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Optimization of Dynamic Headspace Extraction of the Edible Red Algae *Palmaria palmata* and Identification of the Volatile Components

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A new extraction method was applied to the volatile compounds of *Palmaria palmata*. Dynamic headspace was optimized according to an experimental design, and descriptive sensory analysis and intensity and similarity tests were performed for each extract to assess their respective representativeness. Results showed that extract obtained with crushed algae after a 30 min purge was the most representative. GC-MS analysis was then performed on this extract to identify the volatile components. Seven halogenated compounds, seven aldehydes, two ketones, three alcohols, and four miscellaneous compounds were identified. Among them, halogenated compounds were the most characteristic of red algae, and more particularly, iodoethane and iodopentane, which had yet been found in other seaweeds.

KEYWORDS: *Palmaria palmata*; dynamic headspace extraction; sensory analysis; volatile compounds; halogenated compounds.

INTRODUCTION

In France, 11 macroalgae, including the red alga *Palmaria palmata*, have been authorized for human consumption since 1988 (*1*, *2*), while their utilization in food in eastern countries dates back several centuries (*3*). Consequently, the use of seaweeds in France is not yet well developed. In fact, their important nutritional qualities, due to their high mineral content, nutritional value, and high protein content (especially in red algae) have made them mainly appreciated in dietetics. However, seaweeds also have interesting organoleptic properties, such as aroma, that could enable them to be included in processed foods, as condiments, or vegetables, and could lead to the development of new marine foodstuffs.

Few studies have been carried out on algae aroma. Most of the work has been done by Japanese teams, such as Kajiwara et al. in 1988 (*4*) and Sugisawa et al. in 1990 (*5*), on brown and green algae, respectively. In 1997, Michel et al. (*6*) studied the influence of two drying methods on the volatile compounds of *Ulva* sp. and *Palmaria palmata*. Recently, Le Pape et al. (*7*) studied the effects of two means of preservation on the odor of *Palmaria palmata* and optimized a vacuum hydrodistillation method, using sensory analysis, to obtain a characteristic aroma extract of the alga. Crushing, extraction temperature (20 or 30 °C) and addition of salt were studied using a factorial design. Crushing led to extracts characterized by green notes; increase in temperature gave extracts with a cooked aroma. Extracts obtained with salted water were badly defined by the panelists. The most representative extract was the one obtained with whole algae, at 20 °C, and without salt addition.

Most of the extraction techniques, such as simultaneous distillation−extraction (SDE), require heating and even boiling of the initial sample to collect the volatile compounds in an organic solvent. In the case of the study of raw products such as seaweeds, these methods lead to odorous artifacts.

An alternative is extraction by vacuum hydrodistillation. This technique does not require high temperatures, and efficiently extracts compounds with both low and high boiling points. It is particularly adapted for the analysis of the volatile compounds of raw products such as wine (*8*) or oysters (*9*). This technique has also been used for the extraction of *Palmaria palmata* volatiles (*7*). Nevertheless, some volatile compounds can be lost or modified during the concentration process that follows the vacuum hydrodistillation (*10*).

Another way to extract volatile compounds without heating the initial product is dynamic headspace extraction. It is generally carried out at a temperature close to that of vacuum hydrodistillation, but only low-boiling-point compounds are extracted. This technique is frequently used for the analysis of crude material such as honey (*11*) and oysters (*9*, *12*). It has also been used for the extraction of volatile compounds of dried Kombu, an edible Japanese brown alga (*13*), but it has never been used for the extraction of fresh seaweed volatiles.

When optimizing an aroma extraction method, an essential step is the assessment of the representativeness of the odor of

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the extracts. Indeed, this step is necessary before all quantitative, qualitative, and olfactometric analyses to ensure that the extract aroma smells such as the aroma of the raw product. Most of the studies on aroma authenticity have been done on beverages (Abbott et al. (*14*) on beer, Bernet et al. (*8*) on Gewurztraminer wine, and Escudero et al. (*15*) on Champagne). However, other studies have also been carried out on sea products such as mussels (*16*) and oysters (*9*). All these authors have shown the importance of reincorporating the extract in a matrix with the same characteristics as the original product. In fact, to reconstitute the product aroma, it is necessary to restore the interactions between volatile compounds and the other components of the matrix.

