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Authors	Garicano Vilar, Elena;O'Sullivan, Maurice G.;Kerry, Joseph P.;Kilcawley, Kieran N.
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A chemometric approach to characterise the aroma of selected brown and red edible seaweeds/extracts

Aroma of brown and red edible seaweeds

Elena Garicano Vilar[a,b], Maurice G O'Sullivan[b], Joseph P Kerry[c], and Kieran N Kilcawley[a,*].

[a.] Food Quality & Sensory Science Department. Teagasc Food Research Centre, Moorepark, Fermoy, County Cork, Ireland, P61 C996.

[b.] Sensory Group, School of Food and Nutritional Science, University College Cork, Cork, Ireland, T12 R229.

[c.] Food Packaging Group, School of Food and Nutritional Sciences, University College Cork, Cork, Ireland, T12 R229.

***Corresponding author:** Kieran N Kilcawley. Food Quality & Sensory Science Department. Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland.

Email: kieran.kilcawley@teagasc.ie

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ABSTRACT

Background: Information pertaining to the aromatic profile of seaweeds and seaweed extracts can provide information in relation to their potential suitability as an ingredient in processed foods. To date only limited information is available on the volatile profiles of some seaweed species, with others in this study not previously described. The volatile profiles of dried brown (*H. elongata, U. pinnatifida, A. esculenta*) and red (*P. umbilicalis, P. palmata*) seaweeds, and a brown seaweed extract (fucoxanthin) from *L. japonica,* were investigated using a chemometric approach to collate volatile GC-MS, direct sensory aroma evaluation and GC-O data in order to obtain a better understanding of their volatile profile and sensory perception. . **Results:** Greater than one hundred volatile compounds were identified by HS-SPME and TD GC-MS. Brown seaweeds were characterised by 'grassy/herbal/floral', 'fruity' and 'fatty' aromas, red seaweeds by 'green/vegetable', 'mushroom/earthy' and 'sweet/buttery' aromas, and the fucoxanthin extract by 'rancid' and 'nutty' aromas with an overall lower intensity. Heptanal appeared to be a major odour active compound in all samples. Other volatiles were more characteristic for each individual seaweed; hexanal, (E,Z)-2,6-nonadienal and 2-pentylfuran for *H. elongata,* ethyl butanoate and 2,3-butanedione for *U. pinnatifida,* 6-dimethylpyrazine, (E,Z)-2,6-nonadienal and sulactone for *P. palmata,* 1-octen-3-ol for P. umbilicalis, heptanone for *A. esculenta,* and 2-furanmethanol for fucoxanthin. **Conclusion:** Brown and red seaweeds had distinct sensory properties with individual seaweeds having differing volatiles and odorants. This study provides additional

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information that can contribute to the development of products incorporating dried seaweeds/extracts which are more acceptable to the consumer.

Keywords: seaweeds, volatile compounds, aroma, GC-MS, GC-O.

1. INTRODUCTION

Volatile compounds are fundamental for the aroma and odour perception of seaweed [1]. The volatile compounds of brown and red seaweed have been previously investigated [2–4]. However, little information is available on species, such as *Himanthalia elongata*, *Undaria pinnatifida*, *Alaria esculenta*, *Laminaria japonica*, *Porphyra umbilicalis* and *Palmaria palmata*, which are found in abundance around Western Europe coastlines (see www.algaebase.org) [5–9]. From extensive review of the scientific literature, there appears to be no publications pertaining to the volatile profiles and odour characteristics of the seaweeds *A. esculenta* and *L. japonica*.

Gas chromatography–mass spectrometry (GC-MS) is one of the most powerful analytical methods used in flavour chemistry. Complex mixtures are separated by GC, and the components are then identified by MS [10]. Many methods exist for the extraction and pre-concentration of analytes in solid samples [11]. However, no single extraction method is perfect, as each has a degree of bias, mainly due to issues relating to polarity, molecular weight or vapour pressure [12]. A definite movement towards automated approaches has occurred due to ease of use, time efficiency, and general effectiveness [12]. To date, the extraction of volatile compounds from seaweeds has been carried out by dynamic headspace extraction (purge and trap) [13], distillation-solvent extraction [14] and

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static headspace solid phase micro-extraction (HS-SPME) [11]. In this study, two automated extraction techniques were evaluated; (i) HS-SPME a static headspace technique, widely used for the identification of volatile organic carbons (VOCs) because of its ease of use and wide range of fibre's available to target different chemical classes and, (ii) thermal desorption (TD), a dynamic technique that has a much larger sorbent phase capacity with an enrichment capability.

Gas chromatography olfactometry (GC-O) uses human assessors in parallel with a detector (e.g., flame ionization or mass spectrometric (MS) detector) to obtain sensory and chemical responses for aroma compounds which enables the identification of key odorants that exert the greatest impact on sensory perception [15]. GC-O has been extensively applied for the characterization of aroma impact compounds in a variety of matrices, mainly in food-related fields like coffee [16], tea [17], meat [18] and others [19]. Nevertheless, very few studies [20,21] have utilized GC-O to evaluate seaweeds and or seaweed extracts. The volatiles in seaweeds produce a range of different odours that could be categorised as 'marine' or 'seafood'. Other odours such as 'fish', 'fatty', 'honey', 'green', 'floral' and 'spicy' have also been detected in various green, red and brown seaweeds [1,22]. In this study HS-SPME GC-O was employed to elucidate the key aroma compounds in five dried edible seaweed species and in an edible seaweed powder extract. An aroma assessment was also undertaken to provide a more expansive profile, as a potential weakness of GC-O is that assessors can only sniff individual or co-eluted volatiles, and may miss any aromas generated from the combination of volatiles and possibly aspects relating to the composition of the product.

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The primary objective of this study was to use a chemometric approach to investigate aroma of five dried edible seaweed species; four brown (one fucoxanthin, in the form of a powder extract), and two red species in order to determine their main odour active properties.

2. MATERIAL AND METHODS

2.1. Seaweed material

The edible seaweeds used in this study were four brown seaweed species (*H. elongata*, *U. pinnatifida*, *A. esculenta* and *L. japonica*) and two red species (*P. umbilicalis* and *P. palmata*). *H. elongata*, *P. umbilicalis*, *P. palmata* and *A. esculenta* were supplied by Wild Irish Seaweed Ltd. (Co. Clare, Ireland). *U. pinnatifida* was bought from Algamar (Pontevedra, Spain) and *L. japonica* fucoxanthin powder extract was shipped by Nutra Green Biotechnology Co. Ltd. (Shanghai, China). Seaweeds were harvested along the west coast of Ireland, north coast of Spain or in China. The seaweeds from Wild Irish Seaweed were air-dried and dehumidified (Caherush Point, Spanish Point, Co. Clare, Ireland) and Algamar seaweeds were dried at low temperature (<42 °C) at Polígono de Amoedo (Pazos de Borbén, Pontevedra, Spain), to preserve them [23]. The fucoxanthin powder extract was produced by water and ethanol extraction. These seaweed species were chosen on the basis of abundance in Western European waters and potential suitability as ingredients in the formulation of new products. The fucoxanthin extract was included for comparative purposes.

