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Effects of sun/open-air drying and oven-drying on chemical composition and functional properties of dried *Ulva intestinalis*

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ABSTRACT

Seaweeds are important sources of macro- and micronutrients used as food, minerals, and as important sources of many bioactive compounds. Due to high water content, seaweeds are highly perishable and should be immediately subjected to drying process to increase their shelf-life. The present study aimed to investigate the effect of oven-drying in comparison with conventional sun/open-air drying on the chemical composition, functional properties and fatty acid profile of *Ulva intestinalis.* The results showed significantly lower water holding capacity in sun/open-air dried samples (8.83 mL g^{-1}) compared to oven-dried ones (10.83 mL g^{-1}; $p > 0.05$). There was no significant difference between oil holding capacity, emulsifying activity and swelling capacity of dried *U. intestinalis* and two different drying methods ($p > 0.05$). The EPA and DHA contents in sun/open-air and oven-dried samples were 1.282 and 0.806 (g/100g for EPA), while 2.377 and 1.972 (g/100g for DHA), respectively. N-3/n-6 ratio in the above-mentioned samples were 0.959 and 0.340, respectively. Based on the results, oven-drying method is suggested for drying this algae regarding to higher water holding capacity and higher content of highly unsaturated fatty acids.

Keywords: Green algae, Drying methods, Functional properties, Water holding capacity, Fatty acid profile.
Article type: Research Article.

INTRODUCTION

Marine macroalgae that commonly named as seaweeds, are classified according to their color, chemical and nutritional composition (Muraguri *et al*. 2016). They are considered as good sources of food, minerals, phycocolloids and bioactive substances (Mehdipour *et al*. 2014; Muraguri *et al*. 2016). They have been consumed as food for centuries due to their unique protein, carbohydrate, lipid, fiber, and micronutrient contents, so they have been valued as healthy foods (Mehdipour *et al*. 2014, Muraguri *et al*. 2016, Silva *et al*. 2019). Recently, these resources have attracted a great deal of attention for extraction many functional and bioactive components for use in food industries such as food supplement and food enrichment, as well as in non-food industries such as medical, pharmaceutical, cosmetic, paper and textile industries and also in producing fertilizer, fungicide, herbicides and etc. (Muraguri *et al*. 2016, Silva *et al*. 2019). The extraction of different kinds of carbohydrates such as monosaccharides, the sulphated and non-sulphated polysaccharides, hydrocolloids from seaweeds for their attractive functional, physicochemical and bioactive properties are growing, so, many studies are focused on this area (Silva *et al*. 2019). In addition to having high quality and widely applicable protein and carbohydrates, seaweeds have around 1-6% (of dry weight) lipids exhibiting different fatty acid profiles based on seaweed kinds and their environmental condition (Mehdipour *et al*. 2014) that could be considered as important source of long-chain fatty acids in the future. *Ulva intestinalis* (*Enteromorpha intestinalis*), a green macroalgae in the phylum Chlorophyta, is widely found in marine and brackish waters around the world (Afanasyev *et al*. 2016; Srikong *et*

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al. 2017). It has been reported from the Caspian Sea and Persian Gulf (Mehdipour *et al*. 2014; Afanasyev *et al*. 2016; Pirian *et al*. 2017). In *U. intestinalis*, thallus is usually tubular, unbranched, swollen, light or dark green in color and usually quite soft. Height of thallus is from a few centimeters to 30-40 cm, and its width is 0.5-5.0 cm (Afanasyev *et al*. 2016). Actually, the seaweeds have high content of water, making them highly perishable to marketing. In this regard, drying is the best way to maintain their quality during storage and for longer marketing for food, cosmetics and other uses (Silva *et al*. 2019). The main drying methods used for seaweed industry are sun/open-air drying and forced air tunnels. In addition, oven- and freeze-drying methods are also used in many studies in experimental scale. These studies reported different effects on physicochemical, functional properties and bioactivity of different seaweeds based on drying condition (Tello-Ireland *et al*. 2011; Uribe *et al*. 2018; Silva *et al*. 2019). In this context, the present study was intended to investigate the impact of oven-drying in comparison with sun/open-air drying methods on functional properties and fatty acid profile of *U. intestinalis*.

