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Effect of drying techniques on color and bioactive potential of two commercial edible Indonesian seaweed cultivars

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Abstract

Seaweeds (*Kappaphycus alvarezii* and *Sargassum duplicatum*) are potentially rich sources of bioactive compounds and functional constituents that are used in food applications. Sun, oven, vacuum, and freeze-drying techniques are commonly used seaweed drying techniques and could be classified as cheap (sun and oven) and expensive (vacuum and freeze) drying techniques based on ovens' market price. Therefore, the study was designed to investigate the effect of cheap (sun and oven) and expensive (vacuum and freeze) drying techniques based on color and antioxidant potential of *K. alvarezii* and *S. duplicatum* using phenolic and antioxidant assays. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were employed to discriminate the drying techniques by instrumental color analysis. The results revealed that oven-dried seaweed extracts exhibited higher levels of phenolic (0.30 to 0.36 mg GAE g^{-1}) and antioxidant potential (3.59 to 79.68%). PCA and HCA discriminated the drying techniques into two well-defined groups/clusters I (oven, vacuum, and freeze-drying) and II (sun drying) and revealed higher ΔE values of sun-dried seaweed samples, which was interpreted as color was preserved using drying techniques of cluster I. Overall, oven, vacuum, and freeze-drying techniques reported similarities in color characteristics and could be an alternate drying technique to preserve the color of seaweed cultivars. Therefore, oven drying technique is recommended due to low cost (compared with vacuum and freeze-drying) and an affordable alternative to sun drying, the preferred technique in fishing communities of low-and-middle-income countries, for the development of seaweed-enriched functional foods.

Keywords Seaweed cultivars · Drying techniques · Color characteristics · Antioxidant activity · Statistical methods

Introduction

Macroscopic marine algae or seaweed is one of the major interesting research areas due to their extensive medicinal properties and as a source of natural bioactive compounds. These properties of seaweeds have encouraged food manufacturers to exhaustively develop raw or semi-processed seaweed products. Several publications have appeared in recent years documenting the different functional properties of seaweeds; for instance, a study on phenolics and antioxidant potential of seaweed reported the possible biological effects of seaweed against liver injury, antioxidant activity, antiviral activity (Ling et al. 2015), and antimicrobial potential (Manivannan et al. 2011).

Seaweeds, considered a potentially rich source of phenolic compounds, are documented for their natural antioxidants in food applications (Sabeena Farvin and Jacobsen 2013). However, fresh seaweed contains a high amount of water (75 to 80%) that needs to be removed for further industrial applications (Neoh et al. 2016) and to prolong the shelf life of dried seaweed products. Recently, drying of seaweed has received considerable critical attention due to the quality of its dried products. Drying is one of the processing methods commonly used to maintain safety and extend the shelf life of produce (Younis et al. 2018) and several studies have reported on the application of drying techniques and quality changes observed in their bioactive content (Adiletta et al. 2016). For

