



Insights into the volatile profile of a red macroalga (*Gracilaria vermiculophylla*) for future food applications

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ARTICLE INFO

Keywords:

Algae
Seaweed
Volatile profile
HS-SPME
GC×GC-ToFMS
Aroma wheel

ABSTRACT

Gracilaria vermiculophylla has the potential to be used as a food ingredient due to its nutritional and bioactive values, however, the aroma is considered a restrictive factor for its large acceptance by consumers. In-depth knowledge about its volatile profile is crucial for the definition of mitigation strategies to overcome this drawback and to improve its acceptability. This work aims to characterize the volatile profile of *G. vermiculophylla* using an advanced multidimensional chromatographic technique. For the first time, *G. vermiculophylla* volatile profile was unveiled and a total of 136 volatile compounds were determined and grouped by 10 chemical families, including aldehydes, ketones, alcohols, mono and sesquiterpenic compounds, norisoprenoids, esters, lactones, carboxylic acids, and halogenated compounds. From these, monoterpenic compounds were present in higher number (31 out 136) and aldehydes exhibited the largest GC areas (17–27 % of the total). A *G. vermiculophylla* aroma wheel was developed by combining data from the list of the detected volatile components with the respective aroma descriptors available in the literature. This strategy allowed to identify the volatile compounds whose aroma descriptors may potentially explain ca. 70 % of the aroma notes present in the algal aroma wheel. This included undesired fishy and sulfuric notes from dimethyltrisulfide and herbaceous notes from fatty acid derivatives, as aldehydes, and positive citric notes linked to monoterpenic compounds. However, *G. vermiculophylla* volatile compounds related to sweet, almond, beany, fresh, and minty notes, not yet considered in the algal aroma wheel, were also determined. This information may be further used to define mitigation strategies of non-valued algae aromas through volatiles' insights, contributing to the future acceptance of algae food products.

1. Introduction

Macroalgae have been recognized as macroscopic aquatic plants with high nutritional value, associated to their richness in proteins, carbohydrates, minerals, and vitamins [1,2]. In addition, macroalgae are a source of fucoidans and phlorotannins that have been related, respectively, to anti-proliferative [3] and antioxidant activities, among other bioactive properties [4], suggesting that their consumption provides beneficial human health outcomes [5]. In fact, the diverse chemical composition of macroalgae and its potential health benefits are the main reasons for the increasing interest in algae and their global uptake as ingredients in different food products [6]. Overall, macroalgae hydrocolloids have been well accepted as thickener and stabilizer ingredients in the food industry [7,8]. However, the aroma of algae-based

food represents one of the most challenging issues when looking for the acceptance of consumers [9]. To mitigate the undesired impact of the aroma of algae-based food, while benefiting from their nutritional value, it is essential to have a deep knowledge of the volatile compounds contributing to the aroma attributes of the food. This will allow to promote algae for different gastronomic applications in harmony with conventional food, such as soups, pastas, and salads, to increased acceptance [6,10].

Recently, an aroma wheel dedicated to algal aromas [10] was proposed to be used as a tool for sensory evaluation in algae. It is based on data from the green *Ulva pertusa* [11] and marine oils [12] and also considers algae aroma descriptors. It includes two levels of information: the first refers to the aroma type/class and the second one corresponds to the subdivision of each class [10]. However, this tool did not consider

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the algae volatiles responsible for each specific aroma note. The analysis of volatile compounds from seaweeds has been conducted using different extraction/concentration techniques, including the solvent-free solid-phase microextraction (SPME) technique [13,14]. SPME is easy to use and does not require any concentration step prior to analysis, preventing the production of artifacts [15,16]. The extraction step is commonly followed by one dimensional gas chromatography-mass spectrometry (1D GC-MS) analysis [11,13,14,17,18]. Different volatile compounds belonging to the chemical families of aldehydes, alcohols, ketones, esters, carboxylic acids, and terpenic and halogenated compounds were reported in macroalgae [14,19–21]. From these, alcohols, aldehydes, ketones, esters, and carboxylic acids have been considered key volatile compounds in the aroma of algae [11,13,14,17,18]. In addition, halogenated compounds are very specific to algae due to the natural seawater environment rich in halides. However, their contribution to the algae aroma is scarce as a result of the usage restriction of halogen-containing compounds as flavoring agents [22]. Also, terpenic compounds, mainly monoterpenic ones, are potentially important odor compounds in algae since they have rather low odor threshold values [23]. Despite this knowledge, the aroma descriptors explained by each determined volatile compound are still largely unexplored in many macroalgae species, although their introduction into the human diet is a global trend.

Gracilaria vermiculophylla is the most common red macroalgae (Rhodophyta) species on the Portuguese coast, being its presence well-marked in the Ria de Aveiro lagoon ($40^{\circ}38'N$, $8^{\circ}43'W$), an urbanized estuary impacted by oyster cultivation and finfish aquaculture [24]. This macroalga has recognized economic relevance, mainly as a source of agar [25]. In addition, *G. vermiculophylla* is rich in valuable lipophilic components with nutritional and health promoting benefits, such as sterols, like cholesterol, and unsaturated fatty acids [1], as well as proteins (around 15 % dry weight) and photosynthetic pigments, as chlorophylls and carotenoids [2]. Due to its nutritional value, this edible macroalga was already reported as a feed ingredient to be used in ruminant nutrition [26] and in rainbow trout diets [27]. However, for *G. vermiculophylla* inclusion in highly valued markets, like the functional food industry for the human diet, its aroma properties linked to the volatile compounds need to be considered to ensure consumers' acceptance [9]. Although there are studies dealing with the volatile composition of the *Gracilaria* genus (*Gracilaria* sp. [19,28], *G. chilensis* [28], and halogenated compounds of *G. lemaneiformis* [29] and *G. cornea* [30]), to the best of our knowledge nothing is known regarding *G. vermiculophylla* volatile composition and its aroma properties. Thus, this study aimed to establish, for the first time, the volatile profile of *G. vermiculophylla* red macroalga through headspace solid-phase microextraction (HS-SPME) combined with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry detection (GC × GC-ToFMS). This methodology will promote faster run times, increase peak capacity, improve resolution and limits of detection, and enhance mass selectivity when compared to the 1D GC systems [31–34]. Moreover, the volatile profile determined for this alga was combined with the aroma descriptors available in literature from other species and/or matrices, to add a third level of information to the algal aroma wheel [10].

2. Materials and methods

2.1. Samples and sampling

G. vermiculophylla (GV) macroalga was collected at Ria de Aveiro (shallow coastal lagoon located at the northwest Atlantic coast of Aveiro, Portugal), near saltpans, in two locations (L1: $40^{\circ}39'22.18''N$, $8^{\circ}39'50.73''W$ and L2: $40^{\circ}37'44.87''N$, $8^{\circ}39'42.67''W$) and at three moments, corresponding to GV harvest period in Portugal: July (GVL2-1), September (GVL2-2), and October (GVL1-1, GVL2-3, and GVL2-4). The first location (L1) is a wilder environment, in the core of the saltpans

bulk, while the second location (L2) is situated in the surrounding saltpans bulk, close to the urban environment of Aveiro (Fig. 1). The collection strategy herein adopted by considering different locations and harvesting moments allowed the understanding of the intrinsic and natural variability of *G. vermiculophylla* and the validation of the data obtained. Samples were washed in distilled water to remove mud, sand, epiphytes, and dead fragments, and were left to dry, at the laboratory room temperature ($20 \pm 1 ^\circ C$), for 4 days, until reaching a moisture content of 12 %–14 %. The samples were stored in the dark in hermetic bags at the laboratory room temperature ($20 \pm 1 ^\circ C$).

2.2. Determination of the volatile components of *G. vermiculophylla*

2.2.1. HS-SPME experimental conditions

The HS-SPME experimental parameters were established based on the work previously developed for marine salt volatile characterization [35], with some modifications. Briefly, 1.0 g of dried *G. vermiculophylla* was placed into a 135 mL glass vial. The vial was capped with a PTFE septum and an aluminum cap (Chromacol Ltd., Herts, UK) and placed in a thermostated water bath at $60.0 \pm 0.1 ^\circ C$, for 10 min. Then, the 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 1 cm) fiber was exposed to the sample vial headspace for 60 min. For each sample, three independent aliquots were analyzed. The fiber was preconditioned before use according to the manufacturer's recommendations. Blanks, corresponding to the analysis of fiber not submitted to any extraction, were run between sets of three analyses.

2.2.2. GC × GC-ToFMS analysis

After the extraction/concentration step, the SPME coating fiber was manually introduced into the GC × GC-ToFMS injection port at $250 ^\circ C$ and kept for 3 min for desorption. The injection port was lined with a 0.75 mm I.D. splitless glass liner. Splitless injections were used (3 min). LECO Pegasus 4D (LECO, St. Joseph, MI, USA) GC × GC-ToFMS system consisted of an Agilent GC 7890A gas chromatograph, with a dual stage jet cryogenic modulator (licensed from Zoex) and a secondary oven. An HP-5 30 m × 0.32 mm I.D., 0.25 μm film thickness (J&W Scientific Inc., Folsom, CA, USA) was used as the first (1D) column, and a DB-FFAP 0.79 m × 0.25 mm I.D., 0.25 μm film thickness (J&W Scientific Inc., Folsom, CA, USA) was used as the second (2D) column. The carrier gas was helium at a constant flow rate of 2.80 mL/min. The primary oven temperature was programmed from $50 ^\circ C$ (3 min) to $230 ^\circ C$ (1 min) at $5 ^\circ C/min$. The secondary oven temperature was programmed from $65 ^\circ C$ (3 min) to $245 ^\circ C$ (1 min) at $5 ^\circ C/min$. The temperature of MS transfer line and MS source was $250 ^\circ C$. The modulation time was 6 s; the modulator temperature was kept at $20 ^\circ C$ offset (above the primary oven). The ToFMS was operated at a spectrum storage rate of 100 spectra/s. The mass spectrometer was operated in the EI mode at 70 eV using a range of m/z 50–510 and the voltage was $-1500 V$. Automated data processing software ChromaTOF® (LECO) was used to process total ion chromatograms, at signal-to-noise threshold of 100. Contour plots were used to evaluate the separation quality and for manual peak identification.