MATERIALS AND METHODS

Raw material and preparation

The fresh *U. intestinalis* was collected from coastal line of Bandar Anzali (Guilan, Iran) in June-July 2019. The epiphytes were thoroughly removed from fresh plants by rinsing three times with seawater, then placing in plastic bag. On the arrival to laboratory, the seaweed samples were again washed with tap water for 15 min and finally with distilled water to remove the remains of sand and associated fauna. The seaweed after accurate weighing was divided in two homogenous groups. One group was dried in the free air (in shadow with the mean temperature of 27 °C) and the second group at 45 °C in an electrical oven (Behdad, Iran). Afterward, the dried samples of the two methods were weighed again and milled in a mechanical grinder (MX-798S, National, Malaysia) to obtain a fine and homogenous powder, then stored in zip lock plastic bags and kept in a refrigerator for further analyses.

Chemical composition analysis

Crude protein was determined by Kjeldahl's method. The protein was calculated using a nitrogen conversion factor of 6.25 (Yaich *et al*. 2011). The crude fat was extracted from algal samples using the Soxhlet apparatus with n-hexane as the solvent (Carvalho *et al*. 2009). Total ash was determined by calcination in electric muffle furnace at 550 °C until a constant weight (Muraguri *et al*. 2016). The moisture content (dry matter, DM) was determined by oven-drying method at 105 °C (Muraguri *et al*. 2016).

Functional properties

Swelling capacity (SWE): Swelling capacity of samples was analyzed by the method of Yaich *et al*. (2011). So that, 10 mL distilled water was added to 1.0 g dried sample, in a 10-mL measuring cylinder, and then the mixture was stirred vigorously and left to stand for 18 h at room temperature. The SWE volume was measured and expressed as cm^3 swollen sample/g of sample. Water holding capacity (WHC): WHC were determined according to Muraguri *et al*. (2016). The maximum amount of water retained by 1 g sample under low speed centrifugation for 10 min at 25 °C were expressed as grams of water bound per gram of the sample on a dry basis. Oil holding capacity (OHC): This analysis was done according to Yaich *et al*. (2011).

The dried samples (0.5 g) were mixed with 6 mL corn oil in pre-weighted centrifuged tubes, stirred and left for 30 min at room temperature. Afterward, the sample was centrifuged for 30 min at 2500 g, then supernatant oil was removed and measured. The OHC of seaweed samples was expressed as gram oil held by 1 g of sample on dry weight basis. Emulsifying activity (EA): The emulsion activity was determined using the method of Muraguri *et al*. (2016). Two grams of dried sample were dissolved in 20-mL distilled water and vortexed for 10 min. At the 5th minute, 20-mL corn oil was added continuously once stirring. The emulsion was centrifuged at 2100 rpm for 10 min at 25 °C. The emulsified layer volume was recorded and expressed as %.

Fatty acid analysis

For fatty acid analysis, seaweed oil was extracted using Folch *et al*. (1957). Lipid samples were converted to their constituent fatty acid methyl esters by the method of Firestone *et al*. (1998). So that, 5 mL of 2% sodium hydroxide methanol solution was added to extracted oil. Then the tube was sealed, shacked vigorously and then placed in water-bath for 10 min at 100 °C. After cooling, 3 mL BF3 was added to mixture and again placed in water-bath for 2-3 min. Thereafter, 1 mL N hexane was added to the mixture, shacked and then 1 mL saturated salt solution (300 g NaCl in 1000 mL distilled water) was added to the mixture and after vigorous shaking, it was stand to phase separating. The top layer, FAME was then taken for analysis. Analysis of fatty acid methyl esters was performed by a Philips GC with a capillary column and quantified by FID detector. The GC condition was as follow: Injection port temperature was 250 ºC and FID temperature was 300 ºC. The oven temperature program was set at an initial temperature of 160 ºC for 5 min, then raised to 180 ºC at 20 ºC min^{-1}, held for 10 min, again was raised to 200 ºC at 1 ºC min^{-1}, held for 1 min, then temperature was increased to 230 °C at 30 ºC min^{-1} and held for 5 min. The size of the sample injected for each analysis was 0.2 µL. The carrier gas was helium. The samples were manually injected into the GC port. Compounds were identified by comparison with the retention time of known standards.