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example, Roshanak et al. (2016) suggested that drying could alter biochemical reactions, which subsequently affects the product organoleptic characteristics. It appears from the aforementioned investigations that preserving the product quality during drying continues to pose a challenge for food engineers due to consumer demand of safe food products with high nutritional value. As a result, consumer requirements have heightened the need for the development of different drying techniques for novel products and sustainable processes. In general, different drying techniques have been commercialized for seaweed drying including natural (sun and shade drying), mechanical, and/or conventional drying. Orphanides et al. (2013) documented the use of convection oven drying, freeze-drying, and microwave drying to preserve medicinal herbs. Similarly, a comparative study (Neoh et al. 2016) examined the influence of different drying techniques (oven-dried, sun-dried, vacuum dried, and freeze-dried) on chemical constituents of the Malaysian edible red seaweed, *Kappaphycus alvarezii*. These studies highlighted the divergencies of phenolic and antioxidant activities based on the type of drying technique used and demonstrated the influence of drying techniques on the bioavailability of bioactive compounds. More recently, the literature on the role of different drying techniques seems to contradict the impact of drying techniques on bioactivity of food products, which then has attracted many researchers to investigate different drying techniques to understand the conceptual aspects of the drying mechanism and their adverse effect on bioactive compounds. So far, however, there has been little discussion on the effect of drying techniques on phenolic content and antioxidant activity and contribution of phenolic compounds to the overall antioxidant activities of dried seaweed, which remain incomprehensive and contradictory. These discrepancies could be related to seaweed species that contributed to reporting different phenolic and antioxidant activities (Neoh et al. 2016). Despite greater variation in pigment, quality, and availability of phenolic compounds, we selected the farmed *K. alvarezii* (red algae) and wild *Sargassum duplicatum* (brown algae), popularly available seaweed species in Indonesia. Neoh et al. (2016) compared different drying techniques using Malaysian red seaweed and reported the need of in-depth investigation on different classes of seaweeds due to their different chemical composition and varied cell wall polysaccharides, which are impacted by the drying techniques. Although studies have elucidated different mechanical drying techniques, fishing communities from low- and middle-income countries continue to dry seaweed using sun drying due to cost and convenience; for example, seaweed farming communities in Indonesia, where seaweed is traditionally dried under sunlight (Zamroni and Yamao 2011). Another study by de Faria et al. (2014) established the problems associated with seaweed sun drying in Brazil due to fluctuations in weather conditions (high temperature, rainfall, and relative humidity). This indicated that innovations in advanced drying techniques are limited to academic publications and/or to developed countries and highlight their failure to be successfully adapted or introduced in those fishing communities. These observations served as the basis for this study to screen different drying techniques using advanced statistical methods in order to select the best and cheaper drying technique to support the sustainable development of the seaweed industry in those areas.

Therefore, in the study, drying techniques were screened based on color and antioxidant potential of *K. alvarezii* and *Sargassum duplicatum*. Then, we employed multivariate statistical methods (principal component analysis and hierarchical cluster analysis) to discriminate the different drying techniques based on instrumental color analysis of the dried seaweed products. The drying techniques were grouped as cheap drying techniques (sun drying and oven drying) and expensive drying techniques (vacuum drying and freeze-drying) based on oven market price. The findings of the study further assist the practical application and adaptation of suitable drying technique(s) to fishing communities of low- and middle-income countries for the formulation of seaweed-enriched products (e.g., seaweed-enriched meat products).

Materials and methods

Study area and identification of seaweed samples

Two seaweed samples of *Kappaphycus alvarezii* (farmed) and *Sargassum duplicatum* (wild) were collected from the coastal area of Talango Islands, Madura, Indonesia. These cultivars were identified at the Research Center for Oceanography, Indonesian Institutes of Sciences, Jakarta, Indonesia, and were presented as a gift by the Department of Marine and Fisheries, Universitas Airlangga: Campus C, Surabaya, Indonesia. Semi-air-dried samples (packed in transparent food grade LDPE resealable zip lock bags: 28 × 48 cm) were then transported to National Pingtung University of Science and Technology (NPUST), Taiwan, (within 24 h) and were stored in a digital humidity-controller (relative humidity: 50%; temperature: 25 °C) until further experimental analysis (usually within 2 days).

Sample preparation and drying process

The fresh samples (approximately 500 g each) were thoroughly washed with tap water and Milli-Q (ultra-purified) water (Millipore, USA) to remove external adhering salts, epiphytes, microorganisms, and other extraneous foreign impurities. The samples were then cut manually with a stainless-steel food-grade knife and evenly placed in rectangular stainless steel

(23 × 33 cm), and then dried using different drying techniques (sun, oven, and vacuum drying). For freeze-drying, samples were frozen at − 80 °C for 12 h (Freezone Plus 6, USA).

