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Fatty acid composition and nutraceutical perspectives of brown seaweeds from the Atlantic coast of Morocco

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Abstract

Seaweeds are currently considered to be a promising source of polyunsaturated fatty acids (PUFA). However, PUFA content and their composition can vary greatly depending on species and environmental conditions. In this study fatty acid (FA) profiles have been investigated in eight brown seaweeds collected from the Atlantic coast of Morocco, namely *Bifurcariabi furcata, Cystoseira humilis, Cystoseira tamariscifolia, Fucus guiryi, Fucus vesiculosus* var. volubilis, *Laminaria ochroleuca, Sacchorhiza polyschides* and *Sargassum muticum*. The results show that all studied seaweeds are rich in PUFA. The highest content (47.67% of total FA) was observed in *C. humilis* and the lowest (26.12%) in *S. polyschides*. Linoleic acid (C18:2) and arachidonic acid (C20:4) were the PUFA detected in higher abundance. The results of total FA content showed that the species collected from the lower intertidal zone *(L. ochroleuca, S. polyschides)* contain less FA compared to those harvested from the middle and upper intertidal zone (*C. humilis, C. tamariscifolia, F. guiryi* and *S. muticum).* The appropriate n-6/n-3 ratio with a high degree of unsaturation as well as low values of atherogenicity andthrombogenicity indices suggests possible utilization for nutraceutical purposes, especially for *Fucus* and *Cystoseira* species.

Introduction

Algal compounds have recently gained considerable interest due to their multiple applications in the food, phycocolloid, cosmetic and pharmaceutical industries (Kadam and Prabhasankar, 2010; Gupta and Abu-Ghannam, 2011; Sartal *et al*., 2012; Peng *et al*., 2015; Schmid *et al*., 2015). Marine macroalgae have been frequently studied for their bioactive compounds in order to prove antitumor, antiviral, antimicrobial, antioxidant and anti-inflammatory properties (Xu *et al.*, 2004; Chatter *et al*., 2009; Vairappan *et al*., 2010), but also for their FA contents (Kumar *et al.*, 2011; Pereira *et al.*, 2012; Vizetto-Duarte *et al*., 2015). Seaweeds are primary producers of polyunsaturated fatty acids (PUFA) that fish obtain through trophic bioaccumulation (Sijtsma and de Swaaf, 2004; Guschina and Harwood, 2006) and represent a promising new source of PUFA. It has been suggested that seaweeds are of potential value as sources of essential fatty acids (FA), which are important in human and animal nutrition (Floreto *et al*., 1996). PUFA not only play a critical role in nutritional properties, but also show anti-inflammatory, anticancer, and anti-obesity, as well as anti cardiovascular disease effects (Plaza *et al.*, 2008), with important nutraceutical and pharmaceutical applications (Colombo *et al*., 2006; Schmid *et al.*, 2013). Brown seaweeds produce large amounts of long chain polyunsaturated fatty acids (LC-PUFA), such as eicosapentaenoic (EPA, 20:5 n-3) and docosahexaenoic (DHA, 22:6 n-3) acids. The FA composition of algae is related to environmental conditions (Kaneniwa *et al*., 1987), which influence the comparisons between algae from different parts of the world (Stefanov *et al*., 1988).

The Moroccan coastline is rich in seaweed biodiversity, both taxonomic and genetic diversity.As an important climatic refugium (Neiva *et al.*, 2016), it is particularly rich in endemic genetic diversity, though climatically threatened, particularly for fucoid and kelp brown seaweeds (Assis *et al*., 2014, 2016; Neiva *et al*., 2015, 2016, Lourenço *et al*., 2016). However, there are no data on the FA profiles of such genetically rich and distinct seaweeds from Morocco. In this context, the present study investigated for the first time the FA composition of the most abundant brown algal species collected from Doukkala shorelines (Atlantic coast of Northwestern Morocco).