Statistical analysis

Data statistical analysis was performed using SPSS software (version 21). Chemical composition and functional properties measurements were replicated three times and fatty acid profile analysis were done in duplicate. The normality of data was measured using Kolmogorov- Smirnov test before analysis. Significant difference between two methods of drying was determined using t-test. The significance of results was at 5%. All data are expressed as mean ± SD.

RESULTS AND DISCUSSION

Drying time and chemical composition

The drying procedures of *U. intestinalis* seaweed in sun/open air and oven methods took around 240 and 120 h, respectively. The samples were checked twice a day and turned upside down to uniform drying. The drying process yield was 7.997 and 6.478 % for sun/open air and oven methods, respectively. The average compositions of dried *U. intestinalis* by two different drying methods are shown in Table 1.

The protein content found in the present study was relatively high (19.42 and 15.52 % of dry weight by sun/open air and oven methods, respectively). This amount was in the range of values reported for protein content by Mehdipour *et al*. (2104) and Pirian *et al*. (2017) for *U. intestinalis* (*E. intestinalis*) in the Caspian Sea and the Persian Gulf, respectively. The protein content in *Ulva* species was reported between 10-26 % by Fleurence (1999). Variations in the protein content of seaweeds can be attributed to the species, seasonal periods, location and environmental conditions (Yaich *et al*. 2011; Mehdipour *et al*. 2014). The lipid content found in the present study was relatively high (7.14 and 7.10 % of dry weight by sun/open air and oven methods, respectively). This amount was in accordance with the value reported by Yaich *et al*. (2011) in *U. lactuca*. However, this quantity was very higher than those reported in *U. intestinalis* by Mehdipour *et al*. (2014) and Pirian *et al*. (2017). The variations in lipid contents could be attributed to either species types or environmental factors and method used to extract oil (Kumari *et al.* 2010; Yaich *et al*. 2011). The ash content of *U. intestinalis* in the present study was lower than those reported by Mehdipour *et al*. (2014) and Pirian *et al*. (2017) in *U. intestinalis*. However, the ash content of this species was comparable to that of some seaweed species of the same genus, i.e. *U. lactuca* (Ratana-arporn & Chirapart 2006; Yaich *et al*. 2011).

Table1. Chemical composition of dried *U. intestinalis* by sun/open-air and oven method

Treatment	**Dry matter (%)**	**% w/w on dry basis**		
		Protein	**Lipid**	**Ash**
Sun/open air drying	91.17 ± 0.20 ^{a}	19.42 ± 0.20 ^{a}	7.14 ± 0.49 ^{a}	16.17 ± 0.15 ^{b}
Oven drying	89.16 ± 0.15 ^{b}	15.51 ± 0.14 ^{b}	7.10 ± 0.19 ^{a}	19.03 ± 0.09 ^{a}

Values were expressed as the mean ± standard deviation (n = 3). Mean values in column with different small letters indicate significant differences ($P < 0.05$).

In the present study, the drying method exhibited significant effect on yield and also chemical composition of *U. intestinalis*, so that, the yield and protein contents were significantly higher in sun/open air drying method than oven-drying method ($P < 0.05$). The lipid content was slightly higher in sun/open air method. Instead, ash and moisture contents were significantly higher in oven-drying method compared to sun/open air-drying method ($p < 0.05$).

Functional properties

The functional properties of dried *U. intestinalis* by two different drying methods are shown in Table 2.

Table 2. Functional properties of dried *U. intestinalis* by sun/open-air and oven method

Treatment	Functional properties			
	SWE (cm^3/g)	**WHC (g/g)**	**OHC (g/g)**	**EA (%)**
Sun/open air drying	6.77 ± 0.12 ^{a}	8.83 ± 0.21 ^{b}	7.13 ± 0.38 ^{a}	55.61 ± 0.19 ^{a}
Oven drying	6.64 ± 0.06 ^{a}	10.83 ± 0.05 ^{a}	7.09 ± 0.35 ^{a}	56.89 ± 0.80 ^{a}

Values were expressed as the mean ± standard deviation (n=3). Mean values in column with different small letters indicate significant differences ($P < 0.05$).

