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RESEARCH

Effects of cooking on the composition of volatiles, total phenolic compounds, and antioxidant capacity of three Chilean seaweeds

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Abstract

Seaweed consumption is increasing around the world due to consumer demands for sustainable food sources, health benefits derived from bioactive compounds, and a source of novel flavors. Despite all the benefits that come with eating seaweed, consumers still resent of their sensorial properties. Seaweed is traditionally consumed raw or cooked, but there is still not enough information on how the sensory descriptors and bioactive compounds change when cooked. The aim of this study was to determine the change in total polyphenol content (TPC), antioxidant capacity (e.g., DPPH and ORAC values) and changes in volatile compounds of three Chilean seaweeds: *Durvillaea antarctica*, *Pyropia* spp. and *Ulva lactuca* when subjected to traditional cooking for 15 min at 100°C. In all three seaweeds, TPC decreased with cooking and so did the measured antioxidant capacity. Altogether, 46 volatile cpmpounds were identified in *D. antarctica*, 49 in *Pyropia* spp. and 47 in *U. lactuca*. The concentration of these volatile compounds was correlated with aroma sensory descriptors of the same samples. Consumer preferences may be attracted by the herbal notes of *U. lactuca* or the sweet, caramel, and umami flavors of *D. antarctica* and *Pyropia*.

Keywords Seaweeds · Cooking · Volatile compounds · Antioxidants · Aroma descriptors

Introduction

The consumption of seaweeds is increasing in different regions of the world due to their sustainable, low-input requirements for production as well as their nutritional properties and presence of bioactive compounds beneficial to health (Holdt and Kraan 2011; Figueroa et al. 2021). Seaweeds do not require arable land, fertilizer, or freshwater for their growth. Moreover, they contain several bioactive compounds that may have preventive effects on cerebrovascular diseases, exhibit anticoagulant activity and help increase metabolism and reduce obesity (Shannon and Abu-Ghannam 2019). Some studies indicate that since most antioxidants are thermolabile their activity changes when subjected to different hydrothermal treatments. Rajauria et al. (2010) point out that the antioxidant capacity in brown seaweed increases significantly with heating up to 95°C and then it declines. Pina et al. (2014) found that boiling *Chondrus crispus* also increased the antioxidant content. On the other hand, Cox et al. (2012) reported that when *Himanthalia elongata* was exposed to boiling water, the content of antioxidants decreased by 80%. Phenolics are usually the main antioxidants in seaweeds and this can be measured in a quantitative way such as total phenolic content (TPC). This is not a specific method and does not take into regard the effectivity/quality of the phenolics compounds (Getachew et al. 2020). For this reason it is necessary to combine this method with other antioxidant analysis methods. There are different methods to screen for the antioxidant capacity of seaweed such as DPPH (radical scavenging capacity) and ORAC (oxygen radical absorbance capacity), both radical absorbing assays, and they can be used to determine in vitro the antioxidant capacity of the seaweed (Jacobsen et al. 2019).

Seaweeds contain a wide variety of volatile compounds such as phenols, alcohols, aldehydes, ketones, esters, fatty acids, halogenated and sulfur compounds that vary according to species, seasonality, geographical location, harvest time and

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processing (Santos and Narain 2018; Garicano et al. 2020). Seaweeds release volatile compounds that serve as sexual pheromones, chemical defense against herbivores, inhibitors of bacteria and fungi, and to feed attractants (López-Pérez et al. 2017).

In a previous study we analyzed the main sensory characteristics of the brown seaweed *Durvillaea antarctica*, the green seaweed *Ulva lactuca* and the red seaweed *Pyropia* spp. (Figueroa et al. 2022). Expert panelists found that the aroma changed markedly with cooking, from herbal and marine in rehydrated seaweed to caramel, moldy/earthy, and less marine in cooked samples. However, chemical characterization of volatile compounds was not performed at that time. Other studies have identified different volatile compounds that contribute to the aroma profile of seaweeds, such as dimethyl sulfide (DMS), hexanal, (E)-2-octenal, (E)-2-nonenal, and β-cyclocitral (Santos and Narain 2018; Francezon et al. 2021).

In spite of the environmental and nutritional benefits, the appearance, aroma and texture of seaweeds are major drawbacks to their extended consumption by new consumers (Birch et al. 2018). These authors asked a group of 521 Australians why they did not eat seaweed and 39% of them indicated that aroma was a relevant reason for rejecting them. Prager (2016) argued that the sensory properties of seaweeds in their raw state are a major obstacle to their acceptance among Western consumers. This is possibly the reason why increasing the consumption of seaweed among new consumers usually involves heat treatments (e.g., cooking or roasting) and the addition of seaweed powders and extracts to formulated products (pasta, bread, biscuits, etc.) is limited to very small amounts (Roohinejad et al. 2017; Wendin and Undeland 2020).

There are more than 650 species of seaweed in Chile and only three of them are routinely consumed as direct food, namely, *Durvillaea antarctica* (cochayuyo), *Pyropia* spp. (luche) and *Ulva lactuca* (sea lettuce) (Aguilera 2021; Figueroa et al. 2021). Traditionally, these seaweeds are utilized in hot dishes such as stews, empanadas (pies) and broths (Mansilla et al. 2012). However, there are no studies on how the flavor and bioactive compounds of these seaweeds change during cooking. The objective of this study therefore was to analyze the changes in volatile compounds and antioxidants present in these raw seaweed species when they were rehydrated and cooked at boiling temperature for 15 min (i.e., the traditional cooking method). Additionally, we attempted to find possible correlations between volatile compounds and the sensory descriptors for aroma determined in our previous work (Figueroa et al. 2022).

Materials and methods

Samples

Dried samples of *Durvillea antarctica*, *Pyropia* spp., and *Ulva lactuca* (approximately 2 kg of each species) were purchased from the commercial purveyor Kaiso Spa (Chile). The seaweeds were collected in April 2021 and sun-dried near Puerto Montt (approximately 41°N, 72°W). After purchase, the dry samples were kept in vacuum-sealed plastic bags at room temperature (approximately 20 °C) in darkness. Samples were ground before the experiments and stored again in plastic bags until use. Samples utilized in this study came from the same lot as those in Figueroa et al. (2022) and were brought to Denmark by author VF.