For identification purposes, the mass spectrum and retention times of the analytes were compared with standards, when available. The mass spectrum of each peak was compared to those existing in mass spectral libraries, including an in-house library of standards and two commercial databases (Wiley 275 and US National Institute of Science and Technology (NIST) V.2.0-Mainlib and Replib). Additionally, a manual inspection of the mass spectra was done, combined with the use of the linear retention index (RI) values, which were determined according to the Van den Dool and Kratz RI equation [36]. For the determination of the RI, a C₈-C₂₀ *n*-alkanes series was used, and the calculated RI values were compared with literature for GC columns like the 1D column here used [33,37–45]. A mass spectral match factor with a similarity >900 was used to decide whether a peak was correctly identified or not. The RI parameter calculated also supported the identification, since the calculated retention index (RI_{Calc}) differed from 0 to 1.9 % when compared to

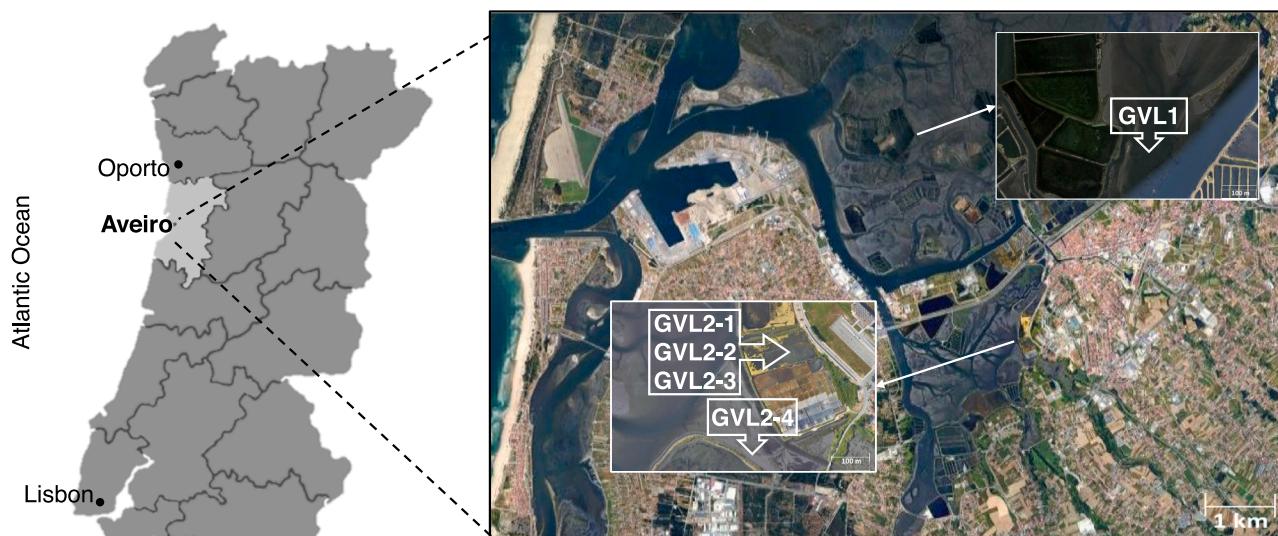


Fig. 1. Ria de Aveiro region (Portugal) with the two locations (GVL1, wilder environment, and GVL2, urban environment) where *Gracilaria vermiculophylla* red macroalga was collected (satellite images adapted from Google maps).

literature (RI_{lit}) for the 1D column or equivalents, corresponding to a median of 0.3 % and a standard deviation of 0.6 %. Also, the majority (>90 %) of identified compounds showed similarity matches >800/1000. Due to matrix complexity, the Deconvoluted Total Ion Current GC \times GC peak areas data were used as an approach to estimate the relative content of each volatile component (Table S1). The reproducibility of the peak area results was determined as relative standard deviation (RSD). Only compounds with a GC peak area $\geq 4 \times 10^4$ arbitrary units were considered for the identification.

2.3. Data processing

Two datasets were used. In one dataset it was considered the five samples of *G. vermiculophylla*, 3 independent replicates, two locations (L1 and L2), 3 collection moments, and 136 variables (total number of volatile components). In the other dataset only the number of variables was different from the first dataset. So, herein it was considered 86 variables instead of 136, corresponding to the 86 volatile components that were common to the five *G. vermiculophylla* samples under study.

A heatmap representation was used to compare each dataset, performing hierarchical cluster (HCA) analyses using MetaboAnalyst 5.0 (web software, The Metabolomics Innovation Centre (TMIC), Edmonton, AB, Canada). Peak areas of the compounds (Table S1) were extracted from the chromatograms and used to build the data matrices. The data (GC peak areas, expressed as arbitrary units, a.u.) were mean-centered and divided by the standard deviation of each variable (autoscaling) using Ward's minimum variance method for clustering analysis. The significance of the variables was determined through a two-sided Mann-Whitney test using the SPSS software 20.0 (IBM, New York, USA) and differences corresponding to $p < 0.05$ were considered significant.

The volatile chemical data from *G. vermiculophylla* samples (Table S1) and their corresponding aroma descriptors tracked for each volatile compound from a range of different sources [46–48] were combined to propose a third level of information in the reported algal aroma wheel [10]. For the construction of the aroma wheel, it was used the sunburst chart of Excel 2016 with three circles, each one corresponding to a different level of information with the inner circle representing the aroma type/class, the middle one representing the subdivision of each class in the correspondent aroma notes, and the outer circle representing the volatile compounds of the *G. vermiculophylla* that were associated to the different aroma notes.

Herein, it was considered the 86 volatile compounds that were common to the five *G. vermiculophylla* samples, thus being representative of this macroalgae, as well as the aroma notes and classes referred in the algal aroma wheel proposed by Francezon et al. [10]. In addition, the aroma descriptors were obtained from a range of different sources and considered representative for neat volatile compounds, since the aroma contribution of each determined volatile compound was not evaluated yet in algae.

3. Results and discussion

3.1. Volatile composition of dried *G. vermiculophylla* red macroalga

The volatile profile of *G. vermiculophylla* samples was evaluated by HS-SPME/GC \times GC-ToFMS. Fig. 2 shows an example of the total ion chromatogram contour plot obtained in full-scan mode for *G. vermiculophylla* illustrating the advantage of using GC \times GC-ToFMS. In this figure, the volatile compounds are spatially distributed based on their chemical structures. The majority of these compounds occur in clusters as revealed for mono and sesquiterpenic compounds and norisoprenoids. This reveals a structured GC \times GC chromatographic behavior based on the differences concerning the volatility and polarity, among the identified compounds. For example, the lower volatile sesquiterpenic compounds (C_{15}) presented higher retention time on the first dimension (1t_R) when compared to monoterpenic ones (C_{10}) since they contain 5 extra carbon molecules. Moreover, within the same chemical group, oxygen-containing compounds have a higher retention time on the second dimension (2t_R) due to their higher polarity (Table S1). This allowed more reliable identifications while avoiding the common co-elution chromatographic problems when only one dimension chromatography, like GC-MS, is used. Moreover, the ToFMS detector in full acquisition mode allowed increasing the sensitivity of the methodology [33,34]. These are relevant characteristics for the analysis of volatile components of complex biological systems [49,50], including algae.

A total of 136 volatile compounds were determined in dried *G. vermiculophylla* macroalga. They were grouped by 10 chemical families, including aldehydes, ketones, alcohols, mono and sesquiterpenic compounds, norisoprenoids, esters, lactones, carboxylic acids, and halogenated compounds. A miscellaneous group was also included containing ethers and sulfur-, nitrogen-, furan-, terpen-, and thiazole-derived compounds (Table 1).

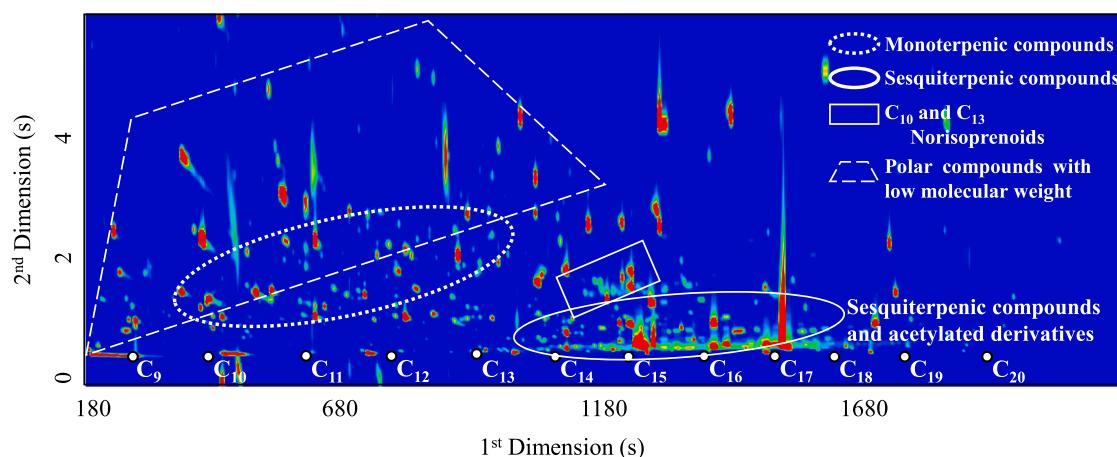


Fig. 2. GC × GC-ToFMS total ion chromatogram contour plot obtained in full-scan mode for *Gracilaria vermiculophylla* collected in July at location 2 (GVL2-1). Examples of clusters formed by structurally related compounds are indicated. The white spots indicate the position of the alkanes from the series used.