Thus, this research first aimed at optimizing the dynamic headspace method for the extraction of the volatile compounds of fresh *Palmaria palmata*, to obtain a representative extract of the initial product. Two parameters were studied, namely crushing and purge time. In fact, they are important as they could act on the liberation of volatile compounds, and consequently, on the extract aroma. They have already been studied in fish by Refsgaard et al. (*17*) and in oysters by Pennarun et al. (*9*). The extracts obtained after optimization were then analyzed by sensory analysis to assess their representativeness. The second purpose of this work was to identify the volatile compounds of *Palmaria palmata* extract to investigate their origin.

MATERIALS AND METHODS

Algae. Algae (*Palmaria palmata*) were handpicked at low tide by the CEVA (Centre d'Etude et de Valorisation des Algues) in Pleubian (Côtes d'Armor, France) and washed with seawater. They were then stored for fifteen days maximum in artificial seawater, which was prepared by dissolving 6 kg of a synthetic salt (Reef Crystals, Aquarium Systems, Sarrebourg, France) in 180 L of water, at 2−4 °C in a 200 L aquarium. It was equipped with an adjustable pump (Maxi-jet ph, MP 1200, Aquarium Systems, Sarrebourg, France) to ensure the stirring of the water and of the algae, and with an internal filter (Rena filstar iV4, Rena, Annecy, France) to permit an efficient oxygenation of the water.

Chemicals. 3-Hexanone was purchased from Sigma-Aldrich Chemical Co. Water was purified using a Milli-Q system (Millipore). Iodoethane, dichloromethane, iodopentane, 3-methylbutanal, heptanal, 2-(*E*)-hexenal, nonanal, 2,3-pentanedione, 3-octanone, 1-butanol, 1-penten-3-ol, 3-methylbutanol were purchased from Aldrich. Dichloromethane was from Panreac Quimica SA, hexanal was from Sigma, and octanal was from Merck.

Dynamic Headspace Volatile Concentration. A purge-and-trap concentrator (LSC 2000, Tekmar Inc., Cincinnati, OH) equipped with a capillary interface for cryofocusing and connected to a gas chromatograph Varian Star 3400 (Varian, Palo Alto, CA) was used. The dynamic headspace concentration was optimized according to a factorial design, to obtain an extract characteristic of the initial product. The two factors studied were the crushing or not of algae and the purge time 30 or 50 min). Crushing was carried out with an Ultra-Turrax grinder (IKA-Labortechnik, Germany), at a rotating speed of 13 500 tr · min^{-1} for one minute. Eight grams of algae was then transferred into a flask with 40 mL of ultrapure water and placed under magnetic stirring. Indeed, as the algae were voluminous, the addition of water ensured a better stirring of the sample. The headspace of the sample was subsequently purged with helium at 60 mL · min^{-1} and swept into a porous adsorbent polymer (Tenax TA, 15 g, 80−100 mesh)) at 25 °C. Volatile compounds were cryofocused at −40 °C using carbon dioxide and thermally desorbed by heating the trap at 195 °C. For sensory analysis, the volatiles were collected, by means of the capillary interface, in 10-mL brown coded flasks (Supleco, Bellafonte, PA), in which a vacuum was created with a syringe, hermetically closed with a silicon cap, sealed with an aluminum cap, and stored at room temperature. For GC analysis, volatile compounsds were directly injected in the gas chromatograph column.

Sensory Analysis. *Panel.* Ten assessors from our laboratory, who were familiar with sea products and test procedures, composed the panel. They were all trained to describe *Palmaria palmata* aroma and to generate descriptors for seaweeds and seaweed extracts (*7*).

Sample Preparation and Presentation. The goal of this study was to optimize the extraction by dynamic headspace. The four trials of the factorial design were performed on the same day and were presented to one panel member at a time. The extracts were stored at room temperature in hermetically sealed 10-mL brown, coded, glass flasks. Just before the sensory analysis, 1 mL of purified water was added to each extract with a 1 mL syringe, and the flasks were agitated.

All the extracts were assessed for odor only and were presented to the panelists in a random order, at room temperature. All the sensory analyses were done in duplicate.

Quantitative Descriptive Analysis. A list of eight descriptors that described the aroma of fresh algae was previously determined by the panelists and subsequently used to describe the different extracts. These consensual descriptors were seaside, iodized, seaweed, fresh, hay, tea, cut grass, and cooked white fish (*7*). Four extracts were presented to the panel members, who were asked to describe the aroma of each sample by evaluating the intensity of each given descriptor on an unstructured scale of 10 cm, anchored at the left end with "no odor" and at the right end with "very strong odor". The intensity notes were given by the distance in millimeters from the left anchor to the marks of the judge. All the results were processed using a Principal Component Analysis (PCA) performed on the total value of each descriptor with Statgraphics Plus software (Manugistics, Inc., Rockville, MD).