(i) Sun drying: Fresh seaweed samples (100 g each) were placed evenly in rectangular stainless steel directly under the open sun until constant weight loss.
(ii) A conventional oven (DOS − 45, Deng Yng, Taiwan) with a galvanized metal sheet was used to dry the seaweed samples. Fresh seaweed samples (approximately 200 g each) were spread in rectangular stainless steel and dried at 50 °C with an air velocity of 1.0 m s^{-1} until the samples reached constant weight.
(iii) Vacuum drying: A vacuum drying oven (Channel vacuum dryer, Taiwan) was used to dry and anneal the samples under vacuum. Briefly, seaweed samples (approximately 200 g each) were placed uniformly in rectangular stainless steel (24 H × 20 W in cm) and kept in the vacuum oven dryer at 50 °C (70 cm Hg) until constant weight.
(iv) Freeze-drying: Freeze dryer was used to dry seaweed samples. Freeze-drying of frozen seaweed samples (200 g each) in freeze-drying flasks was completed in 3 days.

All dried samples were ground to a fine powder using a laboratory-scale grinder (Yu Chi Machinery Co., Ltd., Taiwan) and sieved by universal certified standard sieves (US Standard Sieve Series, ASTME No. 20, and Tyler Standard Sieve Series: 20 Mesh). The powdered seaweed samples (Supplementary Fig. S1) were stored in food grade polypropylene sampling bags (140 × 100 × 0.06 mm) and placed in a digital air humidity controller (relative humidity 50%) at room temperature until further experimental analysis (usually within 3 days).

Color parameters

The color parameters of seaweed samples were determined using a colorimeter (ZE 2000, Nippon 166 Denshoku Industries Co., Ltd.) which was calibrated with a white glossy ceramic tile in terms of CIELAB coordinates: L, a, and b values. Chroma (C_H), hue angle (θ_H), and color difference (ΔE) of seaweed powders were calculated using the following Eqs. (1, 2, and 3).

$$C_H = \left[(a^2 + b^2)^{\frac{1}{2}}\right] \quad (1)$$

$$\theta_H = \left[\tan^{-1}\left(\frac{b}{a}\right)\right] \quad (2)$$

$$\Delta E = \left[\left[(L-L_0)^2 + (a-a_0)^2 + (b-b_0)^2\right]^{\frac{1}{2}}\right] \quad (3)$$

where L and L_0 = difference in lightness; a and a_0 = difference in intensity (red color); b and b_0 = difference in intensity (yellow color).

Extraction procedure

The extraction procedure was performed by adapting the procedure described by Neoh et al. (2016), with slight modification in solvent selection. A sonication bath method (Delta Ultrasonic Cleaner, Taiwan) was used to prepare the seaweed extracts with ethanol (1:20) and sonicated for 1 h in the dark at room temperature followed by vigorous shaking for 4 h at room temperature (25 ± 1 °C). The procedure was repeated twice for each sample and filtered through 90-mm Whatman No.1 filter paper (Toyo Roshi Kaisha Ltd., Japan). The samples were then evaporated using a rotary evaporator (Eyela, A-1000 S, Japan) at 45 °C and stored in brown-colored scintillation vials at 4 °C until further use.

Determination of total phenolic and flavonoid contents

The amount of total phenolic and flavonoid contents in the seaweed extracts were analyzed by a colorimetric method described for seaweed extracts according to Neoh et al. (2016). Total phenolic contents of seaweed extracts were expressed in terms of mg gallic acid equivalent (mg GAE) per gram of sample (wet weight).

The total flavonoid content (TFC) of the seaweed extracts was analyzed using the procedure of Ling et al. (2015). Quercetin standard curve was constructed and the results were expressed as mg of quercetin equivalent (QE) per g of sample (dry weight).

