

Effects of Different Drying Methods on The Bioactive Compunds and Antioxidant Activity of The Edible Algae *Cystoseira Barbata*

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Received: 19.08.2020 Revised in received: 21.09.2020 Accepted: 09.10.2020

Abstract

Thanks to its appreciated antioxidant activity and phytochemical properties, *Cystoseira barbata* has great potential as a functional food. Since algae slurry is perishable and can spoil within a short time, drying of algae is mandatory for storage. Since the drying method can affect chemical content of the finished product, it is critical to determine the appropriate drying method. The comparison of influences of drying techniques on chemical properties and antioxidant activity of edible algae *C. barbata* was investigated. The alga was dried by sun, hot air, and freeze drying. Effects of the drying technique on total phenolic, flavonoid, carotenoid, anthocyanin content and antioxidant activity of *C. barbata* were investigated. The drying technique affected the chemical composition and antioxidant activity of the *C. barbata* samples significantly ($p<0.05$). The results presented in this work indicated that the most appropriate drying method in terms of the flavonoid, carotenoid and anthocyanin content is freeze drying. However, hot air drying at 80°C increased the antioxidant activity and total phenolic content and of *C. barbata* samples.

Key words: *Cystoseira barbata*, drying, antioxidant activity, algae

Farklı Kurutma Tekniklerinin Yenilebilir *Cystoseira Barbata* Alginin Biyoaktif Bileşenleri ve Antioksidan Aktivitesi Üzerine Etkileri

Öz

Yüksek antioksidan aktivitesi ve fitokimyasal özellikleri sayesinde, *Cystoseira barbata* fonksiyonel bir gıda olarak büyük bir potansiyele sahiptir. Algler bulamaç halinde kısa sürede bozulabileceğinden, depolama için kurutulmaları zorunludur. Kurutma yöntemi bitmiş ürünün kimyasal içeriğini etkileyebileceğinden, uygun kurutma yönteminin belirlenmesi son derece önemlidir. Bu çalışmada farklı kurutma tekniklerinin yenilebilir alglerden *C. barbata* alginin kimyasal özellikleri ve antioksidan aktivitesi üzerindeki etkileri karşılaştırılmıştır. Algler güneş, sıcak hava ve dondurarak kurutma teknikleri ile kurutulmuştur. Kurutma tekniğinin *C. barbata* alginin toplam fenolik, flavonoid, karotenoid, antosiyanin içeriği ve antioksidan aktivitesi üzerindeki etkileri araştırılmıştır. Kurutma tekniği, *C. barbata* örneklerinin kimyasal bileşimini ve antioksidan aktivitesini önemli ölçüde etkilemiştir ($p<0.05$). Bu çalışmada sunulan sonuçlar flavonoid, karotenoid ve antosiyanin içeriği açısından en uygun kurutma yönteminin dondurarak kurutma olduğunu göstermiştir. Bununla birlikte, 80°C'de sıcak havayla kurutma yöntemi, *C. barbata* örneklerinin antioksidan aktivite ve toplam fenolik madde içeriğini arttırmıştır.

Anahtar kelimeler: *Cystoseira barbata*, kurutma, antioksidan aktivite, algler

Introduction

Being edible algae, seaweeds are consumed as food in many coastal regions throughout the world (Mabeau & Fleurence, 1993). In many countries algae are important part of regular diet. Since algae are rich source of fiber,

protein, and high levels of omega-3 fatty acids, they have excellent nutritional value (Adharini et al., 2019; Burtin, 2003). Moreover, algae are rich source of many vitamins, and minerals suggesting that they are new potentiality for the food industry (Fleurence et al., 2012). Furthermore, seaweeds

are great source of bioactive compounds that demonstrate several biological activities including cytotoxic, antiviral, antimitotic, antibiotics, and anti-inflammatory activities (Bhosale et al., 2002; Mhadhebi et al., 2011). Thanks to their high antioxidant activity (Farasat et al., 2013), they great potential as a functional food. Moreover, they are nutritionally valuable as fresh or dried vegetables, or as ingredients in a broad range of prepared foods.