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2.2. Sample preparation

Dried seaweeds were milled to a particle size of 1 mm by means of a mechanical grinder. Milled samples were stored vacuum packaged in a -20 °C freezer, for no longer than 30 days.

2.3. Compositional analysis

Moisture of 2 g of seaweed was determined (Table 1) by drying the sample in a preheated (135 °C) oven for 2 h [24]. The fat content of seaweed (5 g) was determined using Foss Soxtec Avanti 2055 Manual system (Foss, Hillerød, Denmark). Seaweed samples were inserted into 33 mm x 80 mm extraction thimbles and extracted with 80 mL of boiling chloroform: methanol (2:1) for 30 min. Subsequently, thimbles were presented in the rinsing position for an additional 38 min. The extraction cups were removed and solvent was evaporated at 103 °C in an oven until a constant weight was obtained. The fat content was determined gravimetrically.

The protein content of 0.5 g of seaweeds was measured (Table 1) using the Kjeldahl method [25]. On completion, the content of the receiver flask was titrated with 0.1 N hydrochloric acid until the green colour reverted back to the original red colour. Finally, the protein was calculated using a nitrogen factor of 6.25. All analysis was performed in duplicate.

Ash content of seaweed was determined (Table 1) in duplicate by a muffle furnace (Nabertherm GmbH, Lilienthal, Germany) [26], which was pre-heated to 600

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°C. Approximately 5 g of blended sample was weighed into porcelain dishes and were placed into the muffle furnace. Samples were heated for 2 h until a white ash was obtained, at which time they were put in a desiccator to cool down. The ash content was calculated by weight of the dishes before and after sample introduction.

The salt content of seaweed was carried out (Table 1), in duplicate, by titration using silver nitrate. Silver nitrate solution was standardised against 0.100% sodium chloride solution (0.1 N). Two g of sample were inserted into the muffle furnace to create ash for ash content analysis. Ash was washed into conical flasks with 20 mL distilled water and 2 mL of added chromate indicator. The flasks were potentiometrically titrated with 0.1N silver nitrate from a clear yellow to an opaque light orange (+255 mV), after cooling to room temperature. Blank titration was performed using 20 mL distilled water.

2.4. Extraction and volatile compounds analysis

2.4.1. Thermal desorption (TD)

The extraction of 10 g of dried milled seaweed by TD-GC-MS was performed as previously described in Garicano Vilar et al. (2020) [27], for 40 min at 72 °C using a. Micro-Chamber/Thermal Extractor (Markes International Ltd, Llantrisant, UK). The set of check standards used were 1-butanol, dimethyl disulfide, butyl acetate, cyclohexanone, benzaldehyde and 2-phenyl-D5-ethanol, at 0.5 g Kg^{-1}.

2.4.2. Headspace Solid-Phase Microextraction (HS-SPME)

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HS-SPME analysis was performed as described by Lamichhane et al. (2018) [28], except that 3 g of dried milled seaweed (or powder extract) were extracted for 20 min at 40 °C, and the mass range scanned was 35-350 amu. Batch processing of samples was carried out using MetaMS, an open-source pipeline for GC-MS-based untargeted metabolomics [29]. A multi-phase SPME 50/30 μm divinylbenzene/carboxen^{TM}/polydimethylsiloxane (CAR/DVB/PDMS) (Agilent Technologies Ltd, Cork, Ireland) was used as the properties of the different phases are used to target chemical classes based on their polarity and volatility and molecular weight. Previous studies have shown that this fibre choice was suitable for the extraction of volatile compounds in seaweeds [5,7].

2.5. Gas Chromatography-Olfactometry (GC-O) analysis for aroma active compounds

The extraction of volatile compounds was carried out by HS-SPME using a Gerstel MPS autosampler (Anatune Ltd, Cambridge, UK). The same SPME fibre (DVB/CAR/PDMS) was used as described earlier. The fibre was pre-conditioned before use at 270 °C for 60 min. Seaweed samples (dried seaweed and extract) (3 g) were weighed in 20 mL amber SPME La-Pha-Pack headspace vials with magnetic screw caps and silicone/polytetrafluoroethylene (1.3 mm 45° Shore A) septa (Apex Scientific Ltd, Ireland). The SPME fibre was exposed to the seaweed headspace at 40 °C for 60 min at a depth of 1 cm. Injections were carried out in splitless mode, with the injector temperature at 250 °C. All samples were analysed in triplicate.

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GC-O analyses were conducted on an Agilent 7890A GC coupled with an Agilent 5975C MSD (Agilent Technologies Ltd, Cork, Ireland). The GC was also equipped with a flame ionization detector (FID) and a Gerstel Olfactory Detection Port ODP3 with heated mixing chamber (Anatune Ltd, Cambridge, UK). A humidifying device was used to reduce nasal mucosa dehydration. Analyses were performed on a DB-624 column (20 m x 1.80 mm i.d. x 1 μm phase thickness). Helium was used as a carrier gas at a flow rate of 1.2 mL min^{-1} and nitrogen as auxiliary gas. The GC oven temperature was programmed to increase from 60 to 180 °C at a rate of 6 °C min^{-1}, with an initial hold time of 2 min, and from 180 to 220 °C at a rate of 15 °C min^{-1}, with a final hold time of 5 min. The total run time was 29.66 min. Column effluent was split equally between the FID, the olfactory port and the MSD. The transfer line into the MSD was kept at 260 °C. The FID temperature was set at 300 °C, with an air flow of 400 mL min^{-1}, a H_2 fuel flow of 30 mL min^{-1} and a N_2 makeup flow of 25 mL min^{-1}. The sniffing port and its exit were maintained at 150 °C and 40 °C, respectively.

A panel of three sensorial assessors (age 26-39 years) carried out the sniffing of the volatile compounds of seaweeds extracted by SPME. Sniffing time was approximately 30 min and each assessor carried out one session per day. The panellists were asked to rate: 1) the intensity of the eluted aroma using a four-point category scale (1= weak, hardly recognizable odour; 2= clear but not intense odour, 3= intense odour, 4= very intense odour), recorded by a Gerstel OID Interface/ODP-Recorder (Anatune Ltd, Cambridge, UK) and 2) the odour perceived, by voice

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recording. The odorants taken as significant were the ones in which at least two assessors were able to detect. None of the assessors were anosmic, as tested by Sniffin' Sticks test (Burghardt Messtechnik, Wedel, Germany) prior to the GC-O analysis [30].

Tentative identifications were based on comparison of the mass spectra of unknown compounds against those of the National Institute of Standards and Technology (NIST), and by comparing the retention index value to values available in the literature and in an in-house library. The odorants were also identified by comparison of their odours with Flavornet database [http://www.flavornet.org], The Good Scents Company database [http://www.thegoodscentscompany.com] and other specified publications. Retention indices for all detected volatile compounds were calculated using an n-alkane series (Merck, Arklow, Ireland) for both GC-MS and GC-O analysis.