Evaluation of in vitro antioxidant capacity

DPPH• (2,2 diphenyl-1-picrylhydrazyl) scavenging assay

The DPPH• scavenging activity of seaweed extracts was estimated in terms of a stable radical DPPH method (Chakraborty et al. 2017). Briefly, extract (2 mL) was mixed with a freshly prepared DPPH• solution (2 mL, 0.16 mM DPPH in methanol). The reaction mixtures were shaken vigorously and allowed to stand for 30 min at room temperature (25 ± 1 °C) in the dark. A control was prepared with DPPH• solution (2 mL) and methanol (2 mL) and the absorbance was read at 517 nm against methanol as blank. The percentage of

radical scavenging activity of seaweed extracts was calculated by following Eq. (4).

$$\%\text{inhibition} = \left[\left(\frac{A_{\text{control}} - A_{\text{extract or standard}}}{A_{\text{control}}}\right) \text{x } 100\right] \quad (4)$$

ABTS˙ (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) scavenging assay

The antioxidant activity of seaweed extracts was measured based on the modified assay in Chakraborty et al. (2017). The ABTS˙ stock solution was prepared by dissolving the same proportion of ABTS aqueous solution (7 mM) and an aqueous solution of $K_2S_2O_8$ (2.45 mM). The reaction mixture was incubated overnight (12–16 h) in the dark at room temperature (25 ± 1 °C). The reaction mixture was diluted with methanol (working solution) to obtain an absorbance of 0.72 ± 0.03 at 734 nm. The ABTS˙ scavenging activity was determined by mixing 0.10 mL of the extract with 5 mL of $ABTS^{\cdot+}$ solution. The reaction mixture without the extract was treated as a control. The final absorbance was measured at 734 nm and the percentage of scavenging activity was calculated by Eq. (4).

FRAP assay

The FRAP (ferric reducing antioxidant power) values for seaweeds was determined following the method of Neoh et al. (2016). Briefly, freshly prepared FRAP (3 mL) reagent (300 mM sodium acetate buffer (pH 3.60), 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in 10:1:1 ratio, v/v) was mixed with seaweed extract or standard (0.30 mL) and allowed to incubate at 37 °C for 30 min (for color development). The absorbance was then read at 593 nm against a blank (distilled water). A calibration curve with $FeSO_4$ was constructed and the antioxidant activity of seaweed extracts was expressed as mM $FeSO_4$ equivalent per gram of seaweed extract.

Multivariate statistical analyses

Principal component analysis

Principal component analysis (PCA) is a multivariate statistical method of dimension reduction used to reveal major dimensions of variations within experimental data. In PCA, experimental data sets were replaced by new and smaller data sets illustrating maximum variations among variables with minimal loss of information by a few principal components (PCs). The number of PCs was determined based on the Kaiser criterion where Eigenvalues greater than 1 are usually considered statistically significant. We performed PCA factor analysis to discriminate drying techniques based on instrumental color by adapting the procedure reported by Sridhar and Charles (2018).

Hierarchical cluster analysis

Hierarchical cluster analysis (HCA) is an exploratory multivariate statistical tool attempted to explain relatively natural groupings or clusters of variables within experimental data using on cluster algorithms (distance measures of similarity or dissimilarity). The decisiveness of HCA is usually represented in the form of a dendrogram, in which the relationship between samples could be shown in a tree form with two main basic grouping strategies: agglomerative or divisive (Granato et al. 2018). We employed the agglomerative (bottom-up) method, which is directed from the leaves to the root of a dendrogram (Zhang et al. 2017). The algorithm method that contains agglomeration schedule and between-group linkage clustering method with squared Euclidean distance interval measurement were used to analyze the drying techniques based on instrumental color characteristics (C_{H}, hue angle θ_{H}, and ΔE) as shown in Eq. (5) according to Sridhar and Charles (2018).

$$D(A_i, B_j) = \text{minimum}_{x,y}\{d(x,y) | x \in A_i, y \in B_j\} \quad (5)$$

where A_i and B_j are a distance between two samples or clusters.

Statistical analysis

All experimental analyses were conducted in triplicate and were reported as mean ± standard deviation ($n = 3$). The data was statistically analyzed among the different drying techniques and the significance levels were set at the 5% probability level using multivariate general linear model and Duncan's post hoc test (IBM SPSS Statistics version 22.0. USA).