Since algae slurry is sensitive and it spoils within a short time, drying is very important for them. Since the drying method can affect the nutritional content and qualitative characteristics of the finished product, it is critical to determine the appropriate drying method. Moreover, the drying technique is a factor very important factor which affects the phytochemical content of seaweeds. Natural drying and hot air drying is widely used for drying algae prior to analysis (Gokulakrishnan et al., 2015; Gómez-Ordóñez et al., 2010; Manivannan et al., 2008; Narasimman, et al., 2012; Ozudogrua et al., 2017; Sellimi et al., 2017). Since it requires only a suitable surface and sunny weather, natural drying is very economical method. However, the dried product is often poor quality and often unhygienic as a result of microorganisms and insects such as flies. Hot-air drying is commonly used because it is practical and generally requires short times. On the other hand, owing to the high temperatures applied, it destroys vitamins, antioxidative compounds and other physicochemical properties of dried products. However, freeze-drying is a very gentle dehydration process used for drying of high-quality and heat-sensitive products.

Cystoseira sp. have many secondary metabolites with biological activity such as terpenoids, carbohydrates, and phlorotannins (de Sousa et al., 2017). Being a traditionally functional food, *Cystoseira barbata* is an edible brown seaweed. (Trica et al., 2019). Thanks to its appreciated antioxidant activity and phytochemical properties, *C. barbata* have been used as a food additive. In order to utilize chemical compounds of *C. barbata*, it is very important to determine appropriate drying method. In previous studies, *C. barbata* dried by using sole dried method and influences of different drying techniques on its chemical characteristics have not been investigated. Therefore, this study aimed to investigate comparison hot-air, sun, and freeze-drying methods on chemical composition and antioxidant activity on *C. barbata* algae.

Material and Method

C. barbata samples were obtained from 0-0.5 m depth in coastal of Big Island (Büyükkada), which is the largest of Istanbul's nine Islands, in December 2019. In order to remove epiphytes and other extraneous matter, the samples were washed with seawater. The cleaned *C. barbata* samples were transported to the laboratory and they were sorted and then thoroughly cleaned by rinsing with distilled water. In order to remove excess water, tissue paper was used.

Drying trials

Hot air-drying trials were performed in an oven (NÜVE FN 500) at 40°C for 24 hours and 80°C for 5 hours. Freeze-drying trials were performed in HeltoHolten DW8 freeze dryer at -80 °C for 24 h and the condenser temperature at -15 °C for 24 h. Sun drying was performed under direct sunlight for 4 days.

Total phenolic content measurements

The total phenolic contents of *C. barbata* samples were measured based on the method described by Djeridane et al. (2006) by using Folin-Ciocalteu reagent. 0.2 ml seaweed extracts were taken in test tubes. 2.5 ml of 10% Folin-Ciocalteu reagent was mixed with 0.5 ml methanolic extract and the mixture was added the test tubes. Then, 2.5 ml of sodium carbonate (7.5%, w/v) was added to the mixture. The mixture incubated at dark. After the incubation at dark for 45 min, the absorbance of the standard (gallic acid) and the extract of biscuit samples was determined spectrophotometrically at 765 nm against blank.

Total Flavonoid Content Measurements

The total flavonoid contents of *C. barbata* samples were determined based on the method proposed by Quettier- Deleu et al. (2000). The results were stated as milligram per gram extract.

Total Carotenoid Content Measurements

The carotenoid contents of the extracts were measured spectrophotometrically based on the method described by Lichtenthaler and Buschmann (2001). The absorbance was measured at 480 nm. The content of carotenoid was calculated by the following equation:

$$A = \alpha \cdot c \cdot l$$

where A represents the absorbance at 480 nm, α represents the specific absorbance coefficient of the solvent, c represents the carotenoids in μg /and l is the path length of the cuvette (1 cm).

Total Anthocyanin Content Measurements

Total anthocyanin (TA) contents of *C. barbata* samples were measured by a spectrophotometric pH differential method based on the method proposed by Giusti and Wrolstad (2001). Briefly, 3.5 mL 0.025 M potassium chloride buffer; pH 1 was mixed with 0.5 mL of extract. After the incubation for 15 min the absorbance was determined at 515 and 700 nm against the blank. Then the extract mixed with 3.5 mL of 0.025 M sodium acetate buffer; pH 4.5. After the mixture was shaken vigorously, it was incubated at room temperature for 15 min. The absorbance was measured at 515 nm. The total anthocyanins content (TAC) was calculated by the following equation:

$$TAC = (A \times MW \times DF) / (\epsilon \times C)$$

Where A is absorbance = (A₅₁₅–A₇₀₀) pH 1.0–(A₅₁₅–A₇₀₀) pH 4.5; MW is molecular weight for cyanidin-3-glucoside = 449.2; DF is the factor of the extract; ϵ is the molar absorptivity of cyanidin-3-glucoside = 26.900; C is the concentration of the buffer in mg mL⁻¹ = 0.025. Result was stated as milligram of cyanidin-3-glucoside (C-3-GE) equivalents in 1 g of dried sample.