Both swelling capacity (SWC) and water holding capacity (WHC) are used for hydration properties of seaweed (Yaich *et al*. 2011). In the present study, the average swelling capacity contents of *U. intestinalis* (6.77 and 6.64 cm^3/g for alga powder in sun/open air and oven methods, respectively) were lower than those reported by Pirian *et al*. (2017) for the same species, However, notably it was higher than that of *U. lactuca* reported by Yaich *et al*. (2011) and Wong *et al*. (2000). The water holding capacity of *U. intestinalis* in the present study was between 8.83 in sun/open air treatment and 10.83 in oven-drying treatment (g water/g dry matter). These quantities were lower than that reported by Pirian *et al*. (2017) for same species. This could be related to effect of temperature in drying process as reported by Yaich *et al*. (2011), who observed that elevation in drying temperature results in higher water holding capacity in algae. Physiochemical and functional properties of seaweeds are related to their dietary fiber and also their protein. Seaweeds were rich in polysaccharide, dietary fiber, particularly in the soluble form and also proteins that are closely related to the cell wall polysaccharides, which may play a role in the physicochemical properties, such as water holding capacity (Darcy-Vrillon 1993; Fleurence, 1999; Yuan *et al*. 2018). Actually, polysaccharides play an important role in functional properties such as water holding capacity, oil holding capacity and emulsion activity in algae (Yuan *et al*. 2018). In addition, protein conformations and the variations in the number and nature of the water binding sites on the protein molecules are also affective (Fleury *et al*. 1991). These functional properties increased the attention to use seaweed in food industry for improving the physical and structural properties of food (Elleuch *et al*. 2011).

The oil holding capacity of *U. intestinalis* were around 7.00 g/g dry matter, which is notably higher than that reported for the same species by Pirian *et al*. (2017) and other species of this genus (Yaich *et al*. 2011). The oil holding capacity was slightly lower in oven-drying than sun/open air drying method. Similar results were reported by Yuan *et al*. (2018) who observed that elevation in the drying process temperature results in the lower oil holding capacity. The mechanism of OHC is mainly due to the physical entrapment of oil by capillary attraction. Moreover, the hydrophobicity of proteins also plays a major role in fat absorption. It has also reported that the OHC of seaweed are also related to the particle size, overall charge density and hydrophilic nature of the individual particles, total content of protein and dietary fiber (Fleury *et al*.1991, Wong *et al*. 2000). The emulsion activities were 55.61 and 56.89 % for *U. intestinalis* in the sun and oven-drying methods, respectively. Muraguri *et al*. (2016) reported higher EA for seaweed species from 59.19 to 75.69 %. The emulsifying activity of seaweeds is related to their phycocolloids and can be used in food processing as emulsifier, thickener and stabilizer agents (Muraguri *et al*. 2016). Finally, the drying method exhibited not significant effect on functional properties of *U. intestinalis* in present study except in water holding capacity.

Fatty acid profile

Table 3 depicts the fatty acids compositions of *U. intestinalis* in the sun/open air and oven-drying treatments. A total of 18 fatty acids were detected using GC. There were some differences in the fatty acid profiles between the two treatments. In the both treatments, SFA was the most abundant group of fatty acids followed by MUFA and PUFAs. A similar pattern of fatty acids for seaweeds were reported by Yaich *et al*. (2011) for *U. lactuca*, by Mehdipour *et al*. (2014) for the same species in some sampling stations and by Muraguri *et al*. (2016) for *U. fasciata*. It is well documented that, in addition to the environmental and the biological factors, processing has detrimental effect on fatty acid composition (Sigurgisladóttir & Pálmadóttir 1993; Zakipour Rahimabadi & Dad 2012; Yaich *et al*. 2011).

In sun/open air-drying treatment, the quantities of fatty acids in descending order were C6:0 >C16:0 > C18:1 (n-9 c)> C8:0 >C14:0, while in oven-drying treatment, were C18:1 (n-9 c)> C16:0> C6:0> C18:2 (n-6 c) and C8:0.

This showed that during drying treatment in sun/open air the contents of medium- and high- length fatty acid declined, while instead, the content of short chain fatty acid was elevated (Table 3). This is the reason for difference between the fatty acid compositions in the two drying treatments that could be related to oxidation procedure in a longer period of drying in sun/open air method. Actually, MUFA are largely represented in neutral lipids and are more prone to migration and oxidation (Badiani *et al*. 2002).