Results

Color analysis

The color values of seaweed powders (*K. alvarezii* and *S. duplicatum*) are shown in Table 1. The statistical analysis indicated that the color parameters (C_{H}, θ_{H}, and ΔE) were significantly ($F_{(18.8,97)} = 5.52$, Wilks' Lambda = 0.001, $p < 0.05$) affected by the drying techniques used for seaweed powders (Table 1). In the *K. alvarezii* samples, the mean values of C_{H} were found to be the highest in samples that were sun-

Table 1 Color characteristics of two seaweed cultivars subjected to different drying techniques[1]

Drying technique	*Kappaphycus alvarezii*			*Sargassum duplicatum*		
	Chroma (C_H)	Hue angle (θ_H)	Color difference (ΔE)	Chroma (C_H)	Hue angle (θ_H)	Color difference (ΔE)
Sun drying	2.77 ± 0.05^{c}	73.53 ± 2.19^{a}	17.32 ± 0.66^{b}	0.92 ± 0.41^{a}	73.53 ± 2.19^{b}	10.52 ± 1.22^{b}
Oven drying	1.87 ± 0.44^{b}	85.66 ± 1.37^{c}	14.95 ± 0.64^{ab}	1.48 ± 0.16^{a}	70.61 ± 0.28^{a}	4.33 ± 0.40^{a}
Vacuum drying	1.57 ± 0.19^{b}	82.63 ± 2.23^{bc}	14.37 ± 0.27^{a}	0.95 ± 0.54^{a}	70.25 ± 0.10^{a}	3.77 ± 1.81^{a}
Freeze-drying	0.78 ± 0.30^{a}	80.54 ± 4.93^{b}	15.68 ± 2.62^{a}	0.83 ± 0.07^{a}	70.25 ± 0.92^{a}	3.48 ± 0.93^{a}

[1] Results expressed as mean ± standard deviation (SD) ($n = 3$). Mean values within a column with different superscripts are significantly different (Wilks' Lambda = 0.001, $p < 0.05$, MANOVA, Duncan)

dried (2.77) and the lowest from freeze-drying (0.78), but a similar trend was not observed in C_H values of *S. duplicatum* samples. The highest values of ΔE were observed in sun-dried samples (*K. alvarezii* 17.32 and *S. duplicatum* 10.52) compared with other drying techniques (oven, vacuum, and freeze) (Table 1). Interestingly, the three drying techniques (oven, vacuum, and freeze) reported statistically nonsignificant differences in ΔE values (Table 1). Moreover, the visual appearance of two seaweed cultivar powders subjected to different drying techniques also confirmed the similarities in color changes among oven, vacuum, and freeze-dried samples, respectively, rather than sun-dried samples (Supplementary Fig. S1).

Total phenolic and flavonoid contents

The impact of drying techniques on total phenolic and flavonoid contents of the two seaweed cultivar extracts is highlighted in Table 2. Oven-dried seaweed samples exhibited the highest TPC (0.36 ± 0.01 and 0.30 ± 0.03 mg GAE g^{-1}) and TFC (0.13 ± 0.01 and 0.36 ± 0.01 mg QE g^{-1}) in *K. alvarezii* and *S. duplicatum*, respectively, compared with other drying techniques, except for sun-dried *S. duplicatum*, where TPC was 1.43 times higher (Table 2).

Antioxidant activity of seaweed extracts

The effect of drying temperatures on DPPH˙ and ABTS˙ scavenging activities and percentage (%) inhibition of seaweed extracts are illustrated in Fig. 1. In the DPPH˙ method, the percentage inhibition ranged from 3.59 to 48.87% with freeze-dried extracts exhibiting the significantly highest percentage inhibition compared with sun, vacuum, and oven-dried seaweed extracts. However, oven- and vacuum-dried extracts reported a similar trend in radical scavenging activities, which was statistically insignificant at the probability level of 0.05. On the other hand, ABTS˙ values of the sample extracts ranged from 32.39 (sun drying) to 80.92% (freeze-drying). We further evaluated the ferric reducing antioxidant power (FRAP) activity and recorded the FRAP values 0.45 (sun drying) to 4.78 (oven drying) mM FeSO4 g^{-1} (Table 3).