DPPH Free Radical Scavenging Assay

1,1-diphenyl-2-picrylhydrazyl (DPPH) activity was determined based on the method proposed by Brand-Williams, Cuvelier, & Berset (1995). Firstly, the samples were diluted in methanol. Then, the DPPH solution was mixed with the diluted sample. The mixture incubated at dark for 30 min and absorbance of the sample measured at 515 nm by a UV–VIS spectrophotometer. The DPPH radical scavenging activity was calculated by using the following equation:

$$(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}$$

ABTS Assay

2,2-azino-bis-(3-ethyl-benzothiazoline-6-sulphonic acid) assay was used based on the method Re et al. (1999). ABTS solution (7 mM) and potassium persulphate (2.45 mM) was mixed. Then the mixture was diluted with 80% methanol. An aliquot (200 μ L) of extract was mixed with 2 mL of ABTS solution, and the mixture was shaken vigorously. The absorbance was determined at 734 nm. Ascorbic acid was used as a standard and calibration. The result was stated as milligrams ascorbic acid equivalents antioxidant capacity (AEAC) in 1 g of dried sample.

Statistical Analysis

SAS software (SAS, 1999) was used for the analysis of data and all statistics. The data were

analyzed in terms of Analysis of Variance (ANOVA). The values were compared by Duncan's Multiple Range Test at a level of $p < 0.05$.

Results and Discussion

Thanks to their healthy properties, phenolics are thought as potential functional ingredients (Kaur & Das, 2011). The phenols have great in vitro antioxidant activity, confirming their importance in the diet. Moreover, they have pharmacological properties including antiviral, anticarcinogenic, antimicrobial, antitumor activities and anti-inflammatory, effects against neurodegenerative pathologies (Esposito et al. 2002; Oueslati et al. 2012). The marine algae species have relatively high phenolic content (Freile-Pelegrín, & Robledo, 2013). Influences of hot air and freeze drying on phenolic content of *C. barbata* samples are shown in Table 1. Total phenolic content of hot air dried *C. barbata* samples was higher than the total phenolic content of sun dried and freeze dried *C. barbata* samples significantly ($p < 0.05$). This may be due to the formation of different compounds in maillard reaction such as melanoidins. The results of the studies which investigated the influences of drying process on the phenolic compounds revealed that heat treatment have a considerable effect on increasing the total phenolic content of food materials (Bolek & Obuz 2014; Chang, Lin, Chang, & Liu, 2006; Sultana, Anwar, Ashraf, & Saari, 2012).

Being one of the most important group of polyphenolic compounds (Caf, Özdemir, Yılmaz, Durucan & Ak, 2019), flavonoids have free radical scavenging properties (Kähkönen et al., 1999). Influences of hot air and freeze drying on total flavanoid content of *C. barbata* samples are given in Table 1. Total flavanoid content of *C. barbata* samples dried at 80°C was significantly lower than the total phenolic flavanoid content of dried *C. barbata* samples at 40°C ($p < 0.05$). Flavonoids are heat sensitive (Chaaban et al., 2017) so, they degraded during hot air drying. However, heating may destroyed enzyme activity and block the synthesis pathway of flavonoids (Zhang et al., 2019).

Many epidemiological research have showed that carotenoid intake may cause reduction in risk of degenerative illness (D'Evoli, Lombardi-Boccia & Lucarini, 2013). Structural properties of carotenoids that are health benefits also cause these compounds highly susceptible to heat and oxidation (Boon, McClements, Weiss, & Decker, 2010). As seen in Table 1, total carotenoid content of freeze dried *C. barbata* samples was higher than the total phenolic flavanoid content of

hot air dried *C. barbata* samples significantly ($p<0.05$). This result could be explained by degradation of carotenoids of *C. barbata* due to heat and oxidation during hot air drying. Freeze drying provided high amounts of anthocyanins in

all samples than other drying methods. Indeed, these results are in accordance with the fact that the freeze drying is the most efficient method to preserve nutritive value and chemical constituents (Ratti, 2001).