From the 136 volatile compounds determined in the headspace of the dried macroalga, a total of 86 were common to all *G. vermiculophylla* samples, being independent of the location and collection moments (names highlighted in bold in Table 1). A graphical representation of these 86 compounds was performed in a dendrogram and a heatmap to allow fast visual access to the volatiles' pattern of dried *G. vermiculophylla* macroalga (Fig. 3). Here, the samples were represented with different colors: GVL1-1_in red, GVL2-1_in green, GVL2-2_in dark blue, GVL2-3_in light blue, and GVL2-4_in pink.

According to the dendrogram in Fig. 3, two main clusters can be observed, corresponding to samples from locations 1 (L1) and 2 (L2), which were separated according to their volatile composition differences. In addition, no relevant clusters were formed when considering the *G. vermiculophylla* collection moments (July, September, and October), thus revealing similarities in their volatile composition along the harvest period of *G. vermiculophylla*, in Ria de Aveiro. In addition, in ca. 60 % of the volatile compounds determined in the headspace of this red macroalga, corresponding to 52 out of 86 analytes, it was observed a significant difference ($p < 0.05$, Fig. 3) between the two locations under study, highlighting their different composition. These results suggest that the macroalga location is the most influential factor for this macroalga volatile composition, following literature for other macroalgae harvested in the North and South of Spain [19,28]. However, the spring and autumn harvesting season of Spain was also considered significant for the volatile composition of the studied macroalgae, which was not here observed for *G. vermiculophylla* collected in July, September, and October. This can be justified by the fact that in this study only the summer and autumn seasons were considered, which correspond to the harvesting period of this red macroalga in Portugal.

The heatmap allows observing the chemical family pattern of *G. vermiculophylla* samples. The major difference related to the volatile composition determined for dried red macroalga from L1 and L2, was that the L1 sample (GVL1-1) had a greater amount of almost all determined volatile compounds (Fig. 3). This suggests that the wilder environment of the first location (L1), in the core of the saltpans bulk (Fig. 1), seems to be favorable to the formation of those volatiles by this macroalga. It is known that the volatile compounds of algae are produced in response to environmental stress [51,52] promoted by a diversity of biotic (vegetation and microorganisms) and abiotic (water constituents) stressors [53]. Thus, the wilder environment of L1 when compared to the more urbanized environment of L2, which suffers higher human interference due to oyster cultivation and finfish aquaculture production [24], can explain the different volatile composition of *G. vermiculophylla* samples. However, further work on the link between the perception of the environmental stress and the algae volatile release may be an important step for the understanding of the adaptive context of

seaweeds.

These results were also followed when considering the total volatiles determined in the headspace of *G. vermiculophylla* samples (136 volatiles, Fig. S1), reinforcing the evidence observed for the common ones (86 volatiles, Fig. 3). In addition, the strategy herein used by considering different locations and collection moments to *G. vermiculophylla* has contributed to the understanding of the intrinsic and natural variability of this red macroalga and allowed the validation of the data obtained.

3.1.1. Mono and sesquiterpenic compounds

Covering all the identified chemical families, monoterpene compounds (C_{10} compounds) represent the largest one with a total of 31 volatile compounds determined (Table 1, Fig. 3). These compounds are secondary metabolites, derived from isoprene, well-known for their fruity, floral, wood, and sweet notes (Table 1). They can contribute to the complex algae aroma [10] since their odor threshold values are rather low [23]. Monoterpene compounds represent ca. 6 % (GVL2-1) to 11 % (GVL2-3) of the total GC areas of the dried *G. vermiculophylla*, being limonene, safranal, verbenone, menthol, and endo-borneol the ones that exhibited higher chromatographic areas. From these, limonene [11,17] and safranal [19], related to citrus and saffron-like notes (Table 1), were identified in different marine algae, including *Capsosiphon fulvescens* and *Ulva pertusa* green ones. Several monoterpene compounds here determined in *G. vermiculophylla* macroalgae have been related to different bioactive activities. For example, limonene revealed to have anticarcinogenic and/or antitumoral potential [54], terpinen-4-ol and menthol are potential anti-inflammatory agents [55,56] and bornyl acetate has antimicrobial activity [57]. Thus, compounds from this chemical family can play an important role in the development of innovative functional foods, while following the demands on healthy and sensorially accepted food resources for the future [58].

Like monoterpene compounds, sesquiterpenic ones (C_{15} compounds) constituted by 3 isopene units) were determined in the headspace of dried *G. vermiculophylla*. They correspond to a total of 15 volatile compounds that represent ca. 3 % (GVL2-3) to 8 % (GVL2-2) of the total GC peak areas (Table 1). Sesquiterpenic compounds, although considered smaller algae aroma contributors than the monoterpene compounds due to their lower volatility and higher odor threshold values [23], can contribute to the global macroalga aroma with woody, cloves, and turpentine notes (Table 1) if present in amounts above their odor threshold values [23,59]. In addition to their relevant aroma properties, terpenic compounds (mono and sesquiterpenic ones), can also be linked to several biological effects [60], broadening the application range of the red macroalga here studied. For example, it was described that terpinen-4-ol and l-menthol have the potential to be used as anti-inflammatory agents [55,56], and bornyl acetate as an antimicrobial one [57],

Table 1

Volatile compounds determined in *G. vermiculophylla* dried red macroalga collected at two locations (Ria de Aveiro, Portugal) and organized by chemical families, using HS-SPME/GC × GC-ToFMS.

Number	1t_R ^a (s)	2t_R ^a (s)	Compound	RI _{lit.} ^b	RI _{cal} ^c	GVL1-1 GVL2-1 GVL2-2 GVL2-3 GVL2-4					Aroma descriptor ^e													
						Peak area $\times 10^6$ and RSD (%) ^d																		
Aldehydes																								
<i>Aliphatics</i>																								
1	264	0.930	Heptanal*	899	904	14.89 (33.8)	21.23 (26.6)	53.74 (25.9)	14.55 (31.0)	23.42 (23.8)	Oily-fatty, rancid, pungent, fermented-fruit-like													
2	354	1.320	2-Heptenal	956	960	1.82 (3.8)	4.10 (22.0)	4.13 (4.5)	3.04 (34.7)	6.77 (21.6)	Pungent, green, fatty													
3	432	0.980	Octanal	1004	1008	15.17 (20.7)	15.91 (21.6)	13.72 (11.1)	12.16 (18.7)	11.76 (4.2)	Fatty, citrus, orange-like, honey													
4	606	1.010	Nonanal	1108	1108	25.53 (10.1)	21.53 (20.7)	20.44 (17.2)	19.77 (11.6)	20.79 (19.1)	Orange and rose odor, floral, fatty, green													
5	420	2.050	2,4-Heptadienal	999	1001	0.32 (22.1)	—	0.53 (12.5)	—	0.66 (1.0)	Fatty, green													
6	522	1.350	2-Octenal	1056	1059	4.56 (19.6)	0.17 (18.6)	4.94 (2.5)	4.82 (20.2)	7.10 (11.5)	Green-leafy, orange, honey-like, cognac-like, seaweed-like													
7	690	1.610	2,6-Nonadienal	1154	1158	1.45 (16.9)	—	0.91 (5.4)	0.36 (37.7)	1.30 (18.9)	Cucumber-like													
8	702	1.320	2-Nonenal	1162	1165	3.21 (15.8)	3.96 (22.2)	4.17 (28.0)	3.29 (13.1)	8.39 (3.3)	Fatty, orris-like, waxy, orange peel-like, paperboard-like, cucumber-like													
9	780	1.010	Decanal	1204	1211	30.04 (33.0)	23.52 (33.9)	19.50 (28.5)	22.42 (36.2)	20.83 (7.1)	Sweet, waxy, floral, citrus, fatty													
10	870	1.290	2-Decenal	1254	1265	0.96 (8.8)	0.81 (9.5)	0.39 (20.3)	0.73 (10.1)	0.75 (0.8)	—													
11	942	1.000	Undecanal	1310	1309	3.13 (23.4)	2.29 (37.2)	2.51 (38.0)	3.18 (16.8)	2.45 (13.9)	Sweet, fatty, orange and rose undertone													
12	1098	1.010	Dodecanal	1407	1413	2.58 (6.8)	1.19 (35.7)	1.60 (10.7)	2.2 (28.9)	1.74 (2.7)	Fatty, violet-like													
13	1248	0.990	Tridecanal	1511	1514	0.64 (4.6)	0.37 (14.7)	0.47 (25.2)	0.49 (28.0)	0.56 (3.2)	—													
<i>Aromatics</i>																								
14	354	3.220	Benzaldehyde	961	961	44.24 (3.5)	19.69 (24.3)	17.75 (13.2)	12.85 (19.4)	17.87 (23.1)	Almond, bitter almond-like odor													
15	498	3.490	Benzeneacetaldehyde	1043	1047	1.69 (16.5)	1.04 (7.3)	1.82 (35.0)	0.84 (11.7)	0.84 (10.7)	Pungent-green, floral, sweet-fruity, honey-like													
16	540	2.790	2-Methylbenzaldehyde	1066	1071	0.42 (14.2)	—	—	—	0.20 (25.1)	Sweet, beany, fresh pea													
Subtotal (GC Peak Area)						150.65	115.84	146.61	100.72	125.54														
Subtotal (Number of Compounds)						16	13	15	14	16														
Aldehydes Subtotal (%)						31.9	27.2	37.0	28.9	28.4														
Ketones																								
<i>Aliphatics</i>																								
17	246	0.850	3-Heptanone	859	849	0.09 (17.3)	0.09 (26.7)	0.08 (15.3)	0.23 (17.0)	0.09 (29.6)	Green powerful odor, fruity													
18	252	0.940	2-Heptanone	882	877	2.88 (14.6)	4.29 (32.8)	5.85 (21.3)	2.59 (24.4)	3.79 (23.5)	Penetrating fruity banana odor, slightly spicy, banana-like fruity odor													
19	312	3.410	2,5-Hexanedione	933	935	0.99 (10.6)	0.72 (31.7)	0.32 (27.9)	0.14 (17.1)	—	Sweet-ethereal, medicinal													
20	372	1.230	2-Methyl-1-hepten-6-one	966	971	0.46 (20.7)	—	0.17 (33.3)	—	0.13 (0.2)	—													
21	390	1.110	1-Octen-3-one	979	982	2.99 (23.5)	2.61 (7.9)	3.40 (31.1)	4.68 (5.8)	6.09 (22.0)	Mushroom													
22	402	0.920	3-Octanone	986	989	0.64 (24.9)	3.77 (6.1)	1.66 (26.0)	0.64 (29.6)	0.54 (23.1)	Mild fruity odor													
23	402	1.250	6-Methyl-5-hepten-2-one	985	990	12.17	16.63	13.99	7.67 (13.4)	10.88	Powerful fatty-green odor, citrus odor													
24	492	1.330	3-Octen-2-one	1036	1042	1.18 (14.9)	4.05 (26.2)	1.00 (31.0)	1.09 (6.5)	1.07 (22.4)	Fruity, lemon, citrus													
25	738	1.830	4-Decanone	—	1187	0.44 (13.3)	0.59 (11.0)	0.23 (17.4)	0.43 (30.2)	0.69 (38.2)	—													
26	924	1.020	2-Undecanone	1291	1297	1.85 (22.8)	0.80 (23.0)	0.48 (23.7)	0.77 (30.6)	0.72 (15.6)	Strong odor, rose, orris-like, citrus													
27	1080	1.010	2-Dodecanone	1401	1401	0.56 (20.5)	0.58 (1.5)	0.33 (38.3)	0.54 (38.6)	0.51 (14.4)	—													
28	1680	0.940	Phytone	1845	1837	23.13	15.35	4.06 (13.7)	8.09 (5.0)	8.70 (12.2)	—													
Subtotal (GC Peak Area)						(14.8)	(20.2)																	
Subtotal (Number of Compounds)						16	15	16	15	15														
<i>Aromatic and Cyclic</i>																								
29	480	0.990	2,2,6-Trimethylcyclohexanone	1035	1035	1.15 (9.8)	0.80 (22.9)	1.44 (13.8)	0.82 (18.1)	2.25 (24.2)	—													
30	534	3.210	1-Phenylethanone	1065	1067	2.76 (17.3)	1.89 (23.0)	1.69 (35.6)	2.22 (33.8)	2.43 (32.9)	—													
31	672	2.490	2-Cyclohexene-1,4-dione	1142	1148	3.09 (24.5)	2.17 (14.6)	1.13 (24.8)	1.47 (8.3)	2.13 (35.9)	—													
32	1398	4.010	Benzophenone	1621	1626	9.82 (18.7)	4.89 (20.1)	3.45 (12.1)	7.86 (12.1)	7.49 (18.0)	Geranium-like odor, sweet, rose-like odor													
Subtotal (GC Peak Area)						64.24	55.88	39.28	39.23	47.53														
Subtotal (Number of Compounds)						16	15	16	15	15														

(continued on next page)

Table 1 (continued)

Number	${}^1\text{H}_\text{R}^\text{a}$ (s)	${}^2\text{H}_\text{R}^\text{a}$ (s)	Compound	RIlit. ^b	RIcal ^c	GVL1-1	GVL2-1	GVL2-2	GVL2-3	GVL2-4	Aroma descriptor ^e
						Peak area $\times 10^6$	and RSD (%) ^d				
Ketones Subtotal (%)											
						13.6	13.1	9.9	11.2	10.7	
Alcohols											
Aliphatics											
33	264	1.590	2-Heptanol	905	905	–	–	0.14 (24.5)	0.16 (7.4)	0.14 (29.2)	Fresh, lemon-like, grassy-herbaceous, sweet-floral undertone
34	378	2.190	1-Heptanol	969	975	1.81 (33.9)	1.43 (18.9)	3.02 (24.4)	–	–	Fragrant faint, aromatic, fatty
35	390	2.090	1-Octen-3-ol	980	983	10.23 (6.1)	25.60 (14.1)	24.94 (23.5)	39.66 (3.3) (27.7)	38.99	Mushroom, herbaceous, savory, brothy, meaty
36	420	1.480	3-Octanol	993	1001	2.85 (17.4)	–	–	0.22 (22.9)	–	–
37	474	1.930	2-Ethyl-1-hexanol	1029	1032	8.92 (11.4)	3.71 (5.0)	4.49 (6.5)	2.69 (22.4)	3.19 (15.1)	Sweet, slightly floral rose-like
38	552	2.080	1-Octanol	1070	1077	6.23 (22.2)	5.82 (32.9)	2.47 (12.1)	3.13 (24.1)	2.75 (6.5)	Fresh, orange-rose, sweet
39	564	1.940	1-Nonen-3-ol	1088	1084	0.92 (30.8)	0.29 (22.3)	0.17 (39.3)	0.29 (8.6)	0.46 (32.8)	–
40	714	0.980	2-Nonenol	1145	1172	2.28 (31.4)	13.96 (36.5)	9.33 (17.9)	1.89 (23.9)	1.27 (7.9)	–
41	726	1.910	1-Nonanol	1171	1180	3.99 (18.1)	3.72 (19.2)	2.66 (4.6)	4.11 (29.4)	2.94 (21.5)	–
42	1194	1.610	1-Dodecanol	1473	1477	16.25 (0.7)	10.53 (6.8)	6.48 (8.0)	8.64 (28.1)	12.89 (29.6)	Fatty odor, unpleasant at high concentration but delicate, floral on dilution
43	1470	1.490	1-Tetradecanol	1676	1678	6.54 (8.2)	3.98 (7.8)	3.22 (14.8)	2.72 (18.3)	4.52 (10.9)	–
44	1722	1.410	1-Hexadecanol	1879	1869	1.27 (17.9)	0.99 (1.1)	1.42 (14.3)	1.05 (20.2)	1.51 (19.7)	Faint odor, odorless
Aromatic and cyclic											
45	498	5.120	Benzinemethanol	1032	1048	0.24 (20.7)	3.33 (23.0)	1.47 (32.5)	0.66 (31.4)	1.65 (16.0)	–
46	606	2.080	2,6-Dimethyl-Cyclohexanol	1112	1108	12.65 (27.8)	8.84 (14.1) (18.1)	13.19 (27.3)	17.68	27.14 (6.2)	–
Subtotal (GC Peak Area)						74.16	82.22	72.96	82.90	97.45	
Subtotal (Number of Compounds)						13	12	13	13	12	
Alcohols Subtotal (%)						15.7	19.3	18.4	23.8	22.0	
Monoterpene compounds											
47	312	0.530	α -Pinene	938	934	0.56 (28.5)	0.66 (28.2)	2.76 (31.5)	0.41 (17.8)	1.19 (13.7)	Characteristic odor of pine, turpentine odor
48	378	0.610	β -Pinene	980	974	0.22 (38.4)	0.28 (18.0)	0.41 (30.7)	0.22 (15.2)	0.21 (6.9)	Turpentine odor, dry, woody or resinous aroma, piney, terpene odor
49	426	0.680	α -Phellandrene	1005	1004	0.23 (32.3)	–	0.19 (37.2)	–	–	Fresh, citrus, peppery, discrete mint, minty, herbaceous
50	462	0.880	σ -Cymene	1020	1025	0.74 (17.5)	0.83 (31.7)	0.75 (19.5)	0.85 (14.8)	0.71 (11.0)	–
51	468	0.700	Limonene isomer	1031	1028	3.49 (8.7)	4.03 (24.7)	2.36 (29.8)	13.50 (22.8)	4.94 (39.1)	Citrus odor
52	474	0.700	1,8-Cineole	1033	1031	0.57 (4.4)	–	0.79 (9.8)	0.35 (25.1)	1.61 (8.9)	–
53	480	0.690	Limonene isomer	1027	1035	–	–	–	0.19 (20.1)	–	Citrus odor
54	522	0.740	γ -Terpinene	1062	1059	0.24 (33.1)	0.13 (12.3)	0.14 (11.7)	0.59 (27.1)	0.04 (21.5)	Lemon
55	546	1.310	Linalool oxide	1074	1073	0.21 (26.4)	–	–	–	–	Sweet, woody, floral, woody-earthly, undertone, pungent
56	552	1.440	Dihydromyrcenol	1075	1077	–	0.66 (27.5)	0.54 (8.4)	0.75 (19.9)	0.56 (4.4)	–
57	594	1.130	Tetrahydrolinalool	1097	1101	0.13 (40.1)	0.11 (17.0)	0.29 (28.7)	0.15 (34.1)	0.10 (8.7)	–
58	600	1.730	Linalool	1098	1105	–	–	1.01 (25.1)	0.99 (28.1)	–	Floral-woody (powdery), faintly citrussy note, floral, sweet-fruity
59	654	1.690	m/z 91, 79, 107	1142	1137	0.61 (25.0)	0.46 (21.8)	0.38 (2.3)	0.44 (20.5)	0.31 (10.4)	–
60	672	1.720	Fenchone	1106	1148	0.52 (15.8)	0.29 (21.5)	–	0.59 (21.6)	0.34 (17.5)	–
61	672	1.960	Dihydrocarveol	1180	1148	0.69 (7.0)	–	–	–	–	–
62	684	1.090	Menthone	1164	1154	–	–	0.56 (39.4)	–	0.86 (7.3)	Slight peppermint odor, characteristic odor like menthol
63	696	1.480	Pinocarvone	1162	1162	0.75 (33.8)	0.48 (18.1)	–	0.34 (13.9)	0.26 (31.5)	–
64	708	2.310	Endo-borneol	1165	1169	3.49 (22.4)	1.98 (38.5)	0.26 (12.8)	1.97 (34.9)	1.09 (38.1)	Camphor-like
65	708	1.570	Isomenthol	1179	1169	–	–	0.41 (3.0)	–	–	–
66	720	1.780	Menthol	1173	1176	1.26 (29.5)	1.31 (27.5)	4.74 (2.9)	1.05 (38.8)	3.54 (13.4)	Peppermint odor
67	726	1.540	Terpinen-4-ol	1177	1179	0.73 (5.8)	1.05 (29.6)	–	0.46 (7.4)	1.39 (11.6)	Pine
68	744	3.870	p-Cymen-8-ol	1183	1192	2.42 (6.2)	0.73 (6.6)	–	1.34 (14.3)	0.82 (27.6)	–
69	762	1.660	Safranal	1201	1201	4.25 (8.6)	2.79 (33.7)	5.84 (12.9)	2.88 (8.7)	4.82 (21.1)	Saffron-like
70	780	1.930	Verbenone	1204	1212	9.39 (5.0)	2.91 (29.0)	0.51 (6.1)	5.59 (8.6)	2.76 (20.6)	–