Principal component analysis

The data from instrumental color analysis as affected by drying techniques were evaluated by PCA to determine the effect of each drying technique on color characteristics of two seaweed cultivars. The variance among the active variables of seaweed cultivars was accounted by the principal components (PCs) based on Eigenvalues. Hence, two successive components (PCs 1 (73.81%) and 2 (26.19%)) explained the cumulative variance (100%) of *K. alvarezii* as affected by the drying techniques (Fig. 2) except for *S. duplicatum*. *Sargassum duplicatum* samples extracted one component (Supplementary Fig. S2) with 84.48% of the total variance (Supplementary Table T1), which consequently failed to produce component plots for *S. duplicatum*. Therefore, through

Table 2 The total phenolic and flavonoid contents of two seaweed cultivar extracts as affected by different drying techniques[1]

Drying technique	*Kappaphycus alvarezii*		*Sargassum duplicatum*	
	TPC (mg GAE g^{-1})	TFC (mg QE g^{-1})	TPC (mg GAE g^{-1})	TFC (mg QE g^{-1})
Sun drying	0.22 ± 0.01^{b}	0.09 ± 0.001^{b}	0.43 ± 0.01^{c}	0.35 ± 0.01^{c}
Oven drying	0.36 ± 0.01^{d}	0.13 ± 0.01^{c}	0.30 ± 0.03^{b}	0.36 ± 0.01^{d}
Vacuum drying	0.25 ± 0.01^{c}	0.07 ± 0.001^{a}	0.14 ± 0.01^{a}	0.12 ± 0.01^{b}
Freeze-drying	0.14 ± 0.01^{a}	0.09 ± 0.001^{b}	0.13 ± 0.01^{a}	0.11 ± 0.01^{a}

[1] Results expressed as mean ± standard deviation (SD) ($n = 3$). Mean values within a column with different superscripts are significantly different (Wilks' Lambda = 1.03×10^{-7}, $p < 0.05$, MANOVA, Duncan). *TPC* total phenolic content, *TFC* total flavonoid content, *GAE* gallic acid equivalent, and *QE* quercetin equivalent

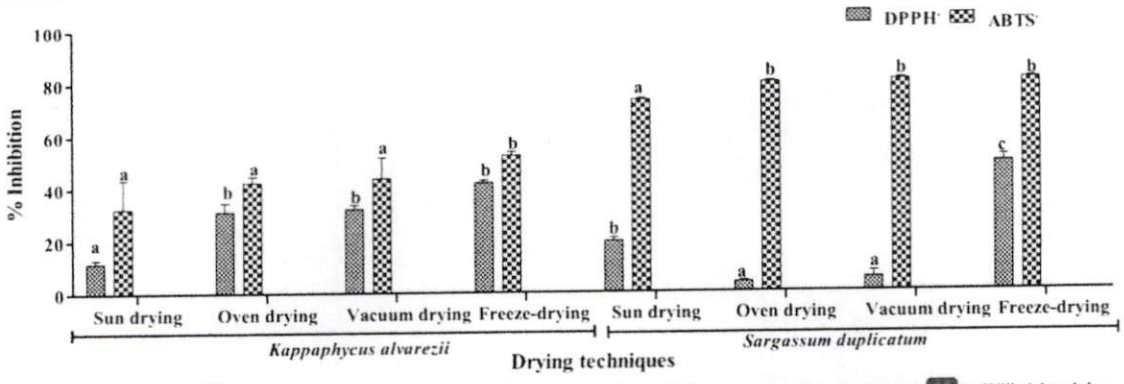

Fig. 1 Effect of drying techniques on antioxidant activities of two seaweed cultivar extracts determined by DPPH˙ and ABTS˙ assays. The error bar represents the standard deviation from the mean of three independent replicates ($n = 3$). Different letters (a, b, and c) represent significant differences among the drying techniques (Wilks' Lambda = 0.09×10^{-4}, $p < 0.05$, MANOVA, Duncan). DPPH˙ = 2,2 diphenyl-1-picrylhydrazyl and ABTS = 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)

the PC score plots of the *K. alvarezii* (Fig. 2), the three drying techniques (oven, vacuum, and freeze) tended to form group I according to their similarities in color characteristics during drying, whereas sun drying alone formed group II (Fig. 2).