Preparation of samples for volatiles analysis

One gram of ground dry seaweed was mixed with 15 g of water and allowed to hydrate for 1 h in Falcon tubes. Rehydrated samples were used as such or cooked for 15 min at 100° C in a pre-heated shaking water bath and cooled at room temperature for 1 h. Then, the uncooked and cooked samples were subjected to extraction of volatiles. Samples were prepared in triplicate.

Analysis of volatile compounds

Collection of volatile compounds was performed by dynamic headspace ″purge and trap″ allowing direct analysis of the sample. Pre-prepared samples (16 g) were weighed into a purge bottle and was added 1 mL of antifoam (Synperonic) (for *Pyropia* spp., 10 mL of antifoam) and 25 mg of internal standard (4-methyl-1-pentanol, 30 µg g^{-1} in water). Samples were warmed at 37°C in a thermoregulated bath, and the volatiles were collected for 30 min into a Tenax tube with a N_2 flow of 340 mL min^{-1}. Finally, the contents of the Tenax tubes were dried for 20 min with N_2 at a flow of 50 mL min^{-1} (Refsgaard et al. 1999).

Afterward, the tubes were placed in an Automatic Thermal Desorber (ATD-350, Perkin Elmer, USA), in which the volátiles were thermally desorbed from the Tenax tube and transferred to a GC (GC Agilent 6890 N, USA). In the ATD, the Tenax tubes were heated to 200° C and the volatiles were transferred to a cold trap at -30°C to concentrate the volatiles. Thereafter, the cold trap was heated to 220°C and volatiles were transferred to the GC. A column DB-1701, 30 m × 0.25 mm × 1.0 µm (J&W Scientific, USA) was used to achieve chromatographic separation using the modified

method of García-Moreno et al. (2016). The oven program had an initial temperature of 45 °C for 5 min, with increments of 1.5 °C min^{-1} to 55 °C, and 2.0 °C min^{-1} to 90 °C and finalized by an increment of 8 °C min^{-1} to 230 °C, where the temperature was held for 8 min. The individual compounds were analyzed by mass-spectrometry with an HP 5973 inert mass-selective detector (Agilent Technologies, USA; Electron ionization mode, 70 eV, mass to charge ratio scan between 30 and 250). The volatile compounds were identified individually by the MS-library Wiley 138 K (John Wiley and Sons, Weinheim, Germany) and quantified by comparison with an external standards calibration curve. Volatile compounds that were present in the seaweed samples were quantified. The spectra were compared with those in MS libraries (XCalibur, WileyRegistry8e and mainlib). Samples were analyzed in triplicate and one injection for each one.

Sample preparation for phytochemical analysis

Ten gram of *D. antarctica*, *Pyropia* spp., and *U. lactuca* was hydrated with 100 mL water and cooked as mentioned in "Preparation of samples for volatiles analysis" section. Phytochemical compounds in rehydrated and cooked samples were extracted using the modified method of Ortiz et al. (2021). This implied the use of water instead of ethanol–water (1:1) and shaking for 12 h at room temperature in the dark. The samples were filtered with a Whatman No. 1 paper and the filtrate was used in the following analyses (in triplicate).

Total phenolic content

Total phenolic content (TPC) was determined according to the methodology proposed by Yildiz et al. (2011), using the Folin-Ciocalteu reagent and measuring the colored solution at 765 nm. The TPC of seaweeds was expressed as mgEAG $(100\ g)^{-1}$ dry seaweed.

DPPH radical scavenging assay

The antiradical activity was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay according to Brand-Williams et al. (1995). Loss of color (purple) in the radical solution when exposed to each extract was measured at 517 nm and results were expressed as % inhibition of DPPH radical according to the following formula:

$$\mathrm{DPPH}(\%) = \left[\left(A_c - A_s\right)/\left(A_c\right)\right] \times 100$$

where, A_c = control absorbance, A_s = Sample absorbance.

Values were expressed as µMeq Trolox g^{-1} of sample according to a calibration curve ($y = -0.0023x + 0.6219$, $R^2 = 0.997$).

ORAC

The ORAC analyses were performed on a multi-detector microplate fluorometer (Synergy/HTX, Biotek Instruments Inc, USA) according to the methodology proposed by Davalos et al. (2004). The antioxidant capacity was expressed as µMeq Trolox g^{-1} of sample.

Statistical analysis

All the experiments were carried out in triplicate. Results are expressed as average ± standard deviation (SD). Analysis of variance (ANOVA) and multiple comparisons were used to find significant differences ($p < 0.05$) between the hydrated and cooked samples using STAT GRAPHICS Centurion XV (StatPoint Technologies Inc., USA). Results of volatiles were analyzed by PLSR (Partial Least-Squares Regression) using the XLSTAT 2015.6 software (Addinsoft, France), where matrix X represented the mean concentration of volatile compounds shown in Tables 1, 2, 3, and matrix Y consisted of the results obtained from the ranking descriptive analysis (RDA) for the sensorial attributes of the 6 types of hydrated and cooked samples (Figueroa et al. 2022). In RDA the evaluators classified samples using an ordinal scale, 1 to 6, and then the results are expressed as the sum of rankings by the nine panelists for each descriptor: the smaller the sum, the smaller the intensity of the descriptor. The different components t1 (horizontal axis of the graph); together with t2 and t3 (vertical axes of the graph) explain the main differences between seaweeds and treatments.

Results

Effect of cooking on the phytochemical constituents of seaweeds

The TPC of raw rehydrated *D. antarctica, Pyropia* spp. and *U. lactuca* were 260.83 ± 20.05, 621.53 ± 3.58 and 309.28 ± 7.23 mg GAE $(100\ g)^{-1}$, and decreased after thermal processing by 6%, 16%, and 21%, respectively. However, changes were statistically significant ($p < 0.05$) only for *Pyropia* spp. and *U. lactuca* (Fig. 1).

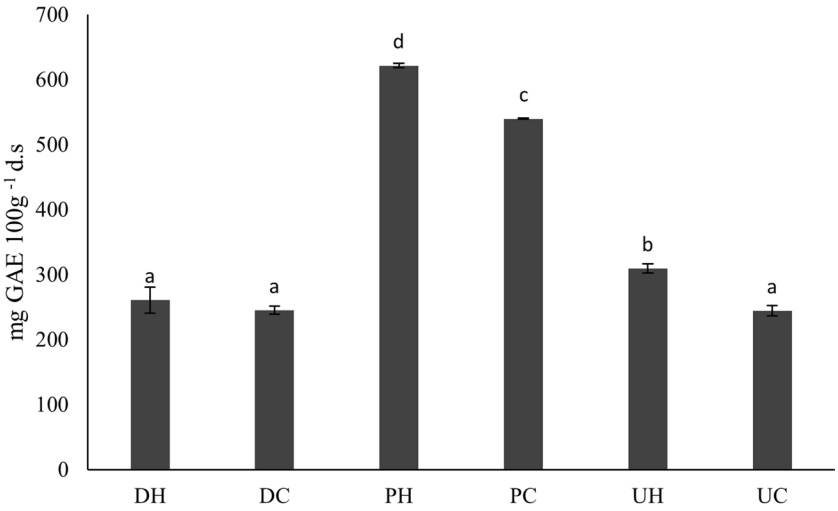

Fig. 1 TPC of raw and cooked seaweeds: *D. antarctica, Pyropia spp. and U. lactuca*. The results are expressed as mg GAE (100 g)$^{-1}$ of dry seaweed. Letters above the bars are the significative difference between the samples. **DH:** Hydrated *D. antarctica*, **DC:** Cooked *D. antarctica*, **PH**: Hydrated *Pyropia* spp., **PC**: Cooked *Pyropia* spp., **UH**: Hydrated *U. lactuca*, **UC**: Cooked *U. lactuca*

As shown in Table 1, *Pyropia* spp. had the highest DPPH value, followed by *D. antarctica* and *U. lactuca*, and significant differences were observed between rehydrated and cooked samples for *Pyropia* spp. and *D. antarctica* but not for *U. lactuca*. Regarding the ORAC values, no significant changes were observed between hydrated and cooked samples in *Pyropia* spp. and *D. antarctica*. On the other hand, there was a significant decrease in ORAC values in *U. lactuca* when it was subjected to cooking.

Effect of cooking on the volatile compounds of seaweeds

Volatile compounds identification

A total of 64 volatile compounds were well identified in the raw rehydrated and cooked seaweeds. The number of volatile compounds varied with the species: 46 for *D. antarctica*, 49 for *Pyropia* spp. and 47 for *U. lactuca*.

Table 1 DPPH and ORAC values for hydrated and cooked seaweeds. Values expressed as mean ± SD (standard deviation) of triplicate samples

Sample	DPPH μM eq Trolox g^{-1} (d.s)	ORAC μM eq Trolox g^{-1} (d.s)
DH	4.42 ± 0.20 ^{c}	26.95 ± 0.83 ^{de}
DC	2.79 ± 0.84 ^{b}	27.06 ± 1.75 ^{e}
PH	4.49 ± 0.11 ^{c}	23.73 ± 1.81 cd
PC	2.74 ± 0.82 ^{b}	23.18 ± 0.08 ^{c}
UH	1.22 ± 0.08 ^{a}	14.15 ± 1.12 ^{b}
UC	1.02 ± 0.11 ^{a}	± 0.07 ^{a}

DH Hydrated *D. antarctica*, *DC* Cooked *D. antarctica*, *PH* Hydrated *Pyropia* spp., *PC* Cooked *Pyropia* spp., *UH* Hydrated *U. lactuca*, *UC* Cooked *U. lactuca.*

Table 2 lists the volatile compounds identified in rehydrated and cooked seaweeds. Among them are 25 aldehydes, 11 alcohols, 11 ketones, 6 hydrocarbons, 2 norisoprenoid derivatives, 4 furans, 1 sulfur compound and 1 ester. This great variety of volatile compounds is responsible for the aromas present in the different seaweeds, which vary between species and treatments.

Effect of species and cooking on the concentration of volatiles

Mean values and standard deviations of volatile compounds before and after cooking are presented in Table 3. Among the compounds, hexanal was found in the highest concentration in the three species of seaweeds, but the highest amount was found in *Pyropia* spp. Also, in *Pyropia* spp., pentanal was found in a high relative concentration in relation to the other seaweeds (Table 3). Butanal was not found in *D. antarctica*, but it was present in the other two species, and increased with cooking. 2,3-butanedione was a compound that increased in the three species of seaweed with cooking. 2-pentylfuran was present just in *Pyropia* spp. and decreased with cooking while 1-pentanol, in the three species of seaweed but did not change with cooking.

Correlation between RDA and concentration of volatile compounds

PLSR described the relationships between the concentration of 25 volatile compounds (X matrix) and the scores obtained by the sensory evaluation for five aroma descriptors (herbal, marine, earthy/moldy, mineral, and caramel) and tastes (bitter, acid, umami, salty and sweet) (Y matrix) from our study on sensory descriptors for the same three edible Chilean seaweeds (Figueroa et al. 2022). Moreover, PLSR permitted the

Table 2 Different volatile compounds identified in the three seaweeds (*D. antarctica, Pyropia* spp. and *U. lactuca*), both hydrated and cooked. All compounds were identified by comparison with mass spectra and retention index database

Volatile compounds	Seaweed samples						Odour description	REF
	DH	DC	PH	PC	UH	UC		
Aldehydes								
butanal			✓	✓	✓	✓	Fruity, Ethereal, Meat	a
3-methylbutanal	✓	✓	✓	✓	✓	✓	Chocolate, peach, fatty, vanilla, sweet, floral, butter	a
(E)-2-butenal	✓	✓	✓	✓	✓	✓	Flowerly	b
Pentanal	✓	✓	✓	✓	✓	✓	Fermented, bready, fruity, nutty, berry	a
(E)-2-pentenal	✓	✓	✓	✓	✓	✓	Green, waxy and fruity	a
hexanal	✓	✓	✓	✓	✓	✓	Fresh, green, grassy, leafy, fruity, sweaty, woody, clean	a
(E)-2-hexenal	✓	✓	✓	✓	✓	✓	Green leafy, fishy	a, c
heptanal	✓	✓	✓	✓	✓	✓	Herbaceous, fishy, fatty, pungent	d,e
(Z)-4-heptenal	✓	✓	✓	✓	✓	✓	Green fatty, fishy	b, c
(E)-2-heptenal	✓	✓	✓	✓	✓	✓	Mossy, sulphury, cooked fish, roasted	
octanal	✓	✓	✓	✓	✓	✓	Citrus, Fat, Green, Oil, Pungent	b
(E,E)-2,4-heptadienal	✓	✓	✓	✓	✓	✓	Green, marine, fatty, nutty, fishy	d, c
5-methyl-2-isopropenyl-4-hexenal	✓	✓		✓	✓	✓	-	
2-octenal	✓	✓	✓	✓	✓	✓	Green, grass, pepper, coffe, cucumber, fatty. Dried kombu with soy sauce	a,d
(E,Z)-2,6-nonadienal			✓	✓	✓	✓	Green, cucumber, melon, fatty and vegetative	a
(E)-2-decenal	✓	✓	✓	✓	✓	✓	Fat, Fish, Orange. Stock made from kombu, rice cracker	b,d
2-nonenal	✓	✓	✓	✓	✓	✓	Earthy, fishy, cucumber, green	d
3,4-dimethyl benzaldehyde			✓	✓	✓	✓	-	
5-methylfurfural					✓	✓	Sweet, caramellic, bready, brown, coffee-like	a
α-cyclocitral			✓	✓			-	
β-cyclocitral	✓	✓	✓	✓	✓	✓	Tropical, Hay	b,d
safranal	✓	✓	✓	✓	✓	✓	Herbal, fresh, grassy, woody, spicy, metallic, tobacco	a
2-hydroxy-4-methylbenzaldehyde			✓	✓			Bitter almond phenolic	a
(E,E)-2,4-decadienal	✓	✓	✓	✓	✓	✓	Coriander, Deep Fried, Fat, Oil, Oxidized	b
benzaldehyde	✓	✓	✓	✓	✓	✓	Sweet, oily, almond, cherry, nutty and woody	a
Alcohols								
1-penten-3-ol	✓	✓	✓	✓	✓	✓	Butter, Fish, Green, Oxidized, Wet Earth	b
1-pentanol	✓	✓	✓	✓	✓	✓	Pungent, fermented, bready, yeasty, fusel, winey, solvent, Cotton candy, fruity	a
(Z)-2-penten-1-ol	✓	✓	✓	✓	✓	✓	Cherry, narcissus, fruity, green, sweet, candy	a
(5Z)-octa-1,5-dien-3-ol					✓	✓	Green, marine	b
1-octen-3-ol	✓	✓	✓	✓	✓	✓	Cucumber, Earth, Fat, Floral, Fishy, Mushroom	b
(E)-2,4,4,7-tetramethyl-5,7-octadien-3-ol						✓	-	
3,5,5-trimethyl-2-cyclohexen-1-ol	✓	✓					-	
2,4,4-trimethylcyclohexane-en-1-ol			✓	✓	✓	✓	-	
(E)-2-octen-1-ol	✓	✓	✓	✓		✓	Green, vegetable-like	a
2,6-dimethyl-cyclohexanol	✓	✓	✓	✓	✓	✓	-	
2-(bromomethyl)-2-adamantanol			✓	✓			-	
Ketones								
2,3-butanedione	✓	✓	✓	✓	✓	✓	Sweet, creamy, buttery, pungent, with a pungent caramellic nuance	a
1-penten-3-one	✓	✓	✓	✓	✓	✓	Fish, Green, Mustard, Pungent	b
4-methyl-1-penten-3-one	✓	✓			✓	✓	-	
4-hexen-3-one						✓	Pungent, acrid, metallic and etherial	a
2-heptanone	✓	✓	✓	✓			Cheese, fruity, ketonic, green banana, with a creamy nuance	a

Table 2 (continued)

Volatile compounds	Seaweed samples						Odour description	REF
	DH	DC	PH	PC	UH	UC		
2,2,6-trimethylcyclohexanone	✓	✓	✓	✓	✓	✓	Pungent thujone labdanum honey cistus	a
3-octen-2-one	✓	✓					Dull, Green, Nut, Rose	b
isophorone	✓	✓	✓	✓	✓	✓	Cedarwood, Spice	b
1,2,3,3-tetramethylcyclopenten-4-one	✓		✓	✓	✓	✓	-	
3,5-octadien-2-one	✓	✓	✓	✓	✓	✓	Green	b
6-methyl-3,5-heptadien-2-one	✓	✓	✓	✓	✓	✓	Spice	b
Norisoprenoid derivatives								
alpha-ionone			✓	✓	✓	✓	Grassy, lemon, citrus, sugary, fruity	a
beta-ionone	✓	✓	✓	✓	✓	✓	Floral, violet, woody	b
Furans								
2-ethylfuran			✓	✓	✓	✓	Butter, Caramel	b
2-pentylfuran			✓	✓			Green bean like	c
trans-2-(2-pentenyl)furan			✓	✓	✓	✓	-	
5-isopropyl-3,3-dimethyl-2-methylene-2,3-dihydrofuran	✓	✓	✓	✓	✓	✓	-	
Hydrocarbons								
2-methyl-1,3-dioxane	✓	✓	✓	✓			-	
4,4,6,6-tetramethylbicyclo[3.1.0]hex-2-ene	✓		✓	✓	✓	✓	-	
sabinene	✓	✓	✓	✓	✓	✓	Pepper	b
1-methyl-4-(2-hydroxyethyl)-ccyclohexane	✓	✓	✓	✓	✓	✓	-	
1,1,5-trimethylindan			✓	✓	✓	✓	-	
1,6-dimethyldecahydronaphthalene			✓	✓			-	
Sulfur-containing compounds							-	
methyl disulfide (DMS)					✓		Cabbage, Organic, Sulfur, Wet Earth, fishy	b
Esters								
n-caproic acid vinyl ester			✓	✓		✓	-	

Odour descriptions were cited from: a) http://www.thegoodscentscompany.com/data/rw1007681.html; b) https://www.femaflavor.org/; c) Riad et al. (2021); d) Giri et al. (2010); e) Varlet et al. (2007).

identification of important changes in the attributes between the rehydrated and cooked seaweeds.

Figure 2 shows the outcomes of PLSR analysis where dimensions t1 and t2, and t1 and t3 explained 73.65% and 67.68% of the total variance, respectively. These values are similar to those reported by Cano-Lamadrid et al. (2020) for sensory descriptors and liking of smoothies. From Fig. 2a, the second PLSR dimension (t2) separated the seaweeds according to species. The three seaweeds had different sensorial attributes and were located in different quadrants in the graph. *D. antarctica* (DH and DC) is in the lower right quadrant and *U. lactuca* (UH and UC) in the lower left quadrant. However, samples of *Pyropia* spp. (PH and PC) are in the quadrants over the X-axis, one in each quadrant, but very close to each other. This graph shows the particular difference between *Pyropia* spp. and *U. lactuca*. The seaweeds found in the quadrants to the right of the Y-axis (PH, DH, and DC) are those that correlate most strongly with sweet, caramel and umami flavors. Seaweeds on the left correlate with aromas and flavors that are not very pleasant in Western cultures such as bitter, acid, mineral, earthy/moldy and herbal (Figueroa et al. 2022).

In Fig. 2b, a third PLSR dimension (t3) separated the seaweeds according to treatment (raw rehydrated and cooked). Seaweed samples in the upper quadrants are raw rehydrated and those present in the lower quadrants are the cooked samples. Cooked seaweeds are associated with sweet and umami tastes and caramel aromas; otherwise, rehydrated seaweeds are associated with earthy/moldy, herbal, marine, and mineral aromas, and bitter and acid tastes.

Table 3 Concentration of volatile compounds obtained from hydrated and cooked seaweeds. Values expressed as mean ± SD (standard desviation) of triplicate samples

COMPOUND	CH	CC	PH	PC	UH	UC	Odor threshold
	mg g^{-1}						
butanal	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	0.84 ± 0.10 ^{c}	1.82 ± 0.21 ^{d}	0.18 ± 0.02 ^{b}	1.05 ± 0.37 ^{c}	5.26×10^{-6}
2,3-butanedione	1.56 ± 0.16 ^{a}	11.79 ± 1.77 ^{c}	2.12 ± 0.30 ^{ab}	10.20 ± 0.44 ^{c}	0.69 ± 0.02 ^{a}	4.94 ± 0.84 ^{b}	-
3-methylbutanal	0.70 ± 0.08 ^{a}	1.0 ± 0.20 ^{ab}	1.36 ± 0.12 ^{ab}	2.03 ± 0.12 ^{b}	3.79 ± 0.24 ^{c}	4.13 ± 1.03 ^{c}	2.5×10^{-7}
2-butenal	1.48 ± 0.14 ^{a}	2.48 ± 0.03 ^{a}	2.30 ± 0.39 ^{a}	5.32 ± 0.76 ^{ab}	2.32 ± 0.02 ^{a}	14.19 ± 8.02 ^{b}	0.0005
1-penten-3-one	0.84 ± 0.06 ^{a}	1.53 ± 0.12 ^{a}	2.15 ± 0.18 ^{ab}	3.50 ± 0.56 ^{bc}	0.92 ± 0.02 ^{a}	5.15 ± 1.79 ^{c}	1.2×10^{-6}
pentanal	0.49 ± 0.04 ^{a}	0.96 ± 0.15 ^{a}	8.06 ± 1.42 ^{b}	15.64 ± 1.01 ^{c}	1.59 ± 0.02 ^{a}	5.63 ± 1.79 ^{b}	0.0002
1-penten-3-ol	4.06 ± 0.20 ^{a}	4.03 ± 0.59 ^{a}	21.01 ± 4.81 ^{b}	19.58 ± 2.75 ^{b}	5.15 ± 0.24 ^{a}	8.13 ± 1.25 ^{a}	0.00036
(E)-2-pentenal	0.78 ± 0.02 ^{a}	1.35 ± 0.10 ^{a}	1.94 ± 0.22 ^{a}	4.43 ± 0.22 ^{b}	1.19 ± 0.65 ^{a}	5.71 ± 1.99 ^{b}	0.0001–00015
1-pentanol	0.82 ± 0.00 ^{a}	0.85 ± 0.05 ^{a}	3.52 ± 0.60 ^{b}	3.51 ± 0.38 ^{b}	0.72 ± 0.10 ^{a}	1.09 ± 0.91 ^{a}	0.88–2.65
(E)-2-penten-1-ol	3.57 ± 0.21 ^{a}	3.21 ± 0.25 ^{a}	13.20 ± 2.04 ^{b}	11.97 ± 0.67 ^{b}	1.49 ± 0.21 ^{a}	0.70 ± 0.21 ^{a}	-
hexanal	7.81 ± 0.31 ^{a}	6.96 ± 0. 05 ^{a}	33.28 ± 2.04 ^{b}	30.03 ± 1.48 ^{b}	2.46 ± 0.32 ^{a}	3.80 ± 0.72 ^{a}	0.00021
(E)-2-hexenal	2.44 ± 0.20 ^{a}	4.75 ± 0.35 ^{b}	3.72 ± 0.53 ^{ab}	7.94 ± 1.24 ^{c}	2.45 ± 0.02 ^{a}	7.72 ± 0.57 ^{c}	0.00005
2-heptanone	0.18 ± 0.01 ^{a}	0.15 ± 0.02 ^{a}	2.58 ± 0.05 ^{c}	1.30 ± 0.12 ^{b}	0.11 ± 0.00 ^{a}	0.13 ± 0.01 ^{a}	0.0007
n heptanal	0.46 ± 0.03 ^{a}	0.39 ± 0.06 ^{a}	2.93 ± 0.05 ^{c}	2.73 ± 0.19 ^{c}	1.07 ± 0.16 ^{b}	1.14 ± 0.15 ^{b}	0.00003
(Z)-4-heptenal	0.17 ± 0.02 ^{a}	0.22 ± 0.00 ^{a}	1.98 ± 0.06 ^{c}	1.75 ± 0.17 ^{c}	0.47 ± 0.06 ^{ab}	0.73 ± 0.05 ^{b}	4×10^{-7}
2-pentylfuran	0.00 ± 0.00 ^{a}	0.000 ± 0.00^{a}	3.02 ± 0.10 ^{b}	2.25 ± 0.43 ^{c}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	-
(E)-2-heptenal	4.81 ± 0.66 ^{bd}	6.28 ± 0.84 ^{d}	3.93 ± 0.00 ^{bc}	6.01 ± 0.14 ^{d}	0.40 ± 0.01 ^{a}	2.38 ± 0.77 ^{b}	-
1-octen-3-ol	8.67 ± 0.75 ^{bc}	4.84 ± 0.20 ^{b}	42.39 ± 7.16 ^{d}	18.72 ± 1.84 ^{c}	1.41 ± 0.05 ^{a}	1.46 ± 0.25 ^{a}	0.0002
benzaldehyde	0.98 ± 0.12 ^{ab}	0.76 ± 0.05 ^{a}	2.02 ± 0.15 ^{d}	1.49 ± 0.28 ^{bc}	1.43 ± 0.12 ^{bc}	1.88 ± 0.14 ^{cd}	0.0003
octanal	0.75 ± 0.06 ^{a}	0.68 ± 0.13 ^{a}	1.44 ± 0.06 ^{b}	2.05 ± 0.20 ^{c}	0.38 ± 0.05 ^{a}	0.73 ± 0.00 ^{a}	1×10^{-7}
(E,E)-2,4-heptadienal	1.04 ± 0.04 ^{ab}	0.89 ± 0.12 ^{a}	1.41 ± 0.24 ^{b}	2.13 ± 0.22 ^{c}	1.46 ± 0.09 ^{ab}	5.60 ± 0.18 ^{d}	3.5×10^{-6}
2-octenal	1.19 ± 0.06 ^{ab}	1.20 ± 0.25 ^{a}	2.47 ± 0.42 ^{bc}	3.30 ± 0.60 ^{c}	1.06 ± 0.03 ^{a}	3.16 ± 0.55 ^{c}	8.3×10^{-5}
2-nonenal	1.61 ± 0.22 ^{ab}	0.49 ± 0.04 ^{a}	3.42 ± 0.41 ^{cd}	4.22 ± 0.81 ^{d}	2.31 ± 0.25 ^{bc}	3.47 ± 0.03 ^{cd}	-
(E)-2-decenal	1.55 ± 0.76 ^{b}	0.77 ± 0.07 ^{ab}	0.63 ± 0.05 ^{a}	0.49 ± 0.06 ^{a}	0.22 ± 0.00 ^{a}	0.25 ± 0.03 ^{a}	1.5×10^{-7}
2,4-decadienal	1.77 ± 0.67 ^{b}	0.86 ± 0.15 ^{a}	1.83 ± 0.13 ^{b}	1.31 ± 0.17 ^{ab}	0.56 ± 0.01 ^{a}	0.65 ± 0.05 ^{a}	0.0005×10^{-7}

Odor Threshold based on: A review of volatile compounds in edible macroalgae (Li et al. 2023).

Discussion

TPC and antioxidant capacity

TPC and antioxidant activity in this study, and in most of the seaweed research, decreased by cooking. These results are contrasted with those reported by Cox et al. (2012) and Rajauria et al. (2010). Rajauria et al. (2010) reported an increase in the TPC content of brown Irish seaweeds heated up to 95 °C, but at higher temperatures, the phenolic content decreased continuously in all the tested species. Temperature initially enhances the extraction of bound phenolic compounds, increasing the concentration of phytochemicals and their antioxidant activity in the medium, but afterward, compounds are not stable when exposed at higher temperatures or prolonged times, and they can be degraded in the free-state (Sergio et al. 2020). Alide et al. (2020) and Sergio et al. (2020) showed that the increase or decrease in the antioxidant content can be influenced by many factors, such as temperature, time, and the solvent used in their extraction; thus, it is difficult to generalize the effect of cooking and antioxidant activity. In this study, the extraction was in aqueous media to try to mimic the traditional way of preparing seaweed. This could have affected the concentration of TPC, decreasing the values due to possible degradation of the compounds released by the seaweed in the water (Lavelli and Vantaggi 2009). This degradation could have occurred because the compounds generated or released from the cooking were less stable to oxidation, enzymatic reactions, etc.

ORAC and DPPH are widely used methods to assess antioxidant efficacy in different foods. Unfortunately, both methods have several limitations, which could have affected the results. DPPH assay is very sensitive to the extractant milieu, pH, oxygen, and light exposure (Xie and Schaich 2014). On the other hand, in the ORAC assay, great care must be taken with the reaction temperature, exposure to oxygen, and the concentration of reagents since

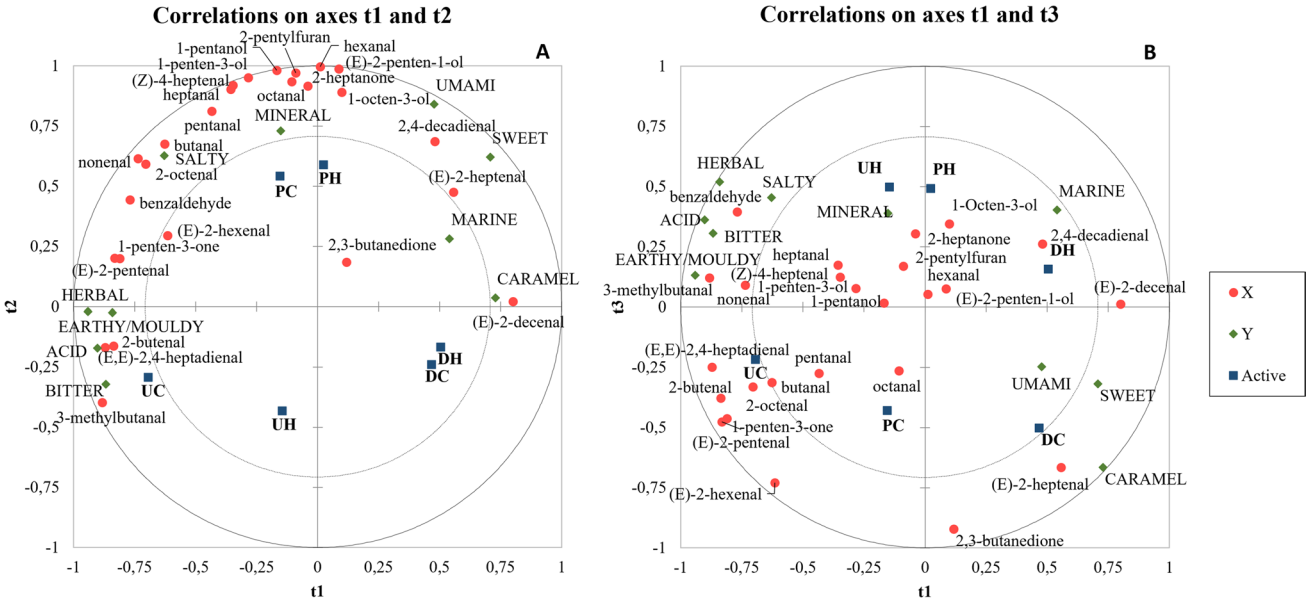

Fig. 2 PLSR Analysis. Correlation between RDA results and volatile compounds. **A)** Correlations between t1 and t2 axes (73.65%). **B)** Correlations between t1 and t3 axes (67.68%). **DH:** Hydrated *D. antarctica*, **DC:** Cooked *D. antarctica*, **PH**: Hydrated *Pyropia* spp., **PC**: Cooked *Pyropia* spp., **UH**: Hydrated *U. lactuca*, **UC**: Cooked *U. lactuca*

all these factors can mean that the results obtained do not correctly interpret the changes generated by cooking in the actual antioxidant content (Schaich et al. 2015). However, although not significant in some samples, Table 1 shows a decrease in antioxidant values after cooking. Amorim et al. (2012) point out that there is a decrease in antioxidant activity in *wakame* and *kombu* seaweeds when they are cooked for 20 min or 1 h at 100°C. Regarding ORAC values, significant differences existed for rehydrated and cooked samples of *U. lactuca,* but not for the other two seaweed species.

In previous studies, the antioxidant capacity has been related to the total content of polyphenols and flavonoids (Rodriguez-Bernaldo de Quiros and Lopez-Hernandez 2021). These compounds act as scavengers of the free radicals produced during oxidation reactions (Uribe et al. 2018). The observed decrease in TPC could be related to the decrease in antioxidant activity (i.e., DPPH values) as shown in Table 1.

The amount of TPC and antioxidant activity cannot be directly compared with those reported in other studies because in our case water was used as the extraction solvent instead of methanol, acetone, or alcohol (García et al. 2020). Moreover, seaweed samples, even those of the same species, vary widely in their content and profile of phytochemicals due to factors such as geographical origin and seasonal, environmental, and physiological variations (Fernández-Segovia et al. 2018).

Identification of volatiles and the effect of cooking on their concentration

The number of volatiles identified for *D. antarctica* was similar to that reported by Moraes et al. (2021) who identified 43 volatiles, but lower than the 93 compounds reported by López-Pérez et al. (2017) for *U. lactuca*. These differences in the number of compounds may be due to the type of subspecies, geographical area of growth, time of harvesting, and storage conditions (Lafeuille et al. 2022). Moreover, the determination of volatiles depends on the analytical procedure where many factors intervene, including the way in which the compounds are extracted, extraction parameters as well as the type of absorbent and adsorbent properties of the different phases. In this investigation, the extraction was carried out using dynamic headspace in water at 37°C in order to imitate the aromas release when eating raw rehydrated or cooked seaweed. Therefore, the extraction of volatiles was not optimized, but it may give an idea of the release of volatile compounds in the oral cavity.

It is possible that prolonged storage of dried seaweeds under vacuum and in darkness may have modified the original composition of chemical compounds and volatiles due to lipid oxidation, antioxidant degradation, and enzymatic reactions, among others. Harrysson et al. (2021) concluded that the quality of lipids, ascorbic acid content and color of oven-dried *Ulva* and *Porphyra* was maintained for up to 150 days of storage in the darkness but after that period they

started to change. In their review, Rodriguez-Bernaldo de Quiros and Lopez-Hernandez (2021) point out that the antioxidant activity of several seaweeds could be reduced during the storage process, but more assays were required. Thus, correlations of sensorial analysis data and volatile content determined a year later have to be taken cautiously and further studies are needed to test the relationship between both sets of data synchronously.

The sensory aroma perception of seaweeds depends on the composition of the mixture of volatile compounds, their concentration and synergism among them as well as on their perception threshold value. Several volatile compounds are well-defined with respect to their contribution to the aroma of several species of seaweeds. Coleman et al. (2022) classified the aromas of seaweeds into three groups. The first group is the volatile compounds generated from the oxidation of polyunsaturated fatty acids. These unsaturated fatty acid derivatives include volatiles such as aldehydes, alcohols, and ketones. A second group corresponds to sulfide compounds (dimethyl sulfide, dimethyl disulfide and methanethiol) that are produced by the degradation of compounds containing sulfides such as dimethyl sulfoniopropionate (DMSP), methionine and taurine. Finally, the third class are nitrogen-containing compounds such as triethylamine (TMA).

Many of the aromatic compounds in seaweeds are aldehydes. These compounds are important due to their low perception threshold (Garicano et al. 2020). The chain length of these molecules affects the threshold of perception. In addition, linear chains of saturated aldehydes contribute to herbaceous and grassy aromas, while unsaturated aldehydes have been suggested to generate green and fishy odors (Sánchez-García et al. 2021; Lafeuille et al. 2022). The three seaweed species analyzed, contained hexanal with herbal, green and fresh notes; heptanal, with a green and citrus aroma; and, octanal with citrus, green and fat aroma. On the other hand, unsaturated compounds such as (2E)-octenal, (E)-2-heptenal and 2-nonenal deliver fishy aromatic notes (Table 2).

Ketones are important compounds in the aroma of marine seaweed due to their abundance and potent odorant activity (Garicano et al. 2020), and their presence is reported for several species of seaweed. Compounds, such as β-ionone, with floral, violet aromas and a woody note, and 1-penten-3-one, which shows fishy, green, mustardy and pungenty aromas, were found in different species such as *Pyropia yezoensis, Undaria pinnatifida, Laminaria digitata*, and have been identified as important contributors to the aroma of these seaweeds (Pan et al. 2004; Garicano et al. 2020; Francezon et al. 2021). Their presence in *Pyropia* spp. and *U. lactuca* may contribute to the aroma of these seaweeds. Another aromatic compound of seaweeds is dimethyl sulfide (DMS) that at high concentrations provides a "sulfur-cabbage" odor with "cooked onions." and "cooked potatoes" tones, while in low concentrations, it is perceived as "fresh seashore" (Francezon et al. 2021). In fact, in this study, DMS was not identified in the brown seaweed *D. antarctica*, nor in the red seaweed *Pyropia* spp. However, DMS was detected in the green seaweed *U. lactuca* and its presence in cooked *Ulva rigida* was associated to herbaceous, grassy and pungent aromas (Sánchez-García et al. 2021).

The volatile compounds present in the seaweeds before and after cooking are presented in Table 3. As previously mentioned, the presence of these compounds during storage and heating in water may be altered due to different factors, including microstructural modifications and reactions such as lipid oxidation or enzymatic breakdown. Moreover, the amount of these compounds may differ from other studies due to the extraction and detection methods.

Some volatile compounds increased after cooking for the three species of seaweeds such as, 2,3-butanedione, 3-methylbutanal, 2-butenal, 1-penen-3-one, pentanal, (E)-2-pentenal, (E)-2-hexenal, (E)-2-heptenal, 2-octenal, 2,4-decadienal. Other volatile components decreased, for example, (E)-2-penten-1-ol and (E)-2-decenal. Lafeuille et al. (2022) found that 2-hexenal and (E)-2-pentenal increased in *Palmaria palmata* during heating, and 2-decenal decreased while Sánchez-García et al. (2021) reported a decrease in several aldehydes related to herbaceous and fresh fish notes when cooking *U. lactuca*, results that are similar to those reported in this study. The concentration of 1-penten-3-ol, (E)-2-penten-1-ol, pentanal, hexanal, and 1-octen-3-ol were considerably higher in *Pyropia* spp. than in *D. antarctica* and *U. lactuca*. Miyasaki et al. (2014) reported that 1-octen-3-ol was a major compound imparting flavor in *Neopyropia* (*Pyropia*) *yezoensis*, and Lafeuille et al. (2022) indicated that hexanal is abundant in seaweeds and gives strong herbal tones.

Some volatile compounds may increase after cooking due to the obliteration of the food matrix by heat and their release from the microstructure (Aguilera 2018; Mateluna et al. 2020). In seaweeds, the matrix scaffold is mostly composed of polysaccharides (and sometimes proteins) that trap or bind volatile compounds making them undetected by analytical methods (Cook et al. 2018).

Correlation between RDA and volatile analysis

A partial least squares regression (PLSR) is an asymmetric method frequently used to understand relationships between two data sets that are possibly correlated. PLSR has been used to relate sensory descriptors in smoothies (Cano-Lamadrid et al. 2020) and chemical and sensory flavor profiles in ice cream (Chung et al. 2003). In this study, PLSR was used to evaluate the relationship between the concentration of volatiles with the RDA results from the sensorial analysis

for the same set of samples (Figueroa et al. 2022). Taste descriptors from the sensorial analysis were incorporated into the PLSR because aromas may interact with some taste components (Noble 1996).

Volatile compounds appear to have great effects on the sensory characteristics of rehydrated and cooked seaweeds, as shown in the PLSR graphs (Fig. 2a and b). RDA results showed that the perceived sensory attributes for caramel, mineral, moldy/earthy, marine, sweet, salty, acid, bitter, and umami were correlated with the average concentration of volatiles for each seaweed analyzed by GC/MS (Table 3). A volatile compound that strongly correlates to caramel aroma is (E)-2-decenal. This compound has been described as having an aroma with hints of orange, dry kombu, and soy sauce (Table 2). *Durvillea antarctica* had the highest amount of this compound and the largest score of caramel flavor in the RDA according to the sensory panel (Figueroa et al. 2022). Thus, (E)-2-decenal may be a key aroma compound in this seaweed. Unsaturated aldehydes butanal, pentanal, hexanal, heptanal and octanal are found in the upper left quadrant of Fig. 2a. These compounds are characterized by conveying a fresh green to oily green aroma (Francezon et al. 2021), correlating with aroma descriptors like earthy/moldy, herbal, and mineral.

Hydrated, uncooked samples are located in the upper quadrants in Fig. 2b, and include different compounds with notes of fish, fresh, green, marine, and cucumber aromas such as 1-octen-3-ol, 2-decadal, 1-penten-3-ol, 2-nonenal, 4-heptenal, and heptanal. On the other hand, compounds such as 2-heptenal, 2,3-butanedione, pentanal and 2,4-heptadienal are associated with fishy, nutty, marine, cooked fish, roasted, and coffee descriptors, are in the lower quadrants, where cooked seaweeds are located. Figure 2b, also suggests that 2,3-butanedione is strongly correlated with the caramel sensorial attribute, the most characteristic aroma of the cooked *D. antarctica* sample.

Conclusions

The identification and quantification of volatile compounds as well as the total phenolic content and antioxidant activity, were determined for rehydrated and cooked Chilean seaweeds *D. antarctica, Pyropia* spp., and *U. lactuca*. TPC and antioxidant activity decreased with cooking, although TPC in *Pyropia* spp. was significantly higher than in the other two species. Sixty-three volatile compounds were identified in the three species of seaweeds. After cooking, the herbal, marine and fresh aromas decreased, while caramel aromas and sweet flavors emerged. Of the volatile compounds, aldehydes were found in the highest concentrations, followed by ketones and alcohols. DMS was only identified in *U. lactuca*. Further studies should evaluate the effects of storage and matrix conditions (e.g., microstructure, particle size) on the contents and extractability of volatile compounds. A correlation was apparent between selected volatile compounds in the rehydrated and cooked seaweeds and some sensory descriptors identified for the same samples. However, to obtain a thorough sensory characterization, more precise aroma descriptors will be required to express the flavor of the seaweeds. Furthermore, the possible interactions between aromas and taste during the sensory evaluation should be resolved. Nevertheless, the information provided may be relevant for researchers and chefs in future developments of products based on seaweeds.

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Data availability The datasets generated during and/or analyzed in the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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