Table 1. Effect of Drying Method on Total Phenol, Total Flavonoid, Total Carotenoid Content and Total Anthocyanin Content of *C. barbata*

Drying Method	Total Phenolic Content (mg GAE/100g)	Total Flavonoid Content (mg/100g)	Total Carotenoid Content (mg/100g)	Total Anthocyanin Content (mg/100g)
Sun drying	28.12±0.52 ^c	22.14±0.71 ^b	0.22±0.04 ^a	1.09±0.01 ^{ab}
Hot air drying (40°C)	49.26±0.42 ^b	16.76±0.68 ^c	0.16±0.02 ^b	0.80±0.01 ^b
Hot air drying (80°C)	55.46±0.32 ^a	12.14±0.25 ^d	0.11±0.03 ^c	0.76±0.03 ^{bc}
Freeze drying	30.21±1.62 ^c	26.76±0.32 ^a	0.26±0.04 ^a	1.17±0.02 ^a

* Values are mean ± standard deviation of three separate determinations (n=3). Values in the row with the same letter in superscript are not significant different from each other at $p\leq 0.05$

DPPH method is based on the reduction of DPPH in the presence of a hydrogen-donating antioxidant thanks to the production of the non-radical form DPPH (Shon et al., 2003). The ABTS radical method is widely used to determine the concentration of free radicals. The antioxidant activity of the *C. barbata* samples were measured by DPPH assay and ABTS given in Table 2. The *C. barbata* samples dried at 80°C had significantly

higher antioxidant activity than freeze-dried and sun dried samples. Maillard reaction and caramelization, which contributed to the antioxidant activity of its products, generally occur at high temperature (Liu et al., 2007; Chen et al., 2009). *C. barbata* samples dried at 40°C had lower antioxidant activity because Maillard reactions occur at higher temperatures.

Table 2. Effect of Drying Method on Antioxidant Activity of *C. barbata*

Drying Method	DPPH assay (mg/mL)	ABTS assay (mg/mL)
Sun Drying	20.36±0.44 ^c	0.50±0.02 ^a
Hot air drying (40°C)	40.36±0.52 ^b	0.27±0.02 ^b
Hot air drying (80°C)	45.22±0.32 ^a	0.23±0.01 ^b
Freeze drying	37.21±0.68 ^b	0.55±0.01 ^a

*Values are mean ± standard deviation of three separate determinations (n=3). Values in the row with the same letter in superscript are not significant different from each other at $p\leq 0.05$

Conclusions

C. barbata algae are rich source of phenolic and flavonoid compounds. Moreover, they have high antioxidant activity. It is vital to determine the proper drying method to prevent damage to these components, which are extremely important for

health. In this study, *C. barbata* algae were dried by sun, hot air and freeze drying. Drying technique affected chemical properties of *C. barbata* ($p<0.05$). Freeze drying was found to be the best method for preserving phytochemical compounds. Sun drying required the longest period of drying, while the shortest time of drying was for hot air

drying at 80 °C. Hot air drying caused a decrease in total flavonoids, carotenoids and anthocyanin, which indicates that some of them were probably destroyed. Sun drying was a better drying method for keeping photochemical contents compared to oven drying method. However, hot air drying at 80°C increased the antioxidant activity of *C. barbata* samples. Thanks to their rich protein, phenolic content and antioxidant activity, *C. barbata* may evaluate for human nutrition. In order to utilize their health benefits, the drying method should be chosen accurately. Therefore, freeze drying is highly recommended for drying of *C. barbata* algae.

Conflict of Interest Statement: The manuscript's authors declare that, they do not have any conflict of interest.

Researchers' Contribution Rate Statement Summary: The authors declare that, they have contributed equally to the manuscript.

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Extraction and Characterization of Alginate from an Edible Brown Seaweed (*Cystoseira barbata*) Harvested in the Romanian Black Sea. *Marine drugs*, 17(7), 405.

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