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Materials and Methods

Sampling and seaweeds preparation

Samples of eightbrown seaweed (Ochrophyta, Phaeophyceae) species *(Bifurcaria bifurcata, Cystoseira humilis, Cystoseira tamariscifolia, Fucus guiryi, Fucus vesiculosus* var. volubilis, *Laminaria ochroleuca, Sacchorhiza polyschides* and *Sargassum muticum)*, were collected in October 2015 during low tide from two locations on the Northwestern Atlantic coast of Morocco. *F. vesiculosus* var. volubilis, was sampled from the lagoon of Oualidia (32°44'52.7" N 9°01'26.8" W), and all other species were collected from one site in the South of El Jadida city (33°14'43.7" N 8°32'35.2" W). For each species, 5 thalli were randomly sampled from intertidal pools of the same tide level, washed with running tap water and subsequently with deionized water to eliminate residues, after which they were freeze-dried, ground to powder and mixed. From this mixture 3 sub-samples were taken for chemical analyses.

Extraction of fatty acid methyl esters (FAME)

FAME of the studied seaweeds were extracted according to the protocol described by Pereira *et al.* (2012) modified from Lepageand Roy (1984). An aliquot of 1.5 mL of methanol/acetyl chloride (20:1, v/v) was added to 50 mg of powdered algal biomass. Afterwards, 1 mL of hexane was added and heated at 90°C for 1 hour. As soon as the samples were placed on ice, 1 mL of distilled water was added to the mixture, after which the organic phase was removed and dried with anhydrous sodium sulfate. Extracts were filtered and the solvent was evaporated using nitrogen gas. Extraction was performed in triplicate for each sample.

Determination of FAME profile by GC-MS

Analysis of FAME was performed as described by Pereira *et al.*, (2012) on a Bruker GC-MS (Bruker SCION 456/GC, SCION TQ MS) equipped with a ZB-5MS (30 m x 0.25 mm internal diameter, 0.25 µm film thickness, Phenomenex). FAME were identified and quantified using a commercial standard (Supelco 37 Component FAME Mix, Sigma-Aldrich, Sintra, Portugal). All analyses were performed in triplicate.

Indexes of lipid quality

The unsaturation index (UI) was calculated by multiplying the percentage of each FA by the number of double bonds followed by summing up their contributions (Poerschmann *et al*., 2004). The atherogenic index (AI) andthrombogenic index (TI) were calculated according to the following equations (Garaffo *et al*., 2011):

$$AI = \frac{(4\times C14:0)+C16:0+C18:0}{\sum MUFA+\sum PUFA\text{-}n6+\sum PUFA\text{-}n3} \quad (1)$$

$$TI = \frac{C14:0+C16:0+C18:0}{0.5MUFA+0.5PUFA\text{-}n6+3PUFA\text{-}n3+PUFA\text{-}n3/PUFA\text{-}n6} \quad (2)$$

Statistical analysis

To test for significant differences in FA profiles among seaweeds species permutational multivariate analysis of variance (PERMANOVA) with one fixed factor (species) was performed in Primer 6. PERMDISP was used to test for homogeneity of variances among species and Monte Carlo simulations (999 replicates) were conducted to identify pairwise differences among species. Multivariate FA profiles across seaweed species were visualized in multidimensional scaling (MDS) plots using square root transformed data and Bray-Curtis similarities.

Results and Discussion

Total fatty acid methyl esters (FAME) concentration

Levels of total FAME present in all tested seaweeds ranged from 2.36 to 8.57 µg/g of dry weight with the highest concentration detected in *F. vesiculosus* var. volubilis followed by *C. tamariscifolia* while the kelp species (*S. polyschides* and *L. ochroleuca*) contained the lowest concentrations (Figure1). According to the obtained results, we suggest that the species collected from the lower intertidal zone *(L. ochroleuca, S. polyschides)* contain less FA while those harvested from the middle and upper intertidal zone (*Cystoseira* species, *F. guiryi*, and *S. muticum*) provide significant concentrations of FA. The obtained results can be explained by the variation of environmental conditions during low tide, which leads to abiotic stresses for seaweeds (collected from the middle and the upper intertidal zone) and consequently affects the FA contents.