Hierarchical cluster analysis

HCA was performed among the drying techniques of two seaweed cultivars based on color characteristics (C_{11}, θ_{11}, and ΔE) to explore the similarities among observations (Fig. 3A and B). Drying techniques used for the two seaweed cultivars relatively showed two well-defined clusters (clusters I and II) in descending order (Fig. 3A and B). In case of *K. alvarezii*, cluster I composed of the oven, vacuum, and freeze-drying techniques, whereas cluster II contained sun drying (Fig. 3A). Cluster I further formed two separate sub-clusters, which indicated the homogeneity of drying techniques based on color characteristics. Moreover, these drying techniques (oven, vacuum, and freeze-drying) reported a similar color change for both seaweed cultivars (Table 1). Similar lines of observations were noticed for *S. duplicatum*, where the oven, vacuum, and freeze-drying techniques collectively formed cluster I, while sun drying formed cluster II (Fig. 3B).

Table 3 FRAP antioxidant values (mM $FeSO_4$ g^{-1}) of two seaweed cultivar extracts as affected by different drying techniques[1]

Drying technique	*Kappaphycus alvarezii*	*Sargassum duplicatum*
Sun drying	0.45 ± 0.01^{a}	2.98 ± 0.01^{a}
Oven drying	0.97 ± 0.01^{d}	4.78 ± 0.06^{d}
Vacuum drying	0.54 ± 0.01^{b}	4.12 ± 0.01^{c}
Freeze-drying	0.91 ± 0.01^{c}	3.57 ± 0.02^{b}

[1] Results expressed as means ± standard deviation (SD) ($n = 3$). Mean values within a column with different superscripts are significantly different (Wilks' Lambda = 0.10×10^{-5}, $p < 0.05$, MANOVA, Duncan). *FRAP* ferric reducing antioxidant power

Discussion

In general, seaweeds are classified into three major categories based on color (e.g., brown, green, and red) (Stévant et al. 2018). In recent years, besides carrageenan, food industries are highly focusing on the development of seaweed products for which color is one of the quality indexes and an important visual parameter (Uribe et al. 2019) that evaluates consumer willingness to purchase. Moreover, seaweeds have been used as a part of the human diet from ancient times to now, predominantly in China, the Korean Peninsula, and Japan. Therefore, various bioactive compounds (e.g., xanthophyll, phycoerythrin, phycocyanin, chlorophyll, and β-carotene) present in seaweeds (Park et al. 2018) presented the potential to develop various kinds of functional foods using seaweed. For example, Sarkar et al. (2017) developed seaweed value-added food products (seaweed jelly, soup, ice-cream, curd; two functional food products: seaweed singara and samucha/samosa; two cosmetic products: seaweed face pack and shampoo). Hence, color evaluation is an essential parameter for commercialization of seaweed value-added products that could promote the lives of seaweed communities in low- and middle-income countries.

The incomparable tendency between the C_{11} values of *K. alvarezii* and *S. duplicatum* was attributed to differences in seaweed species. *Kappaphycus alvarezii* (red algae) and *S. duplicatum* (brown algae) belong to two different Phyla classified based on color. In general, C_{11} values represent the degree of saturation of color that is proportional to the strength of the color (Mphahlele et al. 2016). In this study, higher C_{11} values for *K. alvarezii* were related to higher Hunter "*a*" value,