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Table 1 (continued)

Number	^1H ^a (s)	^2H ^a (s)	Compound	RIlit. ^b	RIcal ^c	GVL1-1	GVL2-1	GVL2-2	GVL2-3	GVL2-4	Aroma descriptor ^e
						Peak area $\times 10^6$ and RSD (%) ^d					
71	786	2.010	Exo-borneol	1173	1216	0.49 (36.7)	2.98 (24.5)	2.01 (35.6)	2.82 (33.4)	1.54 (36.1)	–
72	804	3.210	Exo-2-hydroxycineole	1228	1227	1.37 (34.2)	0.55 (18.7)	–	–	–	–
73	840	1.850	Carvone	1242	1248	0.46 (15.4)	2.22 (24.6)	0.89 (38.3)	0.30 (4.7)	1.79 (29.9)	–
74	888	2.910	2,3-Pinanediol	1313	1278	0.49 (17.9)	–	0.46 (21.7)	0.37 (9.5)	–	–
75	906	1.030	Bornyl acetate	1285	1286	0.96 (21.3)	1.29 (9.3)	1.09 (15.3)	0.82 (11.6)	0.81 (21.7)	–
76	942	1.100	Perilla alcohol	1295	1309	4.06 (7.7)	–	–	–	–	–
77	1122	1.160	<i>m/z</i> 84, 91, 109	1424	1429	0.39 (11.1)	0.25 (24.3)	–	0.22 (13.4)	0.17 (15.4)	–
Subtotal (GC Peak Area)						38.76	25.99	26.40	37.21	29.85	
Subtotal (Number of Compounds)						26	21	21	24	22	
Monoterpeneic compounds Subtotal (%)						8.2	6.1	6.7	10.7	6.7	
7											
Sesquiterpenic compounds											
78	1044	0.690	α -Copaene	1376	1377	0.34 (13.1)	1.36 (8.1)	1.31 (21.4)	1.26 (21.4)	4.24 (18.4)	–
79	1098	0.760	α -Cedrene	1409	1413	0.70 (19.2)	0.69 (22.1)	0.81 (13.7)	0.67 (15.1)	15.96 (34.3)	–
80	1110	0.780	β -Caryophyllene	1418	1421	–	0.57 (7.2)	–	0.46 (5.9)	–	Cloves, turpentine
81	1140	0.790	Allo-aromadendrene	1452	1441	2.69 (6.7)	1.78 (31.6)	5.02 (22.6)	–	–	–
82	1152	0.840	α -Neoclovene	1451	1449	1.24 (23.5)	1.27 (15.4)	20.40 (25.2)	2.58 (17.3)	0.65 (26.2)	–
83	1170	0.810	α -Caryophyllene	1454	1461	0.14 (17.1)	5.23 (19.5)	0.75 (18.6)	–	1.07 (24.5)	–
84	1260	0.880	Δ -Cadinene	1524	1522	0.73 (22.2)	9.86 (9.9)	0.68 (34.6)	–	–	–
85	1260	1.030	δ -Cadinene	1516	1523	1.09 (7.5)	0.39 (27.9)	0.68 (12.6)	0.86 (5.0)	0.89 (8.1)	–
86	1308	1.420	Epiglobulol	1564	1558	1.85 (32.4)	0.67 (5.7)	0.47 (13.4)	1.32 (7.4)	0.80 (29.9)	–
87	1132	1.780	Spathulenol	1576	1575	13.32 (7.5)	–	–	–	1.76 (16.7)	–
88	1344	1.270	Caryophyllene oxide	1581	1584	2.48 (10.8)	2.28 (2.1)	0.62 (17.0)	3.29 (10.2)	3.17 (27.5)	–
89	1344	1.540	Globulol	1580	1584	0.64 (8.8)	–	1.33 (12.9)	–	1.98 (14.2)	–
90	1368	1.630	Cedrol	1596	1601	1.77 (4.3)	–	0.57 (10.2)	–	–	Woody-earthy
91	1440	1.480	α -Bisabolol oxide	1655	1656	11.34 (24.6)	0.67 (19.0)	–	–	4.28 (10.5)	–
92	1584	1.390	Cedryl acetate	1762	1765	0.52 (24.5)	0.24 (15.2)	0.44 (26.0)	0.25 (25.9)	0.27 (10.5)	–
Subtotal (GC Peak Area)						38.85	16.15	33.08	10.67	33.08	
Subtotal (Number of Compounds)						14	12	12	8	11	
Sesquiterpenic compounds Subtotal (%)						8.2	3.8	8.3	3.1	7.5	
Norisoprenoids											
93	1122	1.380	α -Ionone	1426	1429	1.26 (19.5)	0.36 (23.7)	–	–	0.37 (14.1)	Warm, woody, and floral odor with balsamic/sweet tones, violets, sweet-floral
94	1140	1.250	Dihydro- β -ionone	1433	1441	0.43 (4.5)	0.26 (16.6)	0.29 (18.1)	–	0.38 (6.8)	–
95	1164	1.280	Geranyl acetone	1453	1457	10.27 (12.3)	10.40 (6.8)	12.84 (14.6)	10.10 (29.5)	14.17 (17.3)	Green and rosy floral, fresh-floral, sweet-rosy, slightly green
96	1200	1.190	α -Isomethyl ionone	1485	1481	0.91 (16.6)	0.53 (25.5)	0.81 (7.6)	0.59 (10.9)	0.79 (2.6)	–
97	1212	1.460	β -Ionone	1485	1489	12.20 (1.2)	7.48 (28.6)	11.36 (10.4)	9.02 (1.2)	5.69 (30.3)	Warm, woody, and fruity odor, cedar wood-like, violet-like upon dilution
98	1212	1.680	5,6-Epoxyde- β -Ionone	1488	1489	9.02 (4.6)	10.32 (4.6)	6.92 (2.3)	5.75 (1.2)	11.77 (11.2)	–
Subtotal (GC Peak Area)						34.09	29.34	32.24	28.79	33.18	
Subtotal (Number of Compounds)						6	6	5	4	6	
Norisoprenoids Subtotal (%)						7.2	6.9	8.1	8.3	7.5	
Esters											
99	642	0.920	Methyl octanoate	1126	1129	0.46 (19.4)	0.48 (4.6)	0.34 (34.3)	0.41 (18.1)	0.54 (39.8)	Powerful, winey, fruity, orange-like odor
100	1266	0.930	Methyl dodecanoate	1525	1527	2.59 (6.2)	2.43 (29.3)	1.34 (28.8)	2.79 (32.2)	3.09 (40.1)	Fatty, floral odor, reminiscent of wine oily, winey
101	1440	2.160	Methyl dihydrojasmonate	1656	1656	4.23 (24.4)	2.88 (35.3)	4.34 (17.9)	3.78 (6.6)	4.04 (27.1)	–

(continued on next page)

Table 1 (continued)

Number	¹ t _R ^a (s)	² t _R ^a (s)	Compound	RI _{lit.} ^b	RI _{cal} ^c	GVL1-1	GVL2-1	GVL2-2	GVL2-3	GVL2-4	Aroma descriptor ^e
Peak area × 10 ⁶ and RSD (%) ^d											
			Subtotal (GC Peak Area)			7.28	5.78	6.02	6.99	7.67	
			Subtotal (Number of Compounds)			3	3	3	3	3	
			Esters Subtotal (%)			1.5	1.4	1.5	2.0	1.7	
Lactones											
102	294	0.690	Butyrolactone	915	923	15.92 (39.9)	22.50 (23.3)	1.99 (37.7)	1.43 (30.4)	22.19 (11.4)	Pleasant, creamy, faint odor
103	348	4.940	γ -Valerolactone	962	959	1.89 (17.9)	4.68 (30.4)	2.96 (29.4)	2.21 (24.2)	6.75 (11.6)	–
104	516	4.220	γ -Caprolactone	1056	1058	6.61 (8.6)	8.22 (32.8)	3.51 (24.8)	3.59 (23.6)	7.43 (24.9)	–
105	1026	3.020	γ -Nonalactone	1360	1366	5.16 (14.2) (13.8)	29.87	2.82 (12.4)	7.73 (13.6)	9.14 (16.7)	Coconut odor
Subtotal (GC Peak Area)											
			Subtotal (Number of Compounds)			29.59	65.27	11.29	14.96	45.51	
			Lactones Subtotal (%)			4	4	4	4	4	
Carboxylic acids											
106	276	3.510	Pentanoic Acid	911	913	–	1.20 (4.4)	–	1.56 (22.6)	–	–
107	600	4.980	Hexanoic Acid	1085	1107	0.59 (23.2)	2.39 (14.0)	1.19 (22.8)	0.61 (25.1)	0.84 (28.7)	Goat-like odor
108	756	3.400	Octanoic Acid	1191	1199	4.94 (22.9)	2.13 (32.1)	0.49 (36.0)	3.01 (34.6)	0.17 (9.1)	Faint, fruity-acid odor, unpleasant, irritating, slight odor
109	912	1.980	Nonanoic Acid	1280	1291	5.41 (24.4)	6.86 (5.7)	3.61 (7.4)	6.40 (17.7)	3.56 (14.5)	Fatty odor, coconut aroma, slight odor
Subtotal (GC Peak Area)											
			Subtotal (Number of Compounds)			10.95	12.58	5.31	11.58	4.57	
			Carboxylic Acids Subtotal (%)			3	4	3	4	3	
Halogenated compounds											
110	210	0.610	1-Chlorobutane	648	685	–	–	0.53 (38.5)	–	–	–
111	258	0.860	Bromo derivative (<i>m/z</i> 57, 41, 84)	–	901	–	0.19 (12.2)	0.17 (15.8)	–	–	–
112	270	1.240	2-Chlorohexanal	–	908	0.38 (6.0)	–	–	–	–	–
113	294	0.800	1-Iodopentane	–	923	0.29 (32.9)	–	–	–	–	–
114	312	0.750	1-Bromohexane	940	934	–	0.23 (13.1)	0.30 (29.4)	–	0.22 (15.4)	–
115	354	0.700	1-Chloroheptane	962	960	0.30 (28.9)	–	–	–	0.14 (20.3)	–
116	528	0.730	1-Chlorooctane	1064	1038	0.65 (12.7)	0.28 (23.4)	0.26 (8.1)	0.24 (18.8)	0.29 (31.9)	–
117	666	0.810	1-Bromoocetane	–	1143	0.23 (4.4)	0.35 (3.6)	0.54 (39.9)	0.26 (22.3)	0.41 (6.3)	–
118	702	0.750	1-Chlorononane	1167	1165	0.42 (34.0)	0.32 (21.0)	0.33 (15.4)	0.35 (4.5)	0.38 (11.9)	–
119	822	0.890	2-Bromononane	–	1236	0.38 (37.4)	–	–	–	–	–
120	840	0.830	1-Bromononane	–	1247	0.57 (27.6)	0.53 (39.8)	0.44 (13.9)	0.69 (6.4)	0.46 (36.8)	–
121	870	0.770	1-Chlorodecane	–	1268	0.15 (6.5)	–	–	0.15 (8.9)	–	–
122	990	0.880	1-Iododecane	–	1341	0.18 (38.8)	–	–	–	–	–
123	990	1.370	Chloro derivative (<i>m/z</i> 95, 43, 81)	–	1341	–	0.30 (15.8)	–	–	–	–
124	1050	0.880	3-Bromodecane	–	1380	–	–	–	0.22 (37.0)	0.22 (38.8)	–
125	1888	0.790	1-Chlorododecane	–	1473	0.36 (1.9)	0.32 (16.9)	0.21 (18.5)	0.20 (19.4)	0.21 (14.0)	–
126	1308	0.850	1-Bromododecane	–	1557	1.65 (6.6) (30.16)	1.72	1.15 (17.4)	1.47 (10.5)	1.69 (19.1)	–
127	1578	0.870	1-Bromotetradecane	–	1760	0.14 (34.2)	0.17 (14.6)	0.07 (7.5)	0.25 (39.5)	0.21 (25.5)	–
Subtotal (GC Peak Area)											
			Subtotal (Number of Compounds)			5.69	4.44	4.03	3.83	4.21	
			Halogenated compounds Subtotal (%)			13	10	10	9	10	
Miscellaneous											
128	276	2.270	2-Butoxyethanol	909	913	0.58 (24.2)	0.78 (32.6)	0.45 (31.1)	0.56 (34.1)	0.49 (2.9)	–
129	366	1.600	Dimethyltrisulfide	961	968	0.26 (12.0)	0.14 (13.3)	0.48 (29.7)	0.16 (26.6)	0.54 (38.6)	Sulfur, fish, fishy, onion, and cabbage odor, sea-like
130	390	1.680	Heptanenitrile	985	983	–	0.34 (26.5)	0.46 (1.2)	–	–	–

(continued on next page)

Table 1 (continued)

Number	t_{Rk}^a (s)	$2t_{Rk}^a$ (s)	Compound	$R_{lit.}^b$	$R_{calc.}^c$	GVL1-1 Peak area $\times 10^6$ and RSD (%) ^d	GVL2-1 Peak area $\times 10^6$ and RSD (%) ^d	GVL2-2 Peak area $\times 10^6$ and RSD (%) ^d	GVL2-3 Peak area $\times 10^6$ and RSD (%) ^d	GVL2-4 Peak area $\times 10^6$ and RSD (%) ^d	Aroma descriptor ^e
131	408	0.840	2-Pentylfuran	1001	993	10.25 (0.6)	6.92 (30.6)	7.37 (4.7)	5.92 (11.7)	6.80 (19.3)	Green bean, metallic, vegetable
132	504	1.880	1-Nitro-hexane	1050	1049	0.58 (10.2)	0.68 (16.5)	0.86 (18.1)	0.80 (23.0)	0.48 (2.0)	–
133	684	2.400	4-(5-Methyl-2-furanyl)-2-butanone	–	1155	0.47 (34.2)	–	0.34 (32.0)	–	–	–
134	798	1.420	β-Cyclooctal	1120	1221	3.02 (6.5)	1.24 (20.6)	6.84 (10.7)	2.26 (12.1)	3.63 (16.6)	–
135	798	4.620	Benzothiazole	1218	1224	1.94 (1.7)	2.19 (5.1)	1.64 (35.4)	1.65 (3.7)	1.68 (0.6)	–
136	1266	1.700	Lilial	1529	1527	0.73 (13.2)	–	0.58 (4.7)	0.57 (27.8)	0.45 (25.5)	–
Subtotal (GC Peak Area)				17.83	12.29		19.01	11.94	14.06		
Subtotal (Number of Compounds)				8	7		9	7	7		
Miscellaneous Subtotal (%)				3.8	2.9		4.8	3.4	3.2		
Total (GC Peak Area)				472.09	425.79		396.24	348.82	442.65		
Total (Number of Compounds)				122	107		111	105	109		

* Bold compounds' name refers to the 86 volatile compounds detected in all dried *G. vermiculophylla* red macroalgae samples under study.

^a Retention times for first (t_{Rk}) and second ($2t_{Rk}$) dimensions in seconds.

^b $R_{lit.}$: retention index reported in the literature for HP-5 GC column or equivalents [33,37–45].

^c $R_{calc.}$: retention index obtained through the modulated chromatogram.

^d Mean of three independent replicates (available in Table S1) expressed as peak area ($\times 10^6$) and relative standard deviation (RSD), expressed in percentage.

^e Aroma descriptors were tracked for each volatile compound from a range of different sources [46–48]. These are representative of neat volatile compounds since their aroma contribution has not been assessed in algae.

whose properties are linked to the presence of oxygenated functional groups [57,61]. In dried *G. vermiculophylla*, the oxygenated monoterpenic compounds prevail in number when compared to the aliphatic ones (24 against 7, respectively) and in their relative abundance (GC peak area), which is 1.4-fold to 6.1-fold higher (sum of GC peak area of the oxygenated monoterpenoids/sum of GC peak area of the aliphatic monoterpenes). Thus, these results suggest that this red macroalga may be considered a source of volatile compounds with promising health properties.

3.1.2. Norisoprenoids

Other volatile compounds can be produced due to the degradation of carotenoids, tetraterpenoids that are known to be present in algae at different levels, giving rise to norisoprenoids [10]. A total of 6 norisoprenoids were determined in dried *G. vermiculophylla*, representing GC areas from ca. 7 % (GVL2-1) to 8 % (GVL2-3). β -Ionone and geranyl acetone were the major norisoprenoids determined in this macroalga, being related to warm, woody, fruity, cedar wood-like, and violet-like notes, and rosy floral, fresh-floral, sweet-rosy, and slightly green notes, respectively (Table 1). β -Ionone was already identified in different algae, including in the essential oil of the green alga *Capsosiphon fulvescens* [17] and *Rhodomonas*, *Tetraselmis*, and *Chlorella* species [13].

3.1.3. Aldehydes

A total of 16 aldehydes were determined in the headspace of dried *G. vermiculophylla*. This chemical family exhibited the largest GC areas, representing ca. 17 % (GVL2-1) to 27 % (GVL2-3) concerning the total chromatographic areas (Table 1). Among these, higher GC areas of benzaldehyde, decanal, nonanal, octanal, and heptanal were determined. Aldehydes, mainly low-chain aliphatic (C₇ to C₁₃) or 2-enals, could arise from the degradation of algae polyunsaturated fatty acids, either by autoxidation or through the action of endogenous enzymes, such as lipoxygenases [18]. In addition, other exogenous sources might contribute to produce aldehydes, such as cyanobacterium, that have been associated with the production of benzaldehyde [62], or cyanobacterial mat communities that were related to heptanal, octanal, and nonanal production [35,63]. Aldehydes are volatile compounds mainly related to green and fatty notes (Table 1) that, together with the compounds of the other chemical families, can contribute to the overall aroma associated with this red macroalga. Different aldehydes, such as benzaldehyde, benzeneacetaldehyde, 2,4-heptadienal, heptanal, and decanal were determined in red algae, including *Polysiphonia denudata f. fragilis*, *Corallina granifera*, *Callithamnion granulatum*, and *Bangia fusco-purpurea* [20]. In addition, aldehydes can be considered very characteristic of macroalgae aroma, since in *Ulva pertusa* it was found that the sum of aldehydes' odor contributions was ca. 77 % of the total odor contributions of this green algae [11].

3.1.4. Alcohols

A total of 14 alcohols were determined in *G. vermiculophylla*, varying from ca. 16 % (GVL1-1) to ca. 24 % (GVL2-3) of the total GC areas (Table 1). Biochemically, alcohols might be formed due to the decomposition of secondary hydroperoxides of fatty acids, giving rise to, for example, 1-pentanol or 1-hexanol, or by the reduction of the corresponding aldehydes [18]. In dried *G. vermiculophylla*, the major alcohols determined were 1-octen-3-ol, 1-dodecanol, and 2,6-dimethyl-cyclohexanol. The 1-octen-3-ol, a compound related to mushroom, herbaceous, savory, brothy, and meaty notes, was also identified in green algae [11,17], being one of the major alcohols determined in the essential oil of *Capsosiphon fulvescens*.

3.1.5. Ketones

Ketones were also present in the dried *G. vermiculophylla* headspace. A total of 16 compounds were determined, representing ca. 9 % (GVL2-2) to ca. 14 % (GVL1-1) of the total GC areas. Like aldehydes, these

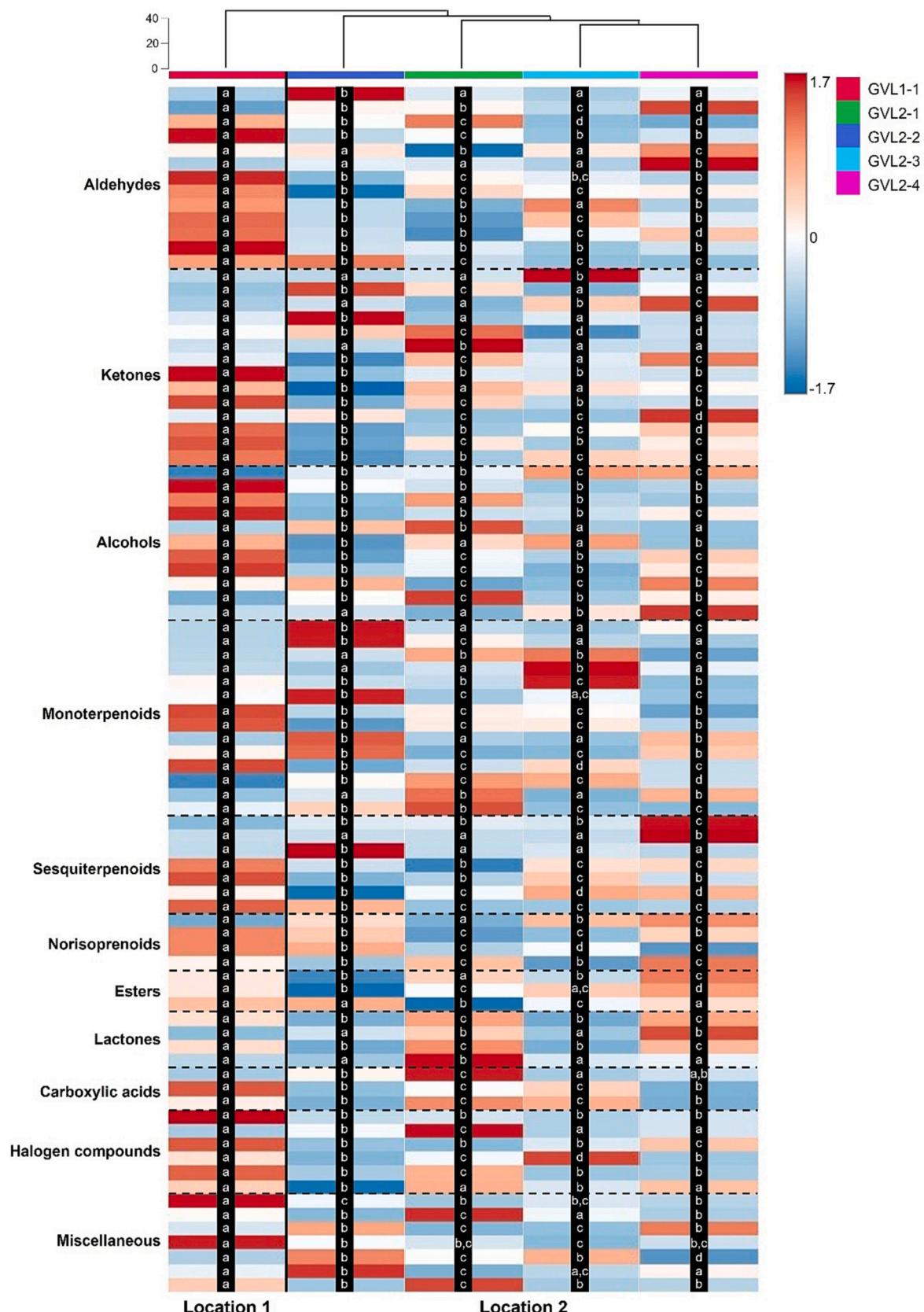


Fig. 3. Dendrogram and heatmap representing the common 86 volatile compounds released from *G. vermiculophylla* collected at two locations (Ria de Aveiro, Portugal) organized by chemical families, considering the GC × GC peak areas (Table 1) after autoscaling: GVL1-1_red, GVL2-1_green, GVL2-2_dark blue, GVL2-3_light blue, and GVL2-4_pink. The relative content of each compound is illustrated through a chromatic scale (from dark blue to dark red, the minimum and the maximum, respectively). In each line, different letters represent values that are significantly different ($p < 0.05$).

compounds are mainly derived from fatty acids' oxidation [18]. Some of them have been identified in green *C. fulvescens* [17] and red *P. tenera* [64] algae, including phytone, 6-methyl-5-hepten-2-one, and 2,2,6-trimethyl-cyclohexanone.

3.1.6. Halogenated compounds

Halogenated compounds are quite common in marine algae due to the high concentration of chlorine and bromine ions in seawater [65,66]. In dried *G. vermiculophylla*, a total of 18 halogenated compounds, mainly haloalkanes, were determined representing less than ca. 1 % of the total GC areas. The volatile halogenated compounds have been considered a characteristic of the algae sea-like notes since they strongly interact with the human receptors, resulting in high odor and flavor intensities [67]. However, as Table 1 shows, scarce information is available on their real contribution to macroalga aroma notes due to the restriction of halogen-containing compounds' usage as flavoring agents [10], although naturally present in algae. This could be relevant for the valuation of *G. vermiculophylla* macroalga usage in innovative food formulation, thus exploring new marine aromas and flavors. The presence of volatile halogenated compounds is well known for marine algae, especially for Rhodophyta such as *Laurencia* red algae [68]. Moreover, they were commonly related to algae defense systems against microorganisms and space competitors, among others [69]. Halogenated compounds have demonstrated to be related to health beneficial properties, including anti-inflammatory and antiproliferative, among other activities [68]. This may open the opportunity for algae biomass exploitation in new applications, such as for pharmaceutical usage.

3.1.7. Esters, lactones, carboxylic acids, and miscellaneous group

Esters (3), lactones (4), carboxylic acids (4), and a miscellaneous class (9) containing ethers, sulfur-, nitrogen-, furan-, terpene, and thiazole-derived compounds were determined in *G. vermiculophylla*. Esters and lactones are likely to be products of the esterification of carboxylic acids, while these acids can result from the thermal decomposition of carotenoids and lipids [6]. Due to their reduced number and GC areas, they can be considered as non-prevailing chemical groups in this macroalga. For esters and carboxylic acids, their relative amount was very similar among *G. vermiculophylla* samples, ranging from 1.4 % (GVL2-1) to 2.0 % (GVL2-3) and 1.0 % (GVL2-4) to 3.3 % (GVL2-3), respectively, while the presence of lactones ranged from 2.8 % (GVL2-2) to 15.3 % (GVL2-1) of the total GC area. This could be explained by the major GC peak areas of butyrolactone in GVL2-1 and GVL2-4 samples, which is a compound with a pleasant, creamy faint odor (Table 1). In addition, the relative GC area of the miscellaneous group ranged from 2.9 % (GVL2-2) to 4.8 % (GVL2-3). This group presents volatile compounds that can be relevant to the aroma of this macroalga, such as dimethyltrisulfide, with sulfur, fish, and cabbage notes, promoting the typical marine aroma, and 2-pentylfuran related to green bean, metallic, and vegetable notes (Table 1). These compounds have been reported in *Codium* sp., *U. lactuca* [19], and *C. fulvescens* [17] green algae.

3.2. *G. vermiculophylla* aroma wheel containing volatile chemical data

Considering the volatile compounds of *G. vermiculophylla* samples and their respective aroma descriptors (Table 1) available in the literature from a range of different sources [46–48], a *G. vermiculophylla* aroma wheel was developed (Fig. 4), based on the global algal aroma wheel [10]. This tool consisted of 3 levels of information where the 1st level refers to the aroma type/class and the 2nd one corresponds to the subdivision of each class, while the 3rd new level corresponds to the volatile compounds of *G. vermiculophylla* that are associated to different aroma notes (Fig. 4). Following the algal aroma wheel, in the 1st level, a total of 8 aroma classes were considered: sulfuric, chemical, floral, marine, woody-spicy, herbaceous, fermented, and fruity. The 2nd level corresponded to the 32 aroma notes included in the main 8 classes, which ranged from 2 to 6 notes per aroma class. From the 32 aroma

notes, fishy, seaweed, sulfuric offensive, and rancid notes are commonly not well accepted by consumers when incorporated into algae-based food. They have been referred as notes that should be reduced and/or mitigated upon storage and processing under specific conditions [6], or by blending with food ingredients in the preparation of novel food formulations or when cooking food [70–72]. This may contribute to find proper algae pairing with ingredients to improve the intake of algae. However, this can only be achieved when considering the volatile compound(s) linked to undesirable aroma notes, as here proposed with the inclusion of the 3rd level of volatile data (Fig. 4).