Table 3. Fatty acid profile of dried *U. intestinalis* by sun/open-air and oven method

Fatty acid (g/100g oil)	**Treatments**	
	Sun/open air drying	**Oven drying**
C6:0	35.687 ± 2.267^{a}	17.143 ± 1.486^{b}
C8:0	7.366 ± 0.420^{b}	8.188 ± 0.071^{a}
C8-10 Caprylic-capric acid	3.595 ± 0.071^{a}	1.378 ± 0.050^{b}
C14:0	6.477 ± 0.612^{a}	4.264 ± 0.438^{a}
C15:0	1.941 ± 0.141^{a}	0.656 ± 0.071^{b}
C16:0	12.706 ± 1.621^{a}	17.745 ± 1.259^{a}
C16:1	4.924 ± 0.692^{a}	3.523 ± 0.743^{a}
C18:0	2.352 ± 0.141^{b}	4.650 ± 0.132^{a}
C18:1 (n-9 c)	9.251 ± 0.670^{b}	23.955 ± 1.485^{a}
C18:1 (n-11 c)	3.165 ± 0.158^{a}	2.544 ± 0.126^{b}
C18:2 (n-6 c)	3.840 ± 0.578^{b}	9.166 ± 0.424^{a}
C18:3 (n-6)	0.569 ± 0.320^{a}	0.172 ± 0.106^{a}
C18:3 (n-3)	1.487 ± 0.273^{a}	0.841 ± 0.078^{a}
C20:0	0.825 ± 0.057^{b}	1.363 ± 0.150^{a}
C20:1	1.166 ± 0.151^{a}	0.317 ± 0.130^{b}
C20:5 (n-3) EPA	1.282 ± 0.530^{a}	0.806 ± 0.374^{a}
C22:4 (n-6) DTA	0.956 ± 0.059^{b}	1.315 ± 0.040^{a}
C22:6 (n-3) DHA	2.377 ± 0.402^{a}	1.972 ± 0.195^{a}
Σ SFA	70.989	55.387
Σ MUFA	18.506	30.339
Σ PUFA	10.511	14.272
Σ n-3 FA	5.146	3.619
Σ n-6 FA	5.365	10.653
n-3/n-6 ratio	0.959	0.340

Values were expressed as the mean ± standard deviation (n=2). Means value in rows with different small letters indicate significant differences ($P < 0.05$).

The *U. intestinalis* fatty acid profile also showed containing some essential fatty acids C18:2 (linoleic acid) and in a lower extent C18:3 (linolenic acid). The occurrence of the C18 PUFAs is important in human nutrition and for fish, which are not capable of synthesizing these fatty acids. These C18 PUFAs have also been reported by Mehdipour *et al*. (2014) for this species (in a lower content) and by Yaich *et al*. (2011) for *U. lactuca*. Despite the higher alterations in fatty acid profile of sun/open air-dried samples in comparison with oven drying method, higher EPA and DHA contents and also higher n-3/n-6 ratio revealed that the sun/open air method keeps better the quality of seaweed lipids.

CONCLUSION

In the present study, the impact of oven-drying on physicochemical and functional properties of *U. intestinalis* was investigated. Oven-drying decreased the dry matter and protein content of this seaweed. Water holding capacity was higher in oven-drying method. Although higher EPA and DHA contents and also higher n-3/n-6 ratio was found in sun/open air method, however, HUFA content was higher in oven-drying method. Seaweeds are important sources of macronutrients. *U. intestinalis* has relatively high levels of protein and lipid. It constitutes a good alternative source of some polyunsaturated fatty acids, such as oleic, linoleic and linolenic acids and DHA that are essential for human nutrition and health. For this reason, *U. intestinalis* could be consumed as food supplement to aid in achieving these valued components.

Water holding capacity and emulsion activity are key functional properties of food ingredients. By the growing interest in functional foods, seaweeds are being intensively considered, where they can used of different functionalities ranging from a simple nutritional improvement to physiologically complex mechanisms. Emulsion activity and other functional properties make them suitable to incorporate to food products to improve their physicochemical and textural properties. This study indicates that this seaweed could be useful in several food and nonfood applications, contributing to more responsible utilization of aquatic bio-resources.

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