Odor Intensity Evaluation. An intensity evaluation was performed to assess the odor intensity of each extract. It was rated on a 10 cm unstructured scale, anchored at the left end with "no odor" and at the right end with "very strong odor". The position of the sample on the scale was read as the distance in millimeters from the left anchor. Results were analyzed with those of the quantitative descriptive analysis for the optimization of the headspace extraction, using a PCA and an analysis of variance (ANOVA).

Similarity Test. The judges were asked to compare the odor of all the extracts with the odor of the fresh algae (reference sample) to determine if the extracts smelled like the product from which they came. The panelists were first instructed to evaluate and memorize the aroma of the reference sample and then to assess the odor of the extract. The similarity was rated on a 10-cm unstructured scale anchored with "very different from the reference" at the left end and with "identical to the reference" at the right end. The corresponding value was given by measuring the distance from the left anchor to the mark of the judge. Results were analyzed with a multiple comparison of means, using a Student−Newman−Keuls test (Statgraphics Plus Software; Manugistics, Inc., Rockville, MD).

Quantitation. Extraction and desorption of volatile compounds was performed as described for the dynamic headspace volatile concentration. A Varian Star 3400 gas chromatograph (Palo Alto, CA) was used. It was equipped with a flame ionization detector and a capillary column (DB−Wax, 30 m × 0.32 mm i.d., 0.5 μM film thickness, J&W Scientific Inc., Folsom, CA). The helium carrier gas flow rate was 1 mL · min^{-1}, and the detector temperature was 250 °C. The oven temperature was first increased from 40 to 57 °C at 1 °C · min^{-1}, and then to 230 °C at 15 °C · min^{-1}, with a final hold at 230 °C for 5 min. 3-Hexanone was used as internal standard (1 mL of a solution at 140 ng · mL^{-1}) for the quantitative analysis of the extract.

Gas Chromatography-Mass Spectrometry (GC-MS). *GC-MS Conditions.* Volatile compounds were extracted and desorbed as described above and then injected into a HP 5890 series II GC/HP 5971 mass selective detector (MSD) (Hewlett-Packard Co., Palo Alto, CA) (splitless mode; 30-s valve delay; injector temperature, 250 °C). The MSD conditions were as follows: electron impact mode, 70 eV; interface temperature, 250 °C; source temperature, 180 °C; mass range, 33−300; scan rate, 2.0 scan · s^{-1}.

Identification. Volatile compounds were identified by comparing their GC retention indices, their mass spectra with those of a commercial spectra database (NBS 75K and the internal library of the laboratory). Some of the identifications were confirmed by the injection of chemical standards into the GC-MS system.

Table 1. Intensity and Similarity Notes Obtained According to the Recovery Support

	0h		8h	
recovery support	intensity notes[a]	similarity notes[a]	intensity notes[a]	similarity notes[a]
air	4.8 ± 1.5	3.7 ± 2.5	4.6 ± 1.3	3.5 ± 1.2
water	3.5 ± 1.7	4.7 ± 1.7	0.5 ± 0.7	0.2 ± 0.3
air, then water[b]	3.7 ± 1.9	5 ± 1.3	3.7 ± 1.4	4.5 ± 2.1

[a] Mean/10 ± standard deviation, $n = 10$, notes in cm. [b] 1 mL of water is added just before the sensory analysis.

RESULTS AND DISCUSSION

Optimization of Dynamic Headspace Extraction. A preliminary study was first performed to determine the recovery support of the extracts (**Table 1**). Thus, the aroma of an extract, obtained with noncrushed algae and after a 30 min purge, was evaluated by the panelists. It was either collected in an empty flask or in a flask containing 1 mL of ultrapure water. This extract was then presented to the judges either immediately or 8 h after the recovery (as we decided to do the four trials of the experimental design within the same day and to present them together to the judges). When presented immediately, the odor of the extract collected in 1 mL of water was found to be less intense than the extract obtained in an empty flask (3.5/10 ± 1.7 against 4.8/10 ± 1.5) but more similar to the fresh algae (4.7/10 ± 2.5 against 3.7/10 ± 1.7). This result has already been observed by Pennarun et al. (*9*) in the case of fresh oysters. When presented 8 h after the recovery, no odor was detected in the extract collected in water (intensity note, 0.5/10 ± 0.7; similarity note, 0.2/10 ± 0.3), whereas no significant changes were observed in the odor of the extract obtained in an empty flask (intensity note, 4.6/10 ± 1.3; similarity note, 3.5/10 ± 1.2). This could be due to a strong adsorption of the matrix and a great solubility of the compounds in water. Ebeler et al. (*18*) explained that, even if they were only slightly soluble in water, volatile compounds decreased in volatility in the course of their time in dilute water solutions. Finally, a last test was performed: The extract was first recovered in an empty flask and 1 mL of ultrapure water was added just before sensory analysis to reproduce the matrix effect first observed. Then, as for the other tests, sensory analysis of the extract was done either immediately or 8 h after the recovery. For the extract assessed just after the recovery, similarity and intensity notes resembled those obtained for the extract immediately recovered in water. Moreover, no significant changes were observed for the extract assessed 8 h after the recovery at a confidence level of 5% (intensity note, 3.7/10 ± 1.4; similarity notes, 4.5/10 ± 2.1). Consequently, all extracts were first recovered in an empty flask, and 1 mL of ultrapure water was added just before sensory analysis.