2.6. Descriptive odour evaluation

Three panellists attended a focus group session on descriptive terms prior to the sensory analysis. The six samples were evaluated in one session, following ISO 6658:2005 recommendation [31]. All samples were assessed only for odour attributes and analysed in duplicate by each panellist. Mean scores for each attribute were calculated. The assessors quantified the attributes using a 10-point scale anchored to the left with "not" and to the right with "very" [32]. Two g of each sample were put in 20 mL amber glass vials with screw caps (Apex Scientific Ltd, Ireland) and the vials

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were kept in a HiSorb Agitator (Markes International Ltd., Llantrisant, UK) at 40 °C for 20 min at an rotational speed of 350 mn^{-1}, to ensure the accumulation of volatiles in the head space. Samples were provided randomly to panellist. The testing area used in this study complied with the ISO 8589:2007 standards [33].

2.7. Data analysis

Normality and homogeneity were examined and One-way analysis of variance (ANOVA) with the Bonferroni test for post hoc analyses was carried out to assess statistical differences between seaweed species in terms of composition and abundance values of volatile compounds, at a significance level of P=0.05, using the SPSS 24.0 statistical package (IBM Corp., Armonk, NY, U.S).

The volatile profile was analysed using R software (RStudio, Inc., Boston, MA) for statistical computing and graphics. PCA was carried out in order to visualize the differences in volatile aroma composition detected by GC-MS, GC-O analysis and sensory panel results and to elicit the main factors contributing to the differences between the six samples.

In addition, volatile compounds and odour intensity values were further analysed using ANOVA-Partial Least Squares Regression (APLSR) using Unscrambler X Software, version 10.3 (CAMO ASA, Trondheim, Norway) (See Figure S1).

3. RESULTS AND DISCUSSION

3.1. Physiochemical analysis

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The composition of the five seaweeds and the extract are summarized in Table 1. The moisture content was statistically different (P <0.01) among all species, with the exception of *U. pinnatifida* and *P. palmata*. The data resembles the proximate composition of three brown seaweeds analyzed by Lorenzo et al. [34], who reported that moisture content ranged from 7.95% in *B. bifurcata* to 11.2% in *A. nodosum* and *F. vesiculosus*. Additionally, Rodrigues et al. [23] reported similar moisture contents, 9.6-10.9% in brown seaweeds and 8.0-11.8% in red seaweeds.

According to Ibañez et al. [35], brown seaweed species (Phaeophyceae) are characterized by a lower protein content in comparison to red seaweed species (Rhodophyta). In effect, the red seaweeds (*P. umbilicalis* and *P. palmata*) presented a higher proportion of protein than the brown seaweeds (Table 1). The protein content was statistically different (P<0.001) among all species tested. These findings are similar those of Lorenzo et al. [34], where the protein content of brown species ranged from 8.7 to 13.0%. However, Rodrigues et al. [23] reported higher (14.4 to 16.9%) protein contents for brown species and lower (20.2 to 23.8%) for red species.

Seaweeds are well known for their low fat content, but values vary considerably between studies. A range of 1.1 and 2.3% of total fat was found in our samples (Table 1). The fat content of red seaweed did not have any significant differences, but significant differences were evident for green seaweed (P<0.01). Higher fat contents of 0.9 and 0.6% were reported in red species of *O. pinnatifida* and *G. gracilis*, respectively, by Rodrigues et al. [23]. Higher fat contents were also observed

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in brown species in this study compared to those reported by Jard et al. [36] for *S. muticum*.

Higher levels of ash are associated with higher amounts of minerals [37]. The ash content of *P. umbilicalis* and *P. palmata* was lower than that of brown seaweed samples. *U. pinnatifida* had the highest ash content (Table 1). Greater variability in total ash content was observed among brown seaweed than red seaweed. The differences between the seaweed species were all statistically significant ($P<0.001$), even between red and brown seaweeds. The ash content values observed in this study are in agreement with published values for *G. turuturu* (18.5%) by Denis et al. [38] and for *G. gracilis* (24.8%) by Rodrigues et al. [23]. In contrast, higher values of ash content were observed in brown species in this study compared to other studies [23,34]. The salt content of all seaweeds followed the same trend as the ash content (Table 1), although no statistically significant difference was observed between *H. elongata* and *P. palmata*.

Table 1.

3.2. Volatile compounds identification

A total of 117 (Table S2) and 109 (Table S3) volatile compounds were detected in the five seaweed species and in the seaweed extract by TD and HS-SPME, respectively. These numbers were lower than the 151 volatile compounds identified by López-Pérez et al. [39] in seven species of dehydrated edible seaweeds. The number

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of volatile compounds identified in each individual seaweed species by TD and HS-SPME, respectively, were 75 and 61 compounds for *H. elongata*, 76 and 58 for *U. pinnatifida*, 65 and 58 for *P. umbilicalis*, 78 and 76 for *P. palmata*, 72 and 60 for *A. esculenta*, and 67 and 53 for the fucoxanthin extract. These numbers were lower than the 129, 140, 131 and 136 volatile compounds found by López-Pérez et al. [39] in *H. elongata*, *U. pinnatifida*, *P. umbilicalis* and *P. palmata*, respectively; but higher than the 23 volatiles in *P. palmata* samples [40] or the 26 and 24 volatile compounds in *Laminaria* spp and *U. pinnatifida*, respectively [13]. The differences may be due to a myriad of reasons; species, geographical area of growth, harvesting, storage and volatile extraction conditions amongst others. As previously mentioned no single extraction technique can provide a complete volatile profile as they have inherent bias due the different adsorbent, absorbent properties of the different phases, molecular sieve characteristics, mechanisms of extraction and the physical parameters used etc. In this study we carried out HS-SPME at 40 °C and extracted for 20 min, where we carried out TD at 72 °C for 40 min in an attempt to extract more compounds as much more sorbent capacity exists for TD than HS-SPME and larger sample amounts of sample were used (10 g vs. 3 g) (Figure S4 and S5). As mentioned the properties of the sorbent materials used in these techniques are different and will therefore extract and concentrate different VOCs. The TD tubes contained Tenax Carbograph which is useful for detection of VOC between C_3 and C_{30} of different volatilities (typically 50 – 450 °C) and polarity. Limitations occur for some low molecular weight compounds lower thanC_3, especially if they are polar,

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such as carboxylic acids, some aldehydes and alcohols [41]. The SPME fibre (DVB/CAR/PDMS) is useful for a wide range of volatiles and semi-volatiles between C_3-C_{20}**.** This fibre affords a greater extraction efficiency for many VOC, in comparison to others fibres (PDMS-DVB, CAR-PDMS, PDMS or polyacrylate) and is particularly useful for very volatile VOCs but less useful for heavier VOCs such as lactones [42–44]. Therefore it is useful to utilise different VOC extraction/concentrationtechniques in order to achieve a more representative VOC profile.

From the TD analysis the highest numbers of volatile compounds were, in decreasing order; 23 alcohols, 20 ketones, 20 aldehydes, 9 acids, 8 esters, 7 furans, 6 benzenes, 5 pyrazines, 5 terpenes, 4 sulphur compounds, 3 ethers, 3 lactones, 2 terpenes, 2 pyridines, 1 phenol and 1 chlorine; with HS-SPME detecting 21 ketones, 20 aldehydes, 16 alcohols, 9 acids, 9 benzenes, 7 esters, 6 furans, 6 pyrazines, 5 terpenes, 3 sulphur compounds, 3 lactones, 2 ethers, 1 phenol and 1 chlorine. The different volatiles detected by both extraction techniques highlight the merits of using more than one extraction technique to encompass a larger volatile profile.