The analyzed samples showed lower total FA concentration compared with those collected from other countries (Rodrigues *et al*., 2015; Vizetto-Duarte *et al*., 2015). This variation could be due to several factors. Previous reports have revealed that the FA content and composition can vary spatially and temporally (Hay and Villoula, 1993; Colombo *et al*., 2006). According to Nelson *et al.* (2002), total macroalgal lipid content increased during winter and spring and declined in summer. Temperature has a large effect on the individual FA in seaweed cell membranes (Peng *et al.*,2015) where at low temperatures FA contents increase (Phleger, 1991) and algae from cold waters are commonly richer in PUFA in comparison to those from warm waters

Table 1. Fatty acid composition of the studied Phaeophycean species.

Fatty acid(%)	*Cystoseira tamariscifolia*	*Cystoseira humilis*	*Fucus vesiculosus*	*Sargassum muticum*	*Bifurcaria bifurcata*	*Fucus guiryi*	*Laminaria ochroleuca*	*Sacchorhiza polyschides*
C14:0	4.42±0.61	1.57±0.24	6.8±0.89	1.96±0.25	3.97±0.53	6.08±0.90	3.41±0.19	4.44± 0.85
C15:0	nd	nd	0.26±0.26	nd	nd	0.35±0.34	nd	0.75±0.75
C16:0	34.55±4.0	29.22±1.68	20.97±0.79	32.62±2.81	34.1±4.26	21.61±2.09	41.56±8.52	43.15±12.01
C18:0	1.39±0.14	1.26±0.09	2.23±1.55	0.31±0.30	nd	1.69±0.25	3.28±0.78	3.23±0.81
C20:0	0.35±0.23	nd	0.89±0.07	nd	nd	0.4±0.40	0.5±0.5	1.06±0.67
C22:0	nd	nd	0.26±0.25	0.34±0.34	nd	nd	nd	nd
C24:0	1.37±0.48	1.39±0.08	2.33±0.48	0.42±0.47	nd	1.7±0.85	nd	nd
∑SFA	**41.9**	**33.44**	**33.74**	**35.65**	**38.07**	**31.83**	**48.75**	**52.63**
C15:1	nd	nd	0.24±0.24	nd	nd	nd	nd	nd
C16:1	7.14±1.13	2.86±0.05	2.18±0.15	12.47±0.72	5.75±0.44	0.71±0.70	5.62±2.81	5.07±3.1
C18:1	13.69±1.9	16.03±1.20	23.34±1.84	13.8±0.78	20.89±1.5	21.87±2.96	15.78±3.21	16.16±2.90
C20:1	nd	nd	nd	1.54±0.19	nd	nd	nd	nd
C22:1	nd	nd	0.43±0.21	1.88±0.94	nd	1.04±0.05	nd	nd
C24:1	nd	nd	2.34±0.32	nd	nd	3.49±0.11	nd	nd
∑MUFA	**20.83**	**18.71**	**28.53**	**29.69**	**26.64**	**27.11**	**21 .4**	**21.23**
C18:2 n-6	6.35±0.86	10.82±0.74	10.2±1.17	10.14±1.29	4.64±0.08	6.29±0.69	8.62±2.92	7.49±3.25
C20:2 n-6	nd	1.34±0.13	0.19±0.18	nd	nd	nd	nd	nd
C18:3 n-6	nd	3.48±0.28	0.3±0.29	nd	nd	0.34±0.34	nd	nd
C20:3 n-6	2.08±0.23	2.14±0.18	0.72±0.36	1.3±0.45	nd	0.69±0.68	nd	nd
C20:4 n-6	24.06±2.90	18.1±1.11	18.54±1.88	16.13±1.38	24.25±1.66	22.68±1.98	13.24±3.92	12.28±4.36
C20:5 n-3	4.6±0.49	11.79±0.91	7.77±0.49	7.09±2.42	6.4±0.72	11.05±0.62	8±3.01	6.35±3.22
∑PUFA	**37.09**	**47.67**	**37.72**	**34.66**	**35.29**	**41.05**	**29.86**	**26.12**