Two parameters were optimized according to a factorial design. The first one was the crushing of algae. This burst the algae cell walls and released intracellular enzymes, such as lipoxygenase, that could act on polyunsaturated fatty acids. Thus, the formation of green note aromas could be observed as well as an increase in total intensity, as shown by Jiang and Kubota (*19*) in the case of Japanese pepper. The second parameter was the purge time. In fact, Refsgaard et al. (*17*) showed that the increase in sampling time in dynamic headspace extraction induced an increase in the level of volatiles collected.

The results of the quantitative descriptive analysis and the intensity test are represented by a principal component analysis (**Figures 1** and **2**). The weight of the two principal components explained 69.3% of the total inertia and could have been sufficient to explain the analysis. However, we decided to take into account the third one, with a weight of 16.4%, as its eigenvalue was greater than 1 (1.476). Therefore, the three components finally represented 86.4% of the total inertia.

Marine descriptors (seaside, iodized, seaweed, and fresh), as well as the descriptor "tea", were strongly related with axis 1 (40.3%). "Cooked white fish" also showed a strong correlation with axis 1, but was anti-correlated with the descriptors previously cited (**Figures 1** and **2**). Only the descriptor "hay" was strongly correlated with axis 2 (29.1%) in **Figure 1**, but it did not discriminate the results in **Figure 2**. Some of the descriptors were also intercorrelated. Thus, "tea" and "seaweed" were closely related (**Figure 1**). In the same way, the intensity note and "cut grass" were strongly correlated (**Figure 2**). Actually, these two descriptors were often associated with the crushing effects, which were an appearance of green notes and an increase in global intensity (*19*). Finally, there was also a strong relation between "seaside" and "iodized" (**Figures 1** and **2**). This relation was more evident than the other two, as the two descriptors were close to each other and often confused by the panelists. Nevertheless, "iodized" was rather described as a salted, piquant, and irritant odor while "seaside" was rather defined by a fresh and marine odor (*7*).

Extracts obtained with noncrushed algae (1 and 1′, 3 and 3′) were placed in the middle of Figures 1 and 2 and were rather undefined by the panelists. They were quite near each other, in particular extracts 1 and 3′, which indicated that there was a very small effect of the purge time on the liberation of volatile compounds for whole algae.

Crushing led to extracts 2, 2′, 4, and 4′ and had a more pronounced effect. They were rather inclined to be characterized by the "cut grass" note and a higher intensity. This observation was particularly illustrated by extracts 2 and 2′ in **Figures 1** and **2**. In fact, crushing induced the liberation of green aromas and intracellular enzymes, which could also have produced new volatile compounds (*19*). This could explain the increase in intensity.

In addition, according to the observations of **Figures 1** and **2**, it seemed that axis 1 described the effect of the purge time on crushed algae. In fact, it appeared that extracts 2 (**Figure 1**) and 2′ (**Figure 2**), obtained with crushed algae after a 30 min purge, were quite well described by marine notes (seaside, iodized, seaweed, and fresh). Inversely, we observed that extract 4′ was strongly described by the note "cooked white fish". *Palmaria palmata* was very sensitive to the heat, even though the temperature fixed here was not high (25 °C). Moreover, Kuo et al. (*20*) explained that some enzymes, like oxidases, that are responsible for cooked aroma, have an optimal temperature of activity between 26 and 35 °C. This phenomenon was also observed for the optimization of the extraction of *Palmaria palmata* aroma by hydrodistillation (*7*). In this case, the increase of the purge time from 30 to 50 min was probably sufficient to give rise to changes in the aroma: the longer the purge, the greater the formation of cooked aroma in the extracts.

According to these results, it seemed that 2 and 2′, both obtained with crushed algae and a purge time of 30 min, were the most characteristic of *Palmaria palmata* odor, and were characterized by marine aromas and great intensity (**Figures 1** and **2**).

Table 2 presents the results of the intensity and similarity tests. With regard to the intensity notes, it can be seen that crushing involved an increase in intensity, which was in

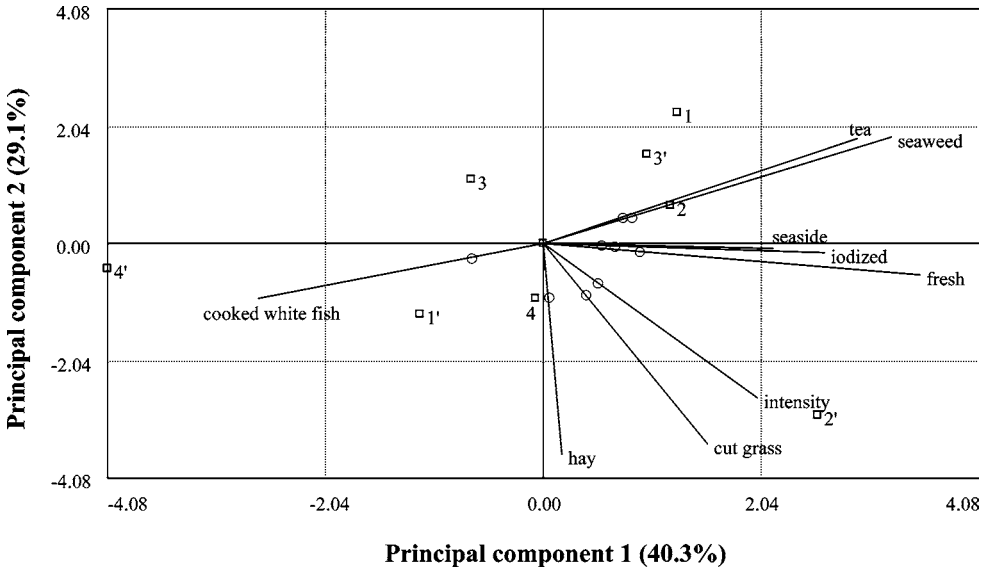

Figure 1. Principal component analysis of dynamic headspace extracts according to principal components 1 and 2.

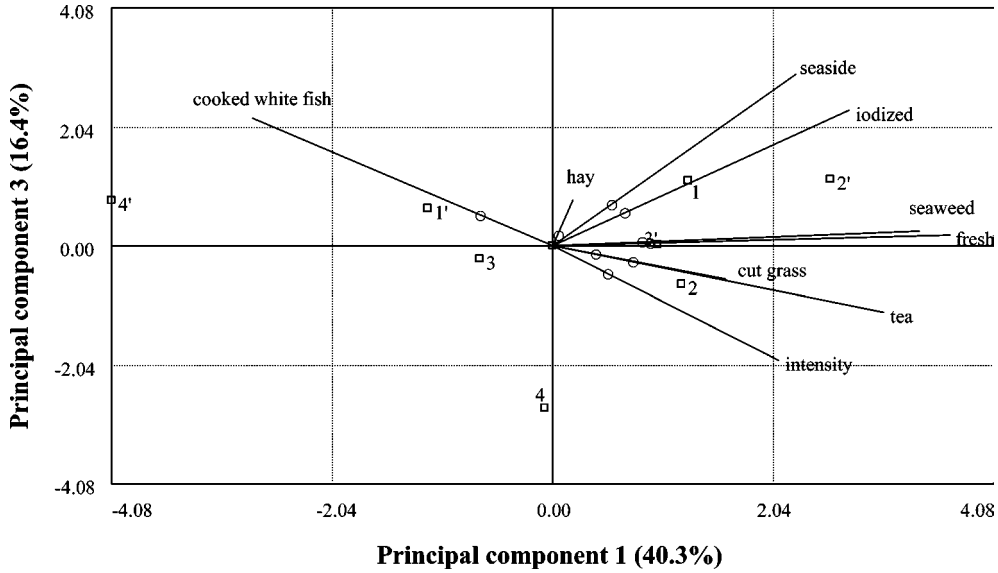

Figure 2. Principal component analysis of dynamic headspace extracts according to principal components 1 and 3.

Table 2. Intensity and Similarity Marks for the Optimization of the Dynamic headspace extraction

extracts	intensity marks	similarity marks
1	3.54[a]	4.23[a]
1′	3.97[a]	3.91[a]
2	5.16[b]	4.11[a]
2′	5.23[b]	4.05[a]
3	3.3[c]	2.96[a]
3′	3.58[a]	3.32[a]
4	4.86[a]	2.59[a]
4′	4.2[a]	2.88[a]

[a,b] Notes with the same superscript letter within the same column were not significantly different at a level of 5% (notes were in cm). 1 and 1′, noncrushed algae, 30 min purge; 2 and 2′, crushed algae, 30 min purge; 3 and 3′, noncrushed algae, 50 min purge; 4 and 4′, crushed algae, 50 min purge.

accordance with the results previously found. However, when the purge time was increased, the intensity decreased, which was unexpected. Actually, Refsgaard et al. (*17*) have studied the influence of sampling time in dynamic headspace extraction of volatiles in fish and have shown that, after 30 min of sampling, there is a loss of volatile compounds probably due to breakthrough of the trap. Sanz et al. (*21*), when optimizing sampling time in static headspace for the extraction of coffee volatiles, observed that the extracted quantity of very volatile compounds, such as aldehydes, ketones and alcohols, was higher for low equilibration times. Nevertheless, these results confirm the tendencies observed in **Figures 1** and **2**, namely that extracts 2 and 2′, with respective intensity notes of 5.16 and 5.23, were the most representative.

This result was also confirmed by the similarity test (**Table 2**). Even though the notes were not significantly different at a level of 5%, they were correlated with the results of the principal component analysis. Thus, extracts 4 and 4′ were found to be less similar (the respective notes were 2.59 and 2.88) whereas extracts 2 and 2′ (4.11 and 4.05, respectively) appeared to be the most representative. These marks were also better than those obtained for hydrodistillation, for which the highest note was 3.76 (*7*). Nevertheless, it was obvious that it was difficult to restore an aroma outside its matrix with volatile compounds only, as previously observed by Pennarun et al. (*9*). In fact, similarity notes were rather weak, and we could have expected marks close to 6 or 7. Nevertheless, fresh algae are a difficult material as they have a very slight aroma. Consequently, the

Table 3. Volatile Compounds of *Palmaria Palmata*[e]

compounds	RI	quantity[a] ng · kg^{-1}	methods of identification	refs
Halogenated Compounds				
iodoethane	818	3580	RI, MS, std	
dichloromethane	847	310	RI, MS, std	
trichloromethane	1014	50	RI, MS, std	a
2-fluoroprop-1-ene	1087	710	RI, MS	
iodopentane	1160	70	RI, MS, std	a
chlorobenzene	1209	3260	RI, MS	
tribromomethane	1418	3300	RI, MS	a
Aldehydes				
3-methylbutanal	840	110	RI, MS, std	a, b
2,2-dimethylpropanal	914	280	RI, MS	
hexanal	1091	80	RI, MS, std	a, b, c
heptanal	1126	130	RI, MS, std	a, b, c
2-(E)-hexenal	1224	660	RI, MS, std	c
octanal	1289	2430	RI, MS, std	b, d
nonanal	1397	1130	RI, MS, std	a, b
Ketones				
2,3-pentanedione	1062	70	RI, MS, std	b
3-octanone	1264	2290	RI, MS, std	a, b, d
Alcohols				
1-butanol	1181	5880	RI, MS, std	a
1-penten-3-ol	1189	7165	RI, MS, std	a, b, c, d
3-methylbutanol	1229	960	RI, MS, std	b
Miscellaneous				
1,3-(*E*)–5-(*Z*)-octatriene	1114	380	RI, MS	d
3-ethylidene-1-methylcyclopentene	1163	7090	RI, MS	
trimethyl-1,3-cyclopentadiene	1176	5470	RI, MS	
1,2,4-trimethylbenzene	1277	450	RI, MS	b, d

[a] Takahashi et al., Nippon Kagaku Kaishi, **2002**, *49* (4), 228–237. [b] Tanchotikul and Hsieh, *J. Food Sci.* **1989**, *54* (6), 1515–1520. [c] Sugisawa et al., *Food Rev. Intern.* **1990**, *6* (4), 573–589. [d] Pennarun et al., *J. Sci. Food Agric.* **2002**, *82*, 1652–1660. [e] RI, retention index on a DB–WAX capillary column; MS, mass spectrometry; std, chemical standard.

panelists found it difficult to evaluate the aromas of extracts and to compare them with the raw seaweed.

Identification and Origin of Volatile Compounds of *Palmaria palmata*. Volatiles of *Palmaria palmata* were first extracted using dynamic headspace sampling, according to the parameters previously optimized (crushed algae with a 30 min purge). They were then injected into the GC-MS system. Twenty-three compounds were identified (**Table 3**), comprising seven halogenated compounds, seven aldehydes, two ketones, three alcohols, and four hydrocarbons. No studies have been reported on volatiles of fresh *Palmaria palmata*, but other teams have worked on green and brown seaweed. Thus, in 1990, Sugisawa et al. worked on the green edible algae *Ulva pertusa*. The volatiles were extracted by simultaneous distillation and extraction (SDE), and 66 compounds were found, of which 6 are in agreement with our findings: 1-penten-3-ol, hexanal, 2-(E)-hexenal, heptanal, octanal, and nonanal. In the same way, Takahashi et al. (*13*) have studied the compounds of dried Kombu (*Laminaria* spp.) by SDE too. Among the 67 compounds they found, 10 are identical to those of *Palmaria palmata*: iodopentane, hexanal, heptanal, 2-(E)-hexenal, nonanal, 1-butanol, 1-penten-3-ol, 3-octanone, trichloromethane, and tribromomethane. In the same study, they also analyzed the volatiles of Kombu by dynamic headspace. The compounds extracted were almost the same, except for trichloromethane and tribromomethane that were not detected, and 3-methylbutanal that appeared. This difference observed between the two extraction methods could be explained by the fact that dynamic headspace extracts low-boiling-point components, whereas SDE extracts medium- and high-boiling-point components. Using this method, low-boiling-point compounds are lost or modified during heating.

Among the volatile components observed in *Palmaria palmata*, the halogenated compounds were the most characteristic. This group included iodoethane, dichloromethane, trichloromethane, 2-fluoroprop-1-ene, iodopentane, chlorobenzene, and tribromomethane, and was the most characteristic of the macroalgae, and more particularly of red algae (*22*). Thus, Burreson and Moore (*23*) have identified in the red Hawaiian seaweed *Asparagopsis taxiformis*, forty-two haloforms and halogenated ethanes, ethanols, aldehydes and ketones. Nevertheless, they can also be found in brown algae: when studying the volatiles of *Laminaria* spp., Takahashi et al. (*13*) extracted four iodides by dynamic headspace and two iodides, chloroform and bromoform, by SDE. In fact, marine macroalgae have a high ability to fix halide ions. By the action of haloperoxidase enzymes that have already been detected in seaweeds, and in the presence of hydrogen peroxide, these ions were oxidized and then could react with organic substrates (*24*, *25*). The more common compounds produced were halocarbons such as trichloro- and tribromomethane or iodoethane, as for *Palmaria palmata*, but halogenated phenols, terpenes, or indoles were also produced(*22*, *26*). Only the presence of 2-fluoroprop-1-ene could not be explained. Naturally occurring fluorinated fatty acids or amino acids have been reported in seaweeds (*27*) and could produce this compound, but no studies have recorded this fact.

Seven aldehydes, 3-methylbutanal, 2,2-dimethylpropanal, hexanal, heptanal, 2-(*E*)-hexenal, octanal and nonanal, constituted the second important group of volatile compounds. Among these seven compounds detected, five could arise from the degradation of polyunsaturated fatty acids (PUFA), either by autoxidation or by the action of enzymes such as lipoxygenases. These aldehydes are widespread, as they have already been found in many other sea products, such as green and brown macroalgae (refs 5 and 13, respectively), lobsters (*28*) and oysters (*12*, *29*). When studying by GC-MS and LC-MS, the aldehydes produced by lipid peroxidation, Enoiu et al. (*30*) demonstrated that hexanal and 2-(E)-hexenal could come from linolenic acid, a ω3 PUFA. They also could arise from other ω3 PUFAs such as C20:5ω3 (*31*), which have been reported as the most likely substrates for enzymatic oxidation, producing mainly aldehydes and alcohols. In addition, Mishra et al. (*32*) and Graeve et al. (*33*) have shown that *Palmaria palmata* is devoid of C18:3ω3, but inversely, that C20:5ω3 is the major PUFA in the alga. Thus, we could conclude that eicosapentaenoic acid was probably the precursor of these two volatile compounds. Hexanal could also be provided by oxidation from linoleic acid (*30*) or other ω6 PUFAs, as well as heptanal. As *Palmaria palmata* contains both linoleic and arachidonic acids (*32*, *33*), the detection of these compounds was not surprising. Octanal and nonanal, which were the major aldehydes extracted (2430 and 1130 ng · kg^{-1}), could originate from ω9 monounsaturated fatty acids (MUFAs) (*12*), among which C18:1ω9 was the only one present in the alga and also from ω6 PUFAs such as linoleic acid (*30*).