Overall, terpenes were the most prominent in *H. elongata*; acids and lactones in *U. pinnatifida*; sulphur compounds in *P. umbilicalis* (HS-SPME extraction only); aldehydes, esters, ketones, pyrazines and pyridines in *P. palmata*; alcohols, ethers and furans in *A. esculenta* while fucoxanthin extract was predominant in benzenes and phenol.

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3.3. Descriptive odour analysis

Descriptive odour analysis was conducted on the five different species of seaweed and the seaweed extract (Fig.1). The samples were classified and characterized by 'rancid, oily/fatty, pungent', 'fruity, citrus', 'grassy, herbal, floral, vegetable', 'fresh, marine, fishy', 'nutty, toasted', 'mushroom, earthy, damp' and 'sweet, buttery, creamy' aroma. A PCA was produced to visualize the differences in the aroma attributes between the species and the extract (Fig.2). The first two principal components of the PCA were able to explain 81% of the variance (Fig.2). Globally, *H. elongata* and *P. palmata* were mostly characterized by 'fresh, marine, fishy' aroma; *U.pinnatifida* and *A. esculenta* by 'grassy, herbal, floral, vegetables' notes; *P. umbilicalis* by 'mushroom, earthy, damp' and 'rancid, oily, fatty' notes and fucoxanthin extractby 'nutty, toasted' aroma (Fig.2).

Figure 1. Figure 2.

3.4. Odour characteristics of aroma active compounds determined by HS-SPME-GC-O

In total 41 aroma active volatiles were identified and 5 unidentified aroma-active compounds were perceived by panellists, consisting of acids, alcohols, aldehydes, ethers, furans, ketones and pyrazines (Table 2). It is common not to be able to identify all peaks by GC-O, which is related to its concentration been below the level of detection, but above its odour threshold. In addition quite a few of the

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compounds were summarised as having odour descriptors different or partially different to referenced descriptors. However, this is also not unusual as odour descriptors can be influenced by the concentration (odour descriptors can change based on concentration for some volatile compounds), the matrix effect (the composition of the sample can influence the release of volatiles into the headspace) and differences in the ability of the human assessors due to genetic and cultural differences [45].

Table 2.

P. palmata had the strongest odour of all samples, as the sum of olfactory scores was the highest at 54.1 [46] (Table 2). On the contrary, the fucoxanthin extract had the lowest score (8.5), resulting in a rather bland aroma profile that was dominated by 'bakery, buttery, sweet, fatty' aromas. This is possibly due to the additional processing required to produce the extract. In addition to heptanal, and taking into account the olfactory scores for each compound individually; (E,Z)-2,6-nonadienal (fresh, cucumber, grass, green) and sulcatone (woody, green, grass, celery, vegetable) appear to be important aroma compounds for *H. elongata* and *U. pinnatifida*. *P. umbilicalis* was characterized by 3 unidentified compounds with (i) 'toasted, yeast, fermented, roasted, nutty', (ii) 'pyrazine, grass, boiled potato' and (iii) 'floral, geranium, green, grass, damp, mouldy' aromas. *P. palmata* was dominated by 2,6-dimethyl-pyrazine (baked, toasted, roasty, caramel), and an unidentified component with 'pyrazine, grass, boiled potato' aroma and 3-heptanone

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(fruity, butter, oily). *A. esculenta* was characterized by 1-octen-3-ol (mushroom, earthy), 3-heptanone (fruity, rancid, butter, oily) and benzaldehyde (green, grass, vegetable, herbal, almond). 2-furanmethanol (cheese, butter, buttermilk, rancid) and 2-methyl-nonane (backed, toasted) appeared to be characteristic aroma notes for only the fucoxanthin extract but at low (<3) intensities.

As mentioned, GC-O analysis showed that 1-octen-3-ol (mushroom, mineral, hay) and heptanal (herbal, grassy) were common in all samples but differed in their intensities. The compound 1-octen-3-ol contributes to 'mushroom' and 'mushroom-metallic' notes [20,47]. Moreover, 1-octen-3-ol has been shown to contribute to the formation of seafood aroma [47]. Heptanal contributes to 'green' and 'fresh' aroma [48]. It has also been described as having 'fat', 'rancid', 'citrus' aromas [49]. Heptanal, which was the potent aroma compound for all samples in this study, exhibited a 'fatty, oily, fishy, seaweed, lake water' aroma and appears to contribute to the characterizing notes of the fish, seaweed, oil aromas of seaweed. Additionally, many short chain aldehydes (e.g. 2-octenal) can provide several aroma's 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 [47].

Many volatile compounds in a food present low aroma intensity or no aroma and thus do not necessarily contribute to the overall odour [50]. Thus, high threshold compounds can present low odorant power, even if present at a high abundance. Conversely, low concentrations of low threshold compounds in the sample can

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present a high odor intensity [19]. Therefore, it is necessary to know if the extracted volatile compounds are actually contributing to the characteristic aroma.

A second PCA was performed on the seaweeds samples in order to determine the relationships between the volatile aroma active compounds as determined by GC-O analysis in the different seaweed species (Fig.3). The first two principal components explain 59% of the variance.

Figure 3.

A clear discrimination exists between these samples based on their olfactometry assessment (Fig.3). Both *H. elongata* and *A.esculenta* are quite similar, on the positive side of F2 and the negative side of F1. These samples are associated with heptanal (fatty, oily, fishy, seaweed, lake water), (E)-2-nonenal (fresh, lemon, grassy, nuts), 1-hexanol (lemon, citrus, grass), benzeneacetaldehyde (tea, floral), 1-octen-3-ol (mushroom, mineral, hay), 2-hexyl furan (fruity, floral, green, tea), 2-pentyl furan (butter, toasted, herbal), acetone (sulphur, boiled corn, rocket), (Z)-2-penten-1-ol (sweet, cherry, fruity, candy), 1-pentanol (cotton candy, fruity) and 2-heptanone (grassy, plastic). Only some of the individual odour descriptors match the overall aroma characteristic by hedonic assessment, however during aroma evaluation the panellists are sniffing a combination of all these odours rather than individual odours by GC-O, thus combined odours may be perceived very differently [51]. It is, worth noting that odour thresholds and abundances are the main factors that impact on