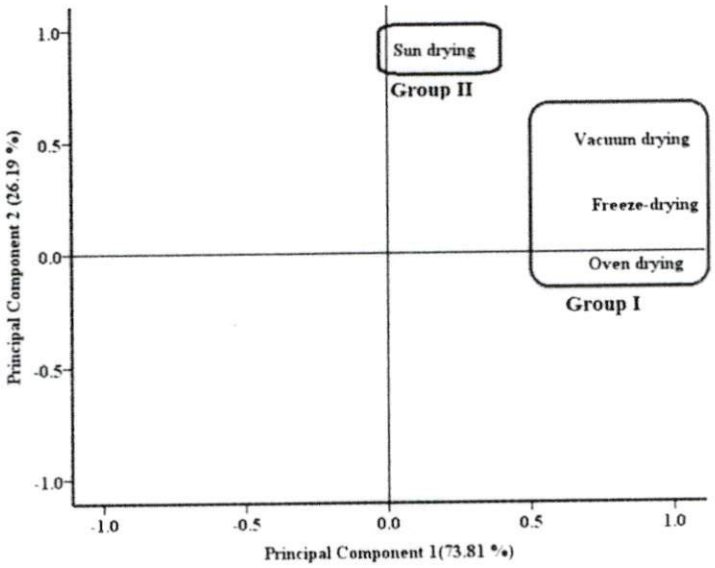

Fig. 2 Principal component analysis (PCA) for the *Kappaphycus alvarezii* extracts as affected by drying techniques based on instrumental color analysis

which was characteristic of the seaweed samples. Moreover, θ_{11} (darkness) values for both seaweed samples were observed in the range of 70.25 to 8[illegible], but no defined trend was observed. On the other hand, ΔE is an important parameter for a dried product, which imitates the human perception to differentiate colors of different samples. In fact, color change phenomenon should be explained by the combination of color parameters (L, a, and b) instead of a single parameter. The ΔE values revealed that sun drying had destroyed the color of seaweed samples that consequently raised the ΔE [illegible]es higher than those of the other three drying techniques. These results are in accordance with the recent studies by Sa[illegible]ti et al. (2019) and Uribe et al. (2019), in which ΔE values were significantly affected by drying conditions (temperature and humidity) and methodology used. Moreover, the color change phenomenon in three drying techniques was slightly negligible and might be related to th[illegible]ell membrane and thermal denaturalization. For example, [illegible]tor studies that have noted the importance of color that might be affected by drying treatments; as a result, pigment reactions (for example, reduction in amino acids and sugars), which led to the development of secondary colored substances (Vairappan et al. 2014; Stévant et al. 2018). Overall, these findings suggested that either oven, vacuum, and freeze-drying techniques could be an alternate drying technique to preserve the color of seaweed cultivars for the development of seaweed enriched functional foods. However, vacuum- and freeze-drying techniques are unaffordable in low- and middle-income fishing communities in developing countries and thus, we recommended oven-drying technique (which is cheaper than vacuum and freeze-drying) to replace sun-drying technique.

Total phenolic and flavonoid contents agreed with earlier observati[illegible] by Ling et al. (2015), who demonstrated TPC and [illegible]FC in the range of 0.26 to 0.53 mg GAE g^{-1} and 0.09 to 0.25 mg QE g^{-1} for *K. alvarezii* using different drying techniques (oven drying with different temperatures, sun, hang, sauna, shade, and freeze-drying). In the same study, the authors reported higher TPC values for oven-dried *K. alvarezii* samples. In our study, higher TPC in *S. duplicatum* was possibly due to fluctuations in temperature (low and high) during sun drying and the complexity of bounded bioactive compounds present in the sample tissue matrix. More recently, Badmus et al. (2019) reported that the bioactive compounds were heat-labile and subjected to loss during [illegible]weed drying. The levels of TPC and TFC observed in this study were lower than those in Neoh et al. (2016), in which they reported higher TPC for vacuum-dried Malaysian red seaweed extracts followed by oven, freeze, and sun-dried extracts. This discrepancy could be the consequences of volatile compounds that lost during the oven drying (Wong and Chikeung Cheung 2001). There are, however, other possible discrepancies that could be related to different cultivars (Malaysian red seaweed) and dr[illegible]g temperatures (sun drying, 32 °C/4 days; oven drying, 60 °C/29 h; vacuum drying, 60 °C/24 h; freeze-drying, −86 °C/48 h) used. Moreover, Neoh et al. (2016) conclusions that shorter drying times in vacuum drying r[illegible]lted in higher TