewer linear aldehydes when compared with species having high PUFA concentrations (e.g., *Chlorella*, *Botryococcus*, *Rhodomonas*). Many of these aldehydes can be

enzymatically produced by the metabolism of living cells.[41,42] Additionally, an important number of the identified aldehydes can be related to fatty acid oxidation and, in particular, to polyunsaturated fatty acid oxidation.[11] Enzymes (lipoxygenases) cleave polyunsaturated fatty acids to produce carbonyls, aldehydes, and ketones.[43] Andreou et al.[44] reviewed that in the brown alga *Laminaria angustata* the majority of C-6 aldehydes originate from C-18 PUFAs. One of the primary products, (*Z*)-3-hexenal, can be converted either enzymatically or spontaneously to other compounds such as (*E*)-2-hexenal and (*Z*)-3-hexenol.[45] Similar processes and compounds have been described in organisms that feed on these PUFA-rich microalgae. Pennarun[46,47] reported that, for example, in oysters the type and content of PUFA and their aldehyde degradation products are modified by the microalgal diet. Similarly, the incorporation of microalgal biomass in the feed of livestock proved to influence the fatty acid composition and hence also the flavor properties, of milk fat,[48] eggs,[49,50] or lamb meat.[51]

Microalgae containing high carotenoid concentrations are typically characterized by high concentrations of ionones that are produced due to reactions with the unstable conjugated double-bound structure of carotenes.[52,53] In the later stages of carotenoid degradation in which their longer chain intermediates are further oxidized, increasing amounts of short-chain mono- and dioxygenated compounds including β-cyclocitral are formed.[54] This can be illustrated by our measurements on *Rhodomonas*, which is known to contain high levels of α-carotene[55] and in which very high concentrations of α-ionone were measured (Table 2). Similar processes also seem to occur in other matrices that contain higher levels of carotenoids. Sun et al.[14] identified β-ionone and β-cyclocitral as one of the main flavor compounds in the green macroalga *Capsosiphon fulvescens*. Donadio et al.[10] described that some important volatiles in specialty sea salts (fleur de sel) are the result of carotenoid degradation.

Although not directly measured in this work, the content of dimethylsulfoniopropionate (DMSP) can explain the presence of some sulfuric volatiles (dimethyl sulfide, dimethyl disulfide) that result from enzymatic degradation of this precursor.[56−58] This is in agreement with our results, showing sulfuric volatiles in *Tetraselmis*, which is known for its high DMSP accumulations.[59] However, more specific studies are needed to investigate the correlation between DMSP accumulation and the flavor and aroma of microalgae. Although this precursor is abundantly produced in the metabolism of phytoplankton,[58] the production of DMSP is confined to a few classes of microalgae, and their production/accumulation is highly dependent on the growing phase and conditions. Groene et al.[57] reviewed the specific roles of DMSP in the physiology of phytoplankton, being a methyl donor, a precursor of acrylic acid, osmolyte,[60] and cryoprotectant. The importance of DMSP and its derivatives has also been described in seafood products. Smit et al.[61] reported that some macroalgae produce high levels of DMSP. Moreover, when these macroalgae are used as feed, they have an important impact on the taste characteristics of aquacultured abalone. Ronald and Thomson[62] described that the characteristic odor of fresh Pacific oyster, *Crassostrea gigas*, is correlated with its content of dimethyl sulfide. Methional has been assigned as one of the major contributors to the characteristic odor of cooked mussels.[63]

Finally, it is also expected that the type of amino acids can have a major impact on the aroma and flavor properties of microalgae. A high content of phenylalanine will, for example, result in a distinctively higher benzaldehyde content. The literature indicates that benzaldehyde, having an almond-like aroma, is produced from phenylalanine in various microorganisms, and several metabolic pathways have been proposed in the literature.[64] Similarly, enzymatic conversion of phenylalanine could result in the formation of phenylacetaldehyde, potentially exhibiting a flowery, honey-like odor note[65] when present in sufficient quantities.

On the basis of the results, and supported by the literature, it is believed that seafood flavors can be mainly attributed to the presence of lower concentrations of sulfuric compounds (dimethyl disulfide, dimethyl trisulfide, methional). At higher concentrations, sulfuric compounds are characterized by the flavor notes of cooked cabbage (dimethyl sulfide) and meaty and cooked onion flavor (dimethyl trisulfide). Because significantly higher scores (p value < 0.05) for the attribute 'cooked shrimp/cooked seafood' were given for *Rhodomonas*, this could be explained by its higher dimethyl trisulfide content. Moreover, 1-octen-3-ol has shown to additionally contribute to the formation of the complex seafood aroma. Indeed, 1-octen-3-ol has been described as an important volatile compound in seafoods.[11,43] This was confirmed by our results showing that the highest concentrations for 1-octen-3-ol were measured in *Rhodomonas* (420 ± 94 ng/g), which was perceived as having a very strong 'cooked shrimp/cooked seafood' aroma.

It is believed that the fruity, vegetable flavors are mainly caused by the presence of shorter chain aldehydes, 2,4-alkadienals and 2,4,6-alkatrienals. However, the fruity notes will also be affected by typical ester compounds such as methyl hexanoate, methyl octanoate, and methyl decanoate.

In conclusion, the authors believe that microalgal biomass will be increasingly integrated into food products, and as such, investigation of their chemical and sensory properties is of importance. In this manner, food products could be enriched with healthy polyunsaturated fatty acids (omega-3), antioxidants, proteins, and valuable minor nutrients while simultaneously enhancing flavor properties. Microalgae possessing typical seafood-like flavors could be, for example, interesting candidates to be used as replacers of omega-3-rich fish oils. Other microalgae having appealing fresh green, fruity flavors are equally interesting for use as additives in non-seafood products.

This study is only a first step in research on the chemical−analytical and sensory properties of microalgal biomass. Further aroma and flavor research is required by evaluating more species to identify the most promising candidates for use as a new generation of food additive. Groene et al.[57] reviewed that in different phytoplankton taxa, variable concentrations of DMSP, the precursor for sulfuric compounds resulting in seafood flavors, are measured. Next to the type of microalga, it is also expected that the growing conditions have a major impact on the aroma and flavor properties. Nutrient limitations, growth phase, and light exposure were reported to strongly influence the DMSP content in microalgae.[57] Abiotic stress during the growth phase, but also during harvesting or processing, can strongly influence the properties of microalgae. It has been reported that in damaged plants fatty acid derivatives, green leaf volatiles, and terpenoids (α-pinene, myrcene, caryophyllene) are produced due to abiotic stress. Vuorinen et al.[66] reported that the total content of volatile organic compounds after herbivore damage was 2.5 times that of an undamaged plant; the same was also described for freshwater diatoms.[67]

Lu et al.[68] described that some sulfuric volatiles can arise from the microbiological degradation of sulfur-containing amino acids under either anaerobic or aerobic conditions. Such microbial

degradation processes were probably limited during this study, because the microalgae were immediately frozen and stored at −20 °C after harvesting and sample preparation. However, one cannot rule out completely some lipid and protein oxidation even if samples are stored at −20 °C. On the other hand, in this study all commercial samples arrived at the same moment and were analyzed together, so the extent of lipid oxidation upon storage should be similar for all samples. Nevertheless, when commercialized, more scientific knowledge should be collected concerning aroma and flavor stability of food products in which microalgae are used. In the future, research attention should also be given to the changes of aroma properties of food products containing microalgal biomass prepared at different food preparation temperatures. In addition, it is highly important to perform research on the evolution of the volatile composition and corresponding aroma properties during the shelf life of microalgal-enriched food products.

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Notes

The authors declare no competing financial interest.

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