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odour perception. It seems plausible that heptanal must be a key odorant in these species as it has an odour similar to their aroma attributes. Heptanal also has a mid to low odour threshold, but had a high odour intensity score (Table 2). The intensity scores for (E,Z)-2,6-nonadienal (Z)-2-penten-1-ol in *P.umbilicalis*, *U.pinnatifida* and the fucoxanthin extract were all on the negative side of F1 and F2 (Fig.3). These were all associated with 'fruity, citrus', 'sweet, buttery, creamy' aroma attributes. Both *P.umbilicalis* and *U.pinnatifida* were strongly associated with acetic acid (vinegar) and *P.umilicalis* was also strongly associated with methyl octanoate (fermented, fresh, green). It appears that methyl octanoate contributes to the overall aroma character of *P.umilicalis* as its aroma is similar. The fucoxanthin extract was differentiated from all the other samples. It was associated more with 2-furanmethanol (cheese, butter, rancid), 2-methyl-nonane (toasted) and dodecane (soap, glue) and less with acetic acid or methyl octanoate. The individual aromas of these volatiles however do not match the overall aroma characteristics. *P.palamata* was also discriminated from all the other samples on Fig. 3, on the positive side of F1 and the negative side of F2. It was associated most with 'mushroom, earthy, damp' and 'rancid, oily, fatty, pungent' aroma attributes, but also with a very wide range of volatiles; 3-methyl-butanal (vanilla, sweet, floral, butter), pentanal (caramel, sugar, spicy, butter), 2-methyl propanal (vanilla, floral), 2,6-dimethyl pyrazine (backed, toasted, roasty, caramel), α-ionone (grassy, lemon, citrus, sugary, fruity), (E)-2-octanal (green, grass, pepper, coffee), 2,3-butanedione (butterscotch, sweet, yeast, butter, vanilla, custard), ethanol (alcoholic), 1-propanol (sweet), methyl

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octanoate (fermented, fresh, green), (E,Z)-2,6-nonadienal (fresh, cucumber, grass, green), 3,5-octadien-2-one (metallic, mineral) and dihydroactinidiolide (damp, floral). It appears that 3,5-octadien-2-one is likely a major contributor to the aroma of *P.palmata* due to its odour characteristics and intensity score (Table 2).

Panellists in the study by Peinado et al. [52] used 'honey-like' odour, 'herbal' odour, 'seaweed-like' odour attributes to describe five different species of brown edible seaweeds (*Laminaria digitata*, *Ascophyllum nodosum*, *Pelvetia canaliculata*, *Fucus vesiculosus* and *Fucus spiralis*). 'Seaweed-like' aroma was, in general, the attribute with the highest score which could be expected as it is the attribute most related to 'seafood-like'. Similarly to our results, only the Laminaria species extract was significantly different from all the others in terms of aroma, being the one with the strongest 'seaweed-like' aroma, and the mildest 'honey-like' aroma. Yamamoto et al. [1] asked panellists to evaluate aroma attributes like animalic, floral, spicy, fatty, green note, marine-like, fresh (watery), powdery and leather-like, in *Ulva prolifera*, *Ulva linza* and *Monostroma nitidum*. They reported that depending on the Japanese prefecture where the seaweed originated, seaweeds were evaluated differently in respect of its green-note, marine-like, fresh and powdery aroma. Some further studies analysed the aroma compounds produced by marine microalgae species [20,47] and flagellates [21], and concluded that they could be described by different aroma characteristics, but microalgae findings are not relevant to this research.

The volatile profiles of *H.elongata*, *U.pinnatifida*, *A.esculenta*, *L. japonica* fucoxanthin extract, *P.umbilicalis* and *P.palmata* were described, and some for the

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first time in this study. This research, based on SPME-GC-MS and TD-GC-MS, has revealed that volatile compounds differ intensely in the five varieties of dried seaweed and the seaweed extract. We also reported key odorants of the different commercial brown and red seaweed samples. This work shows the potential of GC-MS and GC-O coupled with multivariate analysis (PCA) to discriminate between samples of different origins, based on their volatile profiles, and to form the basis for future development of authentication methods and characterization of seaweed containing-products.

Little information is available on species such as *H. elongata*, *U. pinnatifida*, *P. umbilicalis* and *P. palmata*, which are common brown and red species in abundance around Western Europe coastlines. For some species such as *A. esculenta* and *L. japonica* no details on their volatile profiles or odour characteristics have been published before. Therefore, this study addresses significant recent developments of the current understanding of the volatile profile of theses seaweeds and the key aroma compounds that contribute to their unique odours. This characterisation of volatiles in seaweeds may help to develop new products more acceptable to consumers less familiar with seaweed, especially as seaweeds are of growing importance in Western diets, and most recently as components of functional foods. The work has enhanced some fundamental understanding of volatile profiles in seaweeds and in particular our chemosensory knowledge of seaweed odour. Further work is required in relation to VOC changes that occur in processing and storage of

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seaweed species as extracts and in their application in food in relation to sensory perception.

Limitations: GC-O alone does not allow a final conclusion on the contribution of a single compound to the overall aroma. A reason for this is that the entire amount of a compound present in a sample is volatilized during GC-O, whereas, from a food, only the amount present in the headspace above the food is available for the nose receptors and thus is odour-active. Odour activity values (OAVs) are helpful to understand the contribution of aroma compounds, because they correlate quantitative data with odour thresholds in a matrix and thus address the influence of the matrix on the volatility of a given odorant [49].

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Conflict of interest

The authors declare no competing financial interest.

Supporting information

S1 Figure (Partial Least Squares Regression (PLSR) correlation loadings plot); S2 Table (Volatile compounds identified after thermal desorption extraction); S3 Table (Volatile compounds identified after headspace solid-phase microextraction extraction); S4 Figure (Principal Compenent biplot for TD GC-MS analysis); S5 Figure (Principal Component biplot for HS-SPME GC-MS analysis).

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Figure legends

Fig.1 – Spider diagram of sensory evaluation of five seaweed (*H. elongata, U. pinnatifida, P. umbilicalis, P. palmata* and *A. esculenta*) and one seaweed extract (fucoxanthin extract from *L. japonica*). Average scores are shown according to quantitative descriptive odour attributes evaluated by 3 panellists.

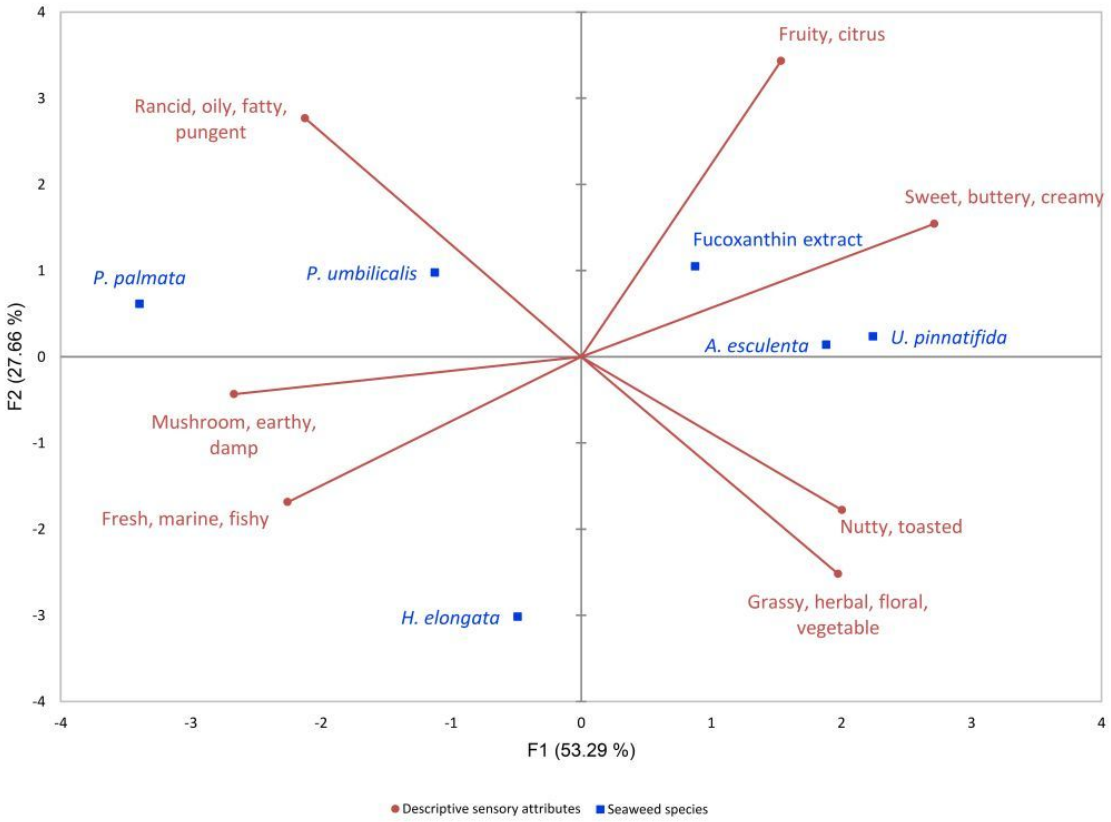
Fig.2 – Principal Component Analysis scores plot of aroma evaluation of five seaweed samples (*H. elongata, U. pinnatifida, P. umbilicalis, P. palmata* and *A. esculenta*) and one seaweed extract (fucoxanthin extract from *L. japonica*), for the first two principal components (PC1 and PC2). Average scores are according to quantitative descriptive odour attributes evaluated by 3 panellists.