According to its structure, 2,2-dimethylpropanal could not come from fatty acids, but its origin remains unknown. This compound has never been detected in seafood or other products. Conversely, the origin of 3-methylbutanal is well known. It is frequently found in many sea products, such as crayfish (*34*), lobster (*28*), or dried (*13*). Actually, this compound is generally associated with products that have undergone heating processes. It is obtained from amino acids, and more particularly from leucine, during the Maillard reaction by the Strecker degradation.

Nevertheless, in the case of the volatiles of *Palmaria palmata*, this route is not valid, as the product was not heated. Thierry and Maillard (*35*) have demonstrated that, in the case of cheese, 3-methylbutanal is produced during leucine catabolism by cheese microorganisms. Thus, we could suppose that the same type of catabolism could occur in *Palmaria palmata*, with specific microorganisms.

The two ketones detected in the algae, 2,3-pentanedione and 3-octanone, had quite undefined origins. 2,3-Pentanedione has already been described as deriving from L-alanine as an intermediate in the Maillard reaction (*36*), but as previously stated, this route is quite improbable, because the seaweeds were not heated. 3-Octanone (2290 $ng.kg^{-1}$) definitely arose from fatty acid oxidation, but its exact origin remains unknown because it can be produced through the oxidation of various fatty acids.

Three alcohols were also detected in *Palmaria palmata*: 1-butanol, 1-penten-3-ol and 3-methylbutanol. They are the most abundant compounds in the algae (5880, 7165, and 960 $ng \cdot kg^{-1}$, respectively). 1-Butanol has previously been detected in brown seaweeds by Takahashi et al., but its origin was not discussed. Nevertheless, according to Tanchotikul and Hsieh (*34*), its formation may be due to the decomposition of secondary hydroperoxides of fatty acids, as for 1-pentanol or 1-hexanol, or by the reduction of the corresponding aldehyde (*37*). The origin of the two other compounds is more evident. 1-penten-3-ol, observed in oysters by Pennarun et al. and in dried Kombu by Takahashi et al. (*13*), is derived from the degradation of $\omega 3$ polyunsaturated fatty acids. As the only $\omega 3$ fatty acid extracted from *Palmaria palmata* was 20:5$\omega 3$ (*32*, *33*), it was probably the only precursor of this compound. The identification of the source of 3-methylbutanol varies from author to author. Thus, according to Thierry and Maillard (*35*), it derives from the corresponding branched aldehyde, which may be produced from leucine either by the action of microorganisms or by heating. Tanchotikul and Hsieh (*34*), in crayfish, and Hartvigsen et al. (*38*), in a fish oil-enriched mayonnaise, found that 3-methylbutanol originated from fatty acid hydroperoxides.

The last group of volatile compounds observed in the algae consisted of cyclic and noncyclic hydrocarbons. 1,3-(E)-5-(Z)-Octatriene has been found in oyster *Crassostrea gigas* (*16*, *29*). It could arise from $\omega 3$ fatty acid degradation, but its exact origin remains unknown. 1,2,4-Trimethylbenzene has been observed in oysters and crayfishes (*29*, *34*). The origin of this compound was discussed by the authors. It was described as coming from polysaccharide degradation by Pennarun et al. (*29*), but Tanchotikul and Hsieh (*34*) explained that carotenoids have been thought to be precursors of benzene derivatives such as alkylbenzene. The last two compounds, which were 3-ethylidene-1-methylcyclopentene and trimethyl-1,3-cyclopentadiene, were the most abundant (7090 and 5470 $ng \cdot kg^{-1}$), but their presence and their origin in other products has never been recorded in the literature.

In this study, we have optimized dynamic headspace extraction for the volatile compounds of an edible seaweed, *Palmaria palmata*, according to the extract representativeness. This method gave an extract similar to the seaweed odor and characterized by marine aromas such as seaside, iodozed, fresh, and seaweed. The GC-MS analysis showed that major volatile compounds that constituted the extract were aldehydes and halogenated compounds. This study on the volatile compounds of *Palmaria palmata* will be soon completed by olfactometric analysis, to characterize odorous compounds responsible for its aroma.

ABBREVIATIONS USED

ANOVA, analysis of variance; GC, gas-chromatography; GC-MS, gas chromatography-mass spectrometry; MUFA, mono-unsaturated fatty acid; PCA, principal component analysis; PUFA, poly-unsaturated fatty acid.

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