Data are represented as a percentage of the total FAME content and presented as mean values of triplicate samples ± SD (n =3); nd: not detected.

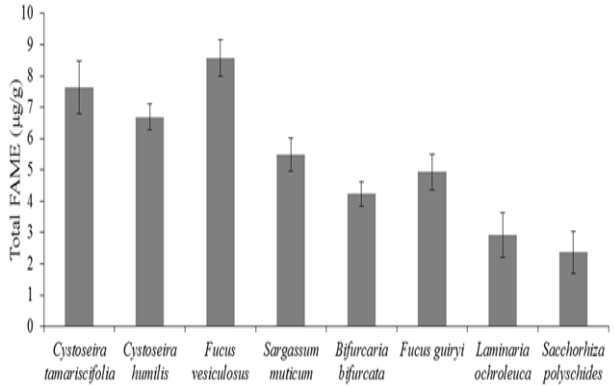

Figure1. Total FAME concentration of the different studied brown seaweed species. Bars represent means ± SD (n=3)

(Colombo *et al.*, 2006).

Fatty acid profiles

Fatty acid profiles of the studied seaweeds are shown in Table 1. Saturated FA (SFA) varied from 31.83% of total FAME in *F. guiryi* to 52.63% in *S. polyschides* and were similar to those obtained previously for other brown seaweeds collected from different regions where total SFA concentrations ranged from 30 to 66% (Li *et al*., 2002, Pereira *et al.*, 2012). The main SFA in all species was palmitic acid (C16:0). Similar results were found in most studied Phaeophyceae (Khotimchenko *et al*., 2002; Pereira *et al*., 2012; Rodrigues *et al.*, 2015). Concerning unsaturated FA, monounsaturated FA (MUFA) concentrations ranged between 18 and 29.6%, where oleic acid (C18:1) was the major MUFA in all species with a maximum of 23.34% in *F. vesiculosus.* Oleic acid is one of the main FA in brown seaweeds (Kaneniwa *et al.*, 1987; Fleurence *et al*., 1994; Silva *et al.*, 2013; Vizetto-Duarte *et al.,* 2015). It can exhibit a high content in some particular brown seaweeds like Macrocystisintegrifoliawith more than 40% of total FA (Khotimchenko *et al.*, 2002). The most characteristic PUFA in all studied species were arachidonic (AA; C20:4) and linoleic (LA; C18:2) acid, in agreement with previous studies (McCauley *et al.,* 2014; Najdek *et al*., 2014). AA content varied from 12.28% to 24.25% of total FA, while LA exhibited levels between 4.64% and 10.68%. The studied species showed higher contents of AA compared to previous reports. For example AA in C. tamariscifoliawas about 24.06%, whereas the levels of this FA did not exceed 15.4% of total FA in the same species collected in Portugal and western Ireland (Schmid *et al*., 2013; Vizetto-Duarte *et al*.,2015). EPA reached a value of 11.79% of total FAME in *C. humilis.* This FA is considered to be one of the critical metabolic precursors of eicosanoids and prostanoids, mediating numerous physiological and biochemical processes (Miyashita *et al.*, 2012). Notably, *S. muticum* was the only species among all those tested that containedeicosenoic acid (C20:1) with a concentration of 1.54% of total FAME. This FA is an intermediate in the human metabolic pathway from palmitic acid (C16:0) to nervonic acid (C24:1). The latter is important in the production of cerebrosides, characteristic components of nerve membranes and the "white substance" of the brain (Guy and Neil, 2002; Carvalho *et al*., 2006). The contents of eicosenoic acid in this study are higher than that reported by Rodrigues *et al*. (2015) for *S. muticum* (0.58%) harvested from the Central West Portuguese coast. FA profiles are highly variable between

Table 2. PERMANOVA analysis of pairwise differences in fatty acid composition of all studied seaweeds based on 999 Monte Carlo simulations

Pairwise species comparison	P-value (MC)
Cystoseira tamariscifolia vs. *Cystoseira humilis*	0.010
Cystoseira tamariscifolia vs. *Fucus vesiculosus*	0.008
Cystoseira tamariscifolia vs. *Sargassum muticum*	0.041
Cystoseira tamariscifolia vs. *Bifurcaria bifurcata*	0.053
Cystoseira tamariscifolia vs. *Fucus guiryi*	0.012
Cystoseira tamariscifolia vs. *Laminaria ochroleuca*	0.218
Cystoseira tamariscifolia vs. *Sacchorhiza polyschides*	0.222
Cystoseira humilis vs. *Fucus vesiculosus*	0.003
Cystoseira humilis vs. *Sargassum muticum*	0.011
Cystoseira humilis vs. *Bifurcaria bifurcata*	0.002
Cystoseira humilis vs. *Fucus guiryi*	0.009
Cystoseira humilis vs. *Laminaria ochroleuca*	0.042
Cystoseira humilis vs. *Sacchorhiza polyschides*	0.038
Fucus vesiculosus vs. *Sargassum muticum*	0.014
Fucus vesiculosus vs. *Bifurcaria bifurcata*	0.019
Fucus vesiculosus vs. *Fucus guiryi*	0.168
Fucus vesiculosus vs. *Laminaria ochroleuca*	0.046
Fucus vesiculosus vs. *Sacchorhiza polyschides*	0.085
Sargassum muticum vs. *Bifurcaria bifurcata*	0.062
Sargassum muticum vs. *Fucus guiryi*	0.008
Sargassum muticum vs. *Laminaria ochroleuca*	0.099
Sargassum muticum vs. *Sacchorhiza polyschides*	0.071
Bifurcaria bifurcata vs. *Fucus guiryi*	0.023
Bifurcaria bifurcata vs. *Laminaria ochroleuca*	0.308
Bifurcaria bifurcata vs. *Sacchorhiza polyschides*	0.346
Fucus guiryi vs. *Laminaria ochroleuca*	0.055
Fucus guiryi vs. *Sacchorhiza polyschides*	0.060
Laminaria ochroleuca vs. *Sacchorhiza polyschides*	0.856

species and strongly influenced by environmental conditions such as light intensity, salinity, and water temperature (Stengel *et al.*, 2011), and interactions between such factors can also contribute to variations in PUFA contents (Floreto *et al*., 1993; Floretoand Teshima, 1998). It has been suggested that responses of marine plants to environmental conditions involve the excess production of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet -}$) and hydroxyl radical (OH−) (Burritt *et al*., 2002). ROS can also generate oxygenated PUFA (Ox-PUFAs) defending against oxidative stress (Foyer and Noctor, 2005). The higher content of PUFA could be an adaptive strategy for maintaining the requisite of greater membrane fluidity needed for the diffusion of the lipophilic compounds and to stabilizing the protein complexes of photosystem II to withstand oxidative stress (Khotimchenkoand Yakovleva, 2005). Free FA like C18:2(n-6) and C18:3(n-6) have also been shown in the defense reactions against hyper salinity-induced oxidative stress (Kumar *et al.*, 2010). Phleger (1991) explained that low temperatures would increase the level of PUFA in polar lipids that would reduce melting points and maintain lipids in a liquid state for normal protoplasmic viscosity. Schmid *et al*. (2013) suggested that life cycle variations with alternation of vegetative and reproductive stages can also affect the PUFA contents of seaweeds.

Our results showed a significant difference in overall FA profile among seaweeds species (p = 0.001; permutational multivariate analysis of variance).The FA profile of *C. tamariscifolia* was

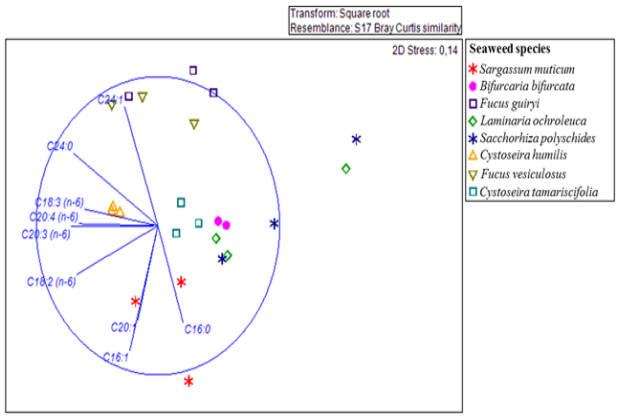

Figure2. Multivariate dimensional scaling plot comparing similarities of fatty acid profiles of thestudied seaweeds based on square root transformed Bray Curtis similarities. Vectors show the correlations with each fatty acid detected; the circle indicates the maximum correlations coefficient of 1.

significantly different from *F. vesiculosus*, but not different from *S. polyschides, L. ochroleuca* and *B. bifurcata* (Table 2) while *C. humilis* had a FA profile significantly different from all other studied species. The FA composition in *F. vesiculosus* was similar to that of *F. guiryi,* whereas the latter species was strictly different from *S. muticum* and *C. humilis*. The kelp *L. ochroleuca* and *S. polyschides* have similar FA profiles, but significantly different to those of *F. vesiculosus* and *C. humilis.* The PERMANOVA outcomes correspond with the clustering in the MDS plot (Figure 2).

Nutritional and pharmaceutical values

Algal lipids, especially those from brown seaweeds, have drawn increased interest due to several health benefits they provide (Miyashita *et al*., 2012). Among the vast array of lipidic molecules present in macroalgae, FA play an important role in human diets, especially the essential FA (Plaza *et al*., 2008) α-linolenic acid (C18:3n-3; ALA) and LA that need to be supplied through the diet (Hamid *et al*., 2015). In this study, all studied species showed high amounts of SFA and PUFA, where PUFA/SFA ratios higher than 1 (Table 3) were detected in *C. humilis* (1.42), *F. vesiculosus* var. volubilis (1.11) and *F. guiryi* (1.28). The other species showed PUFA/SFA ratios close to 1, except *L. ochroleuca* and *S. polyschides*, which demonstrated lower PUFA/SFA ratios (*L. ochroleuca* = 0.61; *S. polyschides*= 0.49). In general, all tested species exhibited PUFA/SFA ratios around or higher than the ratio recommended by the Committee on Medical Aspects of Food Policy in Great Britain (CMAFP, 1994). In all analyzed species, n-6/n-3 PUFA ratios varied from 2.71 to 7.06, corresponding to the range of values discussed in the literature

Table 3. Nutritional indices calculated for the studied brown seaweed species

	Cystoseira tamariscifolia	*Cystoseira humilis*	*Fucus vesiculosus*	*Sargassum muticum*	*Bifurcaria bifurcata*	*Fucus guiryi*	*Laminaria ochroleuca*	*Sacchorhiza polyschides*
PUFA/SFA	0.88	1.42	1.11	0.97	0.97	1.28	0.61	0.49
∑ n-3	4.6	11.79	7.77	7.09	7.09	11.05	8	6.35
∑ n-6	32.49	35.88	29.95	27.57	27.57	30	21.86	19.77
∑ n-6/∑ n-3	7.06	3.04	3.85	3.88	4.51	2.71	2.73	3.11
UI	159.01	191.42	165.14	153.84	164.92	188.75	131.6	117.08
TI	0.17	0.04	0.08	0.1	0.11	0.05	0.17	0.25
AI	0.92	0.55	0.76	0.63	0.63	0.69	1.14	1.35

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UI, unsaturation index; AI, atherogenic index; TI, thrombogenic index.

(Machado *et al*., 2004;Ginneken *et al*., 2011;Pereira *et al.*, 2012). The World Health Organization (WHO) has, however, set recommended daily intakes for n-6 and n-3 FA and, provided that these are met, there is no longer a recommended n-6/n-3 intake ratio (FAO, 2010). Nonetheless, Dawezynski *et al*. (2007) highlighted that European diets are rich in n-6. According to Abreu *et al*. (2014) the advised n-6/n-3 ratio for a healthy diet is 5/1, but most western food products have a 15–17/1 ratio, suggesting a deficiency in n-3 PUFA. It is also suggested that the addition of macroalgae to the diet could be highly beneficial on account of their high n-3 content (van Ginnekin *et al*., 2011; Pereira *et al.*, 2012).

The unsaturation index (UI) showed that the investigated seaweeds were rich in unsaturated FA with a UI varying from 117 *(S. polyschides)* to 191.42 (*C. humilis*; Table 3). Similar values were documented for other brown seaweeds in previous studies (Kumari *et al*., 2013; Vizetto-Duarte *et al.,* 2015). The values of thrombogenic index (TI) ranged from 0.04 to 0.25, whereas the atherogenic index (AI) varied from 0.55 to 1.35. These low AI and TI values are in accordance with those reported by Kumar *et al*. (2011). According to Rajapakseand Kim (2011), seaweeds could be a good source of health-promoting PUFA compared to the other foods derived from plant and animal sources. Concerning pharmaceutical uses, the long chain unsaturated and some FA are claimed to prevent heart disease and have anticancer, anti-obesity, and antioxidant properties (Li and Watkins, 2006). The marine n-3 PUFA have several biological effects: antihypertension, anti-inflammation and immune-regulation (Fleurence *et al.*, 1994; Khan *et al*., 2007; Plaza *et al*., 2008). It has been suggested that n-3 PUFA, EPA and DHA in particular, reduce blood pressure, plasma triacylglycerols, and cholesterol, together with increased blood coagulation time. They also alleviate certain diseases, such as blood vessel disorders and inflammatory conditions (Kim *et al.*, 2011). The essential FA, ALA and LA provide eicosanoids, which regulate diverse body functions (Zhou and Nilsson, 2001; Meschino, 2007). The n-6 PUFA and their derivatives, mainly AA, play an important role in biological systems, such as the immune response, thrombosis, and brain function (Hoffman *et al.*, 2009; Le *et al*., 2009). The high amount of palmitic acid in analyzed samples is interesting since it has been suggested to have preventive effects on metabolic dysfunctions due to retinoid X receptor (RXR) and peroxisome proliferator-activated receptor-alpha (PPAR-α) agonist activity (Hellgren, 2010).

Conclusion

In the present study, we describe FA profiles of the most common brown algal species on the Atlantic coast of Morocco, a hotspot of genetic diversity. The obtained results demonstrate that both FA contents and profiles vary significantly among species. All examined species had a high content of PUFA, which varied from 26.12% of total FA in *S. polyschides* to 47.67% in *C. humilis*. Tested species showed an appropriate ratio of n-6 /n-3 PUFA, with low AI and TI values. The studied seaweeds might represent a potential source of FA for biotechnology and a useful source of food supplement.These interesting results indicated that future work should investigate effects of environmental conditions on FA contents and composition, leading us to a novel study assessing seasonal and spatial variation of macroalgae chemical composition.

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