Fig.3 – Principal Component Analysis scores plot depicting distribution of aroma compounds detected by gas chromatography-olfactometry (GC-O) analysis in five seaweed species (*H. elongata, U. pinnatifida, P. umbilicalis, P. palmata* and *A. esculenta*) and one seaweed extract (fucoxanthin extract from *L. japonica*), for the first two principal components (PC1 and PC2).

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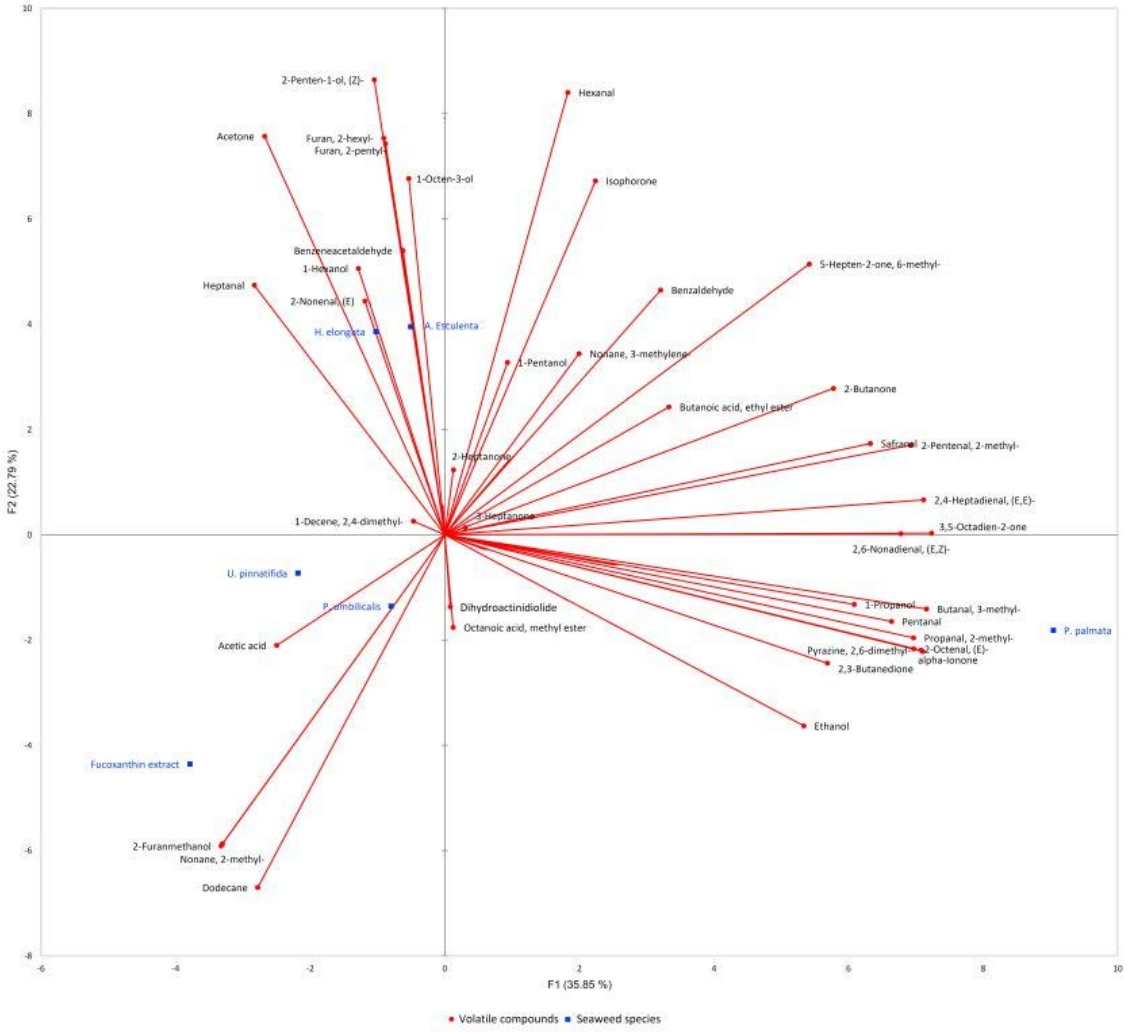
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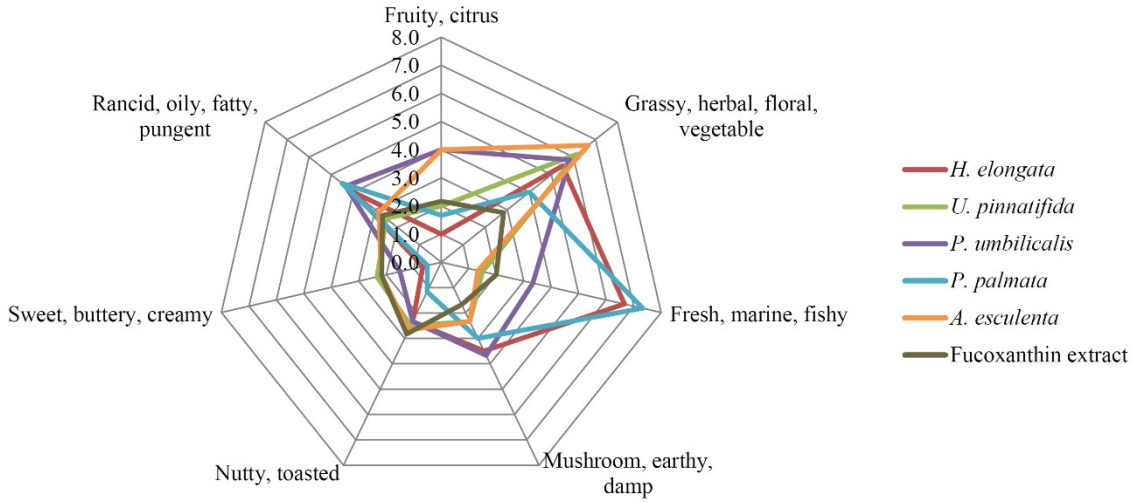


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Table 1. Composition of five dried seaweeds and a seaweed extract.

	HE	UP	PU	PP	AE[†]	LJ	
	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD	*P*-value
% Moisture	16.31 ± 0.43[a]	10.77 ± 0.07[b]	8.99 ± 0.22[c]	10.04 ± 0.38[b]	11.79 ± 0.12[d]	6.68 ± 0.6[e]	<0.001
% Fat	1.42 ± 0.17[a]	2.30 ± 0.12[b]	1.13 ± 0.03[a]	1.69 ± 0.17[a]	2.30 ± 0.07[c]	0.38 ± 0.03[d]	<0.001
% Protein	5.84 ± 0.44[a]	9.02 ± 0.55[b]	33.57 ± 1.4[c]	19.96 ± 1.11[d]	8.82 ± 0.31[e]	0.6 ± 0.14[f]	<0.001
% Ash	35.92 ± 0.45[a]	57.36 ± 0.35[b]	18.94 ± 0.31[c]	28.49 ± 0.75[d]	35.03 ± 0.49[e]	5.93 ± 0.29[f]	<0.001
% Salt	15.16 ± 0.25[a]	36.77 ± 1.93[b]	5.88 ± 0.13[c]	16.34 ± 1.06[a]	17.31 ± 0.21[d]	-	<0.001

HE, *Himanthalia elongata*; UP, *Undaria pinnatifida*; PU, *Porphyra umbilicalis*; PP, *Palmaria palmata*; AE, *Alaria esculenta*; *Laminaria japonica* (fucoxanthin extract); M, mean; SD, standard deviation. [a-f], mean values in the same row bearing different superscripts indicate significant difference (P <0.05) between the seaweed species. [†]Mohammed HO et al. Characterization of the nutritional and bioactive properties of brown and red Irish seaweeds for potential use as functional ingredients in processed meat products. *47th Annual Food Science and Technology Conference* Cork, Ireland; 2018.

Table 2. Aroma-active compounds detected in five dried species of edible seaweeds and a seaweed extract by GC-O.

Compound	**RI[†]**	**LRI[‡]**	**IM[§]**	**Odour[¶]**	**Odour intensity[††]**						**Odour ref. [a-i]**	**Odour threshold (ppb) [j-n]**
					HE	UP	PU	PP	AE	LJ		
ACIDS												
Acetic acid	687	690	A, C	Vinegar	1.6	2.6	-	-	-	-	Vinegar, sharp, sour [a]	480-1000 [j]
Propanoic acid	780	784	A, C	Cheese, rancid, medical	-	2	-	-	-	-	Pungent, acidic, cheesy, vinegar, dairy [a]	20000 [k]
ALCOHOLS												
Ethanol	483	506	A, C	Alcohol	-	1.3	-	-	0.6	-	Strong, alcoholic, ethereal, medical [a]	100000 [k]
1-Propanol	610	615	A, C	Sweet	-	-	1.3	-	-	-	Alcoholic, fermented, tequila, musty, yeasty,	9000 [k]

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											sweet, fruity, apple, pear [a]	
2-Furanmethanol	931	939	B, C	Cheese, butter, buttermilk, rancid	-	-	-	-	-	2.6	Alcoholic, chemical, musty, sweet, caramel, bread, coffee [a]	8000 [l]
2-Penten-1-ol, (Z)-	825		B, C	Sweet, cherry, fruity, candy	3	-	-	-	-	-	Cherry, narcissus, fruity, green, phenolic, nasturtium, ethereal, medicinal, aldehydic, metallic [a]	Nf
1-Pentanol	817	815	A, C	Cotton candy, fruity	-	-	1.6	-	-	-	Pungent, fermented, bready, yeasty, fusel, winey, solvent [a]	4000 [k]
1-Hexanol	919	916	B, C	Lemon, citrus, grass	-	-	-	-	1	-	Fruity, alcoholic, sweet, green, pungent, ethereal, fusel, oily [a]	2500 [k]
1-Octen-3-ol	1028	1030	A, C	Mushroom, mineral, hay	2	2.3	2	2	3.3	-	Mushroom, earthy, green, oily, fungal [a-c]	1 [k]
ALDEHYDES												
Propanal, 2-methyl-	598	592	A, C	Vanilla, floral	-	-	1	1.3	-	-	Fresh, aldehydic, floral, pungent [a]	0.1-2.3 [k]
Pentanal	733	735	A, C	Caramel, sugar, spicy, butter	-	-	1.6	-	-	-	Fermented, bready, fruity, nutty, berry [a]	12-42 [k]
Butanal, 3-methyl-	694	692	A, C	Vanilla, sweet, floral, butter	-	-	1.3	2.3	-	-	Ethereal, aldehydic, chocolate, peach, fatty [a]	12 [m]
Hexanal	837	839	A, C	Herbal, grassy	2.3	-	1.6	1.6	-	-	Fresh, green, grassy, leafy, fruity, sweaty, woody, clean [a,c]	5 [k]
2-Pentenal, 2-methyl-	877		B, C	Sweaty, milk, rancid	-	-	-	2	-	-	Pungent, fruity, juicy, ripe, aldehydic, green, grassy, gassy [a]	290 [n]
Benzaldehyde	1034	1030	B, C	Green, grass, vegetable, herbal, almond	-	-	-	1.3	3	-	Almond, burnt sugar-like, cherry, bitter, sharp, fruity, sweet, bitter almonds [a,c-e]	350-3500 [k]
2,4-Heptadienal, (E,E)-	1074		B, C	Grassy, vegetable, cucumber	-	-	-	2.3	-	-	Fatty, green, oily, aldehydic, vegetable, cinnamon [a]	3600 [m]
Heptanal	942	943	A,	Fatty, oily, fishy,	3.3	3.6	4	3.6	3.3	2	Fresh, aldehydic, fatty,	3-60 [n]

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			C	seaweed, lake water							green, herbal, wine-lee, ozone, citrus, rancid [a,c,f]	
Benzeneacetaldehyde	1109	1119	A, C	Tea, floral	-	0.6	-	-	-	-	Harsh, green, honey, cocoa, sweet, floral, hyacinth, clover, rose, powdery, fermented [a,f,g]	4 [k]
2-Octenal, (E)-	1116		B, C	Green, grass, pepper, coffee	-	-	-	1.6	-	-	Fresh, cucumber, fatty, green, herbal, banana, waxy, leafy, nut [a,f]	3 [k]
2,6-Nonadienal, (E,Z)-	1216		B, C	Fresh, cucumber, grass, green	3.3	3	-	3	-	-	Green, cucumber, melon, fatty, vegetable, dry, violet, leaf [a,c]	0.01 [k]
2-Nonenal, (E)-	1218		B, C	Fresh, lemon, grassy, nuts	2.3	-	-	-	-	-	Green, cucumber, aldehydic, fatty, citrus [a]	0.08-0.1 [k]
ETHERS												
Butanoic acid, ethyl ester	747	828	B, C	Fruity, cherry, strawberry, candy	-	2.6	2	2.3	-	-	Sweet, fruity, tutti frutti, juicy [a]	1 [k]
Octanoic acid, methyl ester	1151	1156	B, C	Fermented, fresh, green	-	-	1	-	-	-	Waxy, green, sweet, orange, aldehydic, vegetable, herbal [a]	200 [k]
FURANS												
Furan, 2-pentyl-	1009	1012	A, C	Fried, melted butter, toasted, herbal	2	-	-	1.3	-	-	Fruity, green, earthy, beany, vegetable [a,c]	6 [k]
Furan, 2-hexyl-	1386		B, C	Fruity, floral, green, tea	1.6	-	-	-	-	-	Floral, pulpy [h]	Nf
KETONES												
Acetone	565	533	A, C	Boiled corn, rocket	-	-	1	-	-	-	Solvent, etheral, apple, pear [a]	500000 [k]
2,3-Butanedione	627	631	A, C	Butterscotch, sweet, yeast, butter, vanilla, custard	-	2.6	1	-	1.3	1	Buttery, sweet, creamy, caramellic, pungent [a,c,f]	2.3-6.5 [k]
2-Butanone	636	639	A, C	Butterscotch, sweet, vanilla, cream, fruity	1.3	-	-	2.3	-	-	Camporheous, fruity, ethereal, acetone, nauseating odor [a,c,e]	50000 [k]
2-Heptanone	934	928	A,	Plastic, grassy	-	-	-	1.3	1.6	-	Fruity, spicy, sweet, herbal,	14-3000 [k]

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			C								coconut, woody, sulfur, pungent, green [a,f]	
3-Heptanone	927		A, C	Fruity, rancid, butter, oily	2	2.6	-	3.3	3.3	-	Green, fatty, fruity [a]	Nf
3,5-Octadien-2-one	1131		B, C	Metallic, mineral	1.6	0.6	-	2	-	-	Fruity, fatty, mushroom [a]	0.15 [n]
Sulcatone	1032		A, C	Woody, green, grass, celery, vegetable	3.3	3	-	3	-	-	Citrus, green, musty, lemongrass, apple, cheesy, banana [a]	50 [k]
Isophorone	1108		B, C	Grapefruit, spicy, wet, rosy, floral, caramel, sugar	-	-	-	2	1.3	-	Cooling, woody, sweet, green, camphor, fruity, musty, cedar, wood, tobacco, leather [a]	200 [l] (air)
PYRAZINES												
Pyrazine, 2,6-dimethyl-	947	944	A, C	Backed, toasted, roasty, caramel	-	-	-	3.6	-	-	Cocoa, roasted, nutty, meaty, coffee [a]	400-1500 [n]
TERPENES												
Dihydroactinidiolide	1701		B, C	Damp, floral	-	-	-	1.6	-	-	Ripe, apricot, fruity, berry, woody [a]	Nf
β-Ionone	1500		B, C	Grassy, lemon, citrus, sugary, fruity	-	-	-	1.6	-	-	Sweet, woody, floral, violet, orris, tropical, fruity [a]	0.6-10 [n]
OTHERS												
Safranal	1260		B, C	Grassy, vegetable, anise, fennel	1.3	-	-	1.6	1	-	Fresh, herbal, phenolic, metallic, rosemary, tobacco, spicy, woody, camphoreous, medicinal, powdery, herbal [a]	Nf
Nonane, 3-methylene-	991		B, C	Incense, spices, earthy, floral, sweet, cherry, strawberry	-	-	1.3	2.3	-	-	Nf	Nf
Nonane, 2-methyl-	965		B, C	Baked, toasted	-	-	-	-	-	1.3	Nf	Nf
1-decene, 2,4-dimethyl-	1229		B, C	Grass, green	-	-	2.3	-	-	-	Nf	Nf
Dodecane	1199		B, C	Soap, glue	2	1.3	-	1.3	-	-	Gasoline-like [i]	Nf

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Unidentified-1	961		C	Toasted, yeast, fermented, roasted, nutty	-	-	3	-	-	-		
Unidentified-2	969		C	Pyrazine, grass, boiled potato	-	-	3	3.6	-	-		
Unidentified-3	1032		C	Floral, geranium, green, grass, damp, mouldy	-	-	3	-	-	-		
Unidentified-4	1143		C	Burning wood, hay, grass	-	-	-	-	1.6	-		
Unidentified-5	1226		C	Rubber, plastic	-	-	-	-	-	1.6		
					Total odour intensity							
					32.9	28.1	32	54.1	21.3	8.5		

HE, *Himanthalia elongata*; UP, *Undaria pinnatifida*; PU, *Porphyra umbilicalis*; PP, *Palmaria palmata*; AE, *Alaria esculenta*; LJ, *Laminaria japonica* (fucoxanthin extract).

[†] Retention index (RI) calculated from GC-O results on a DB-624 UI column.

[‡] Retention index found in the literature (LRI) for a DB-624 UI column.

[§] Identification method (IM): A, identification based on NIST mass spectral database, RI values from the literature and an in-house library created using authentic compounds with target and qualifier ions and linear RI for each compound; B, when only B or RI values were available, it must be considered as a tentative identification; C, identification with GC-O.

[¶] Odour description given by three assessors during GC-O analysis. -, not detected.

[††] Mean odour intensity for each seaweed species evaluated by sniffers. From 1 = low to 4 = high.

[a-i] Odour descriptors found in the literature: **a** [http://www.thegoodscentscompany.com/index.html], **b** [Isleten Hosoglu M. *Food Chem* 240:1210–1218 (2018)], **c** [Narain N. *Examines Mar Biol Oceanogr* 2:195–201 (2018)], **d** [Minteguiaga M et al. *J Sep Sci* 38:3038–3046 (2015)], **e** [Flament I. 1st ed. John Wiley & Sons Ltd., ed. Chichester, England; 2002], **f** [Dong L et al. *J Sci Food Agric* 95:915–921 (2015)], **g** [Lasekan O et al. *CYTA - J Food* 14:154–161 (2015)], **h** [de Sousa Galvão M et al. *Food Res Int* 44:1919–1926 (2011)], **i** [Verma DK et al. 1st ed. Verma DK, Srivastav PP, eds. Sci. Technol. Aroma, Flavor, Fragr. Rice. Oakville, Canada: Academic Press, Inc; 2019]. Nf, not found

[j-n] Odour threshold values in water (unless otherwise designated) found in the literature: **j** [New Jersey Department of Health. Hazardous Substance Fact Sheet Acetic Acid. 2016], **k** [http://www.leffingwell.com/odorthre.htm], **l** [U.S. National Library of Medicine and National Center for Biotechnology Information, 2016], **m** [American Society for Testing and Materials. 2nd ed. Fazzalari FA, ed. Baltimore, Md.: American Society for Testing and Materials; 1978], **n** [Burdock GA. *Fenaroli's Handbook of Flavor Ingredients, Fourth Edition*, 2001]. Nf, not found.

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