On the 3rd level, a total of 38 out of 86 volatile compounds from Table 1 were considered, based on the match between the aroma descriptors reported in Table 1 and the aroma notes of the 2nd level (Fig. 4), previously reported in the algal aroma wheel [10]. Thus, from the 32 aroma notes (2nd level), 22 may be explained based on the set of 38 compounds (estimation of around 70 % of the aroma notes reported in the algal aroma wheel). For instance, it can be observed that dimethyltrisulfide is the compound that can be linked to the undesired marine and sulfuric aroma classes (1st level) since it is related to sea-side/iodized, fresh fish, fishy, sulfur offensive, cabbage, and cooked onion/corn notes (2nd level, Fig. 4). Moreover, in this macroalga, only 2-octenal was also related to undesired seaweed notes from the marine class (Fig. 4). Several volatile compounds were found to be related to herbaceous (9 volatile compounds) and fermented (13 volatile compounds) aroma classes (1st level), which have been considered characteristic of algae [6,10]. These classes were mainly explained by the presence of volatile compounds that belong to the aldehyde and alcohol chemical families (Table 1, Fig. 4). However, the volatile compounds with a fruity aroma, the class formed by only citrus and ripe fruit notes, almost represented a quarter of the proposed aroma wheel. This was mainly due to the citrus notes of monoterpenic compounds and some aldehydes and ketones (Fig. 4), compounds that may contribute to the overall aroma of this macroalga, even if this note was not considered very characteristic in the algal aroma [10]. Moreover, the determined volatile compounds of *G. vermiculophylla* cannot explain the aroma notes related to ink-like, petroleum, shrimp-like, seafood/crustacean, smoky-burnt, hay-dried herbs, green tea, musty, and earthy notes (Fig. 4).

It is noteworthy that from the 86 volatile compounds determined in *G. vermiculophylla*, 48 compounds were not considered in the proposed algal aroma wheel (Fig. 4). This is because for 43 volatile compounds, it was not possible to find their aroma descriptors, in the literature. In addition, the algal aroma wheel used as the base, does not contemplate all aroma notes present in Table 1. For instance, 2-octenal, decanal, undecanal, 2-methylbenzaldehyde, 2-heptanol, 2-ethyl-1-hexanol, 1-octanol, benzophenone, linalool oxide, and α -ionone are related to sweet aroma notes, while benzaldehyde, 2-methylbenzaldehyde, 2-heptanol, and 1-octanol are linked to almond, beany, and fresh aroma notes, and menthol and menthone were related to minty notes. If these compounds are present in concentrations higher than their odor thresholds [6,23], it can be assumed that they are odor active and therefore can play a role in the specific aroma properties of *G. vermiculophylla*. These aroma notes, although not referred in the published algal aroma wheel [10], should be explored to promote the consumption and acceptance of this macroalga, while possibly mitigating the impact of undesired fishy, seaweed, sulfuric offensive, and rancid aroma notes. In this context, the absence of the aroma descriptors for all the volatile compounds determined in *G. vermiculophylla*, combined with their unknown odor threshold values determined in this matrix or even in algae, can justify the fact that a direct connection between the 2nd and 3rd levels of information was not here possible.

4. Conclusions

HS-SPME combined with GC × GC-ToFMS allowed to characterize the volatile compounds released from dried *G. vermiculophylla* red macroalga. A total of 136 volatile compounds were determined and

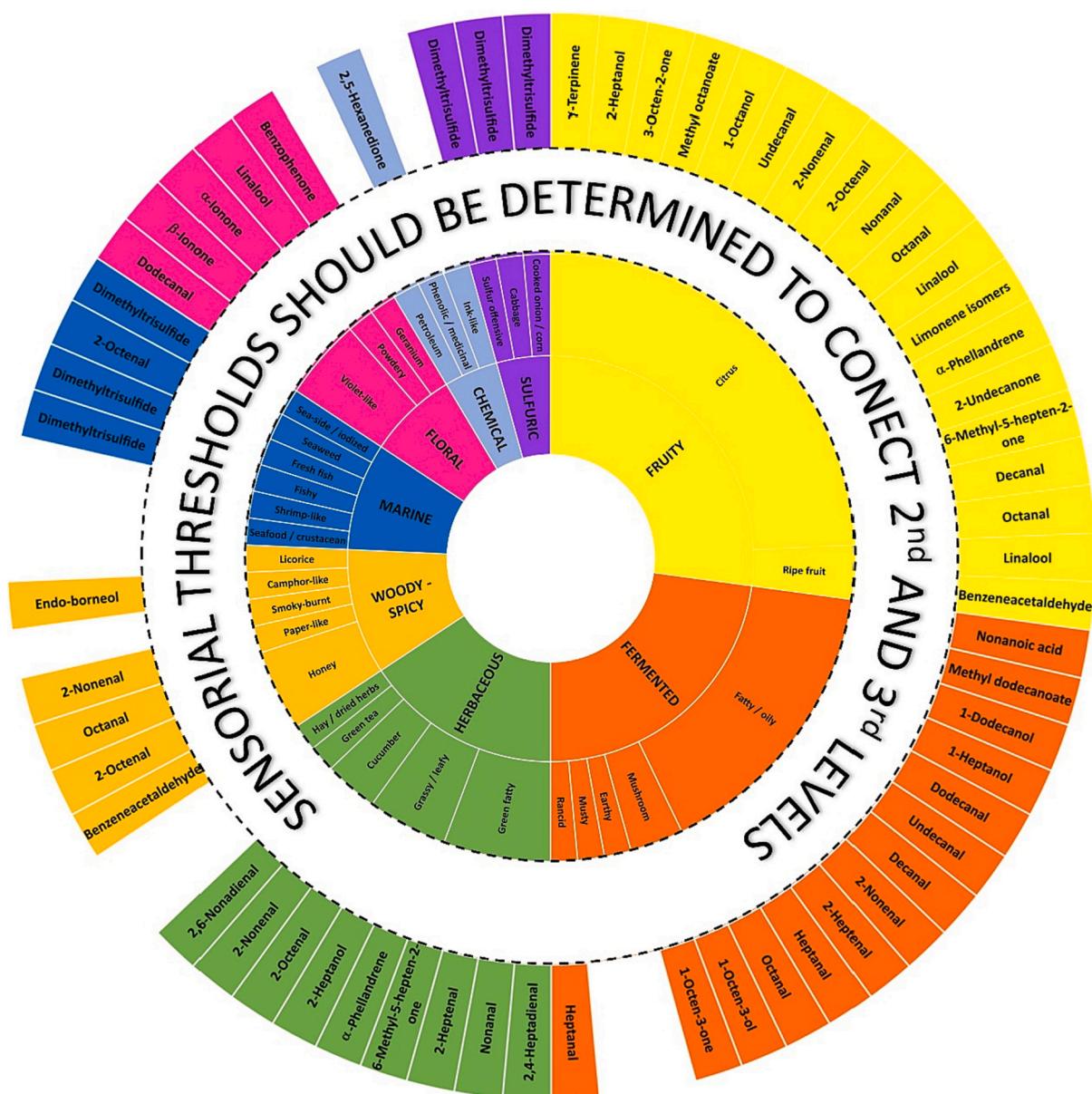


Fig. 4. *G. vermiculophylla* aroma wheel comprising 8 aroma classes and 32 notes reported in the algal aroma wheel [10]. The 3rd level was included based on the match between the aroma descriptors reported on Table 1 for a set of 38 compounds and the aroma notes of the 2nd level. Black dashed lines represent the lack of sensory threshold values determined on algae matrices.

grouped into 10 chemical families, where aldehydes exhibited the higher relative GC peak areas and monoterpenic compounds were determined in higher number. In addition, the volatile chemical data here generated contributed to the development of the *G. vermiculophylla* aroma wheel. This can be used as a tool to estimate the aroma peculiarities of this red macroalgae, where its volatile compounds (38 in total) may potentially explain ca. 70 % of the aroma notes present in the algae aroma wheel. Future improvement of this algae aroma wheel should be done by sensory threshold values determination on algae matrices, particularly in *G. vermiculophylla*, as these values are not available in the literature and are highly matrix-dependent. In fact, to establish the key-aroma contributors, which allowed to estimate its relevance to the algae aroma, it is essential to compare the concentration of each determined volatile with the respective odor threshold value. The findings of this study contribute to the uptake of seaweed biomass, like GV, as an ingredient with an improved aroma profile for future food applications.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.algal.2024.103402>.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Sílvia Petronilho: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation. **Angelo C. Salvador:** Writing – original draft, Investigation, Formal analysis. **Isabel Silva:** Methodology, Formal analysis. **Manuel A. Coimbra:** Writing – review & editing, Supervision. **Sílvia M. Rocha:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Conceptualization.

Declaration of competing interest

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

Data availability

No data was used for the research described in the article.

Acknowledgements

Thanks are due to the University of Aveiro and FCT/MCTES, QREN, COMPETE for the financial support to LAQV-REQUIMTE (UIDB/50006/2020 + UIDP/50006/2020) research unit and CQ-VR at UTAD Vila Real (UIDP/00616/2020) through PT national funds and, when applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement and Compete 2020. Thanks are also given to Norte Portugal Regional Operational Programme (NORTE 2020), under the PT 2020 Partnership Agreement, through the European Regional Development Fund (ERDF) and FSE. FCT is thanked for the post-doc (SP, SFRH/BPD/117213/2016) and Ph.D. (IS, SFRH/BD/31076/2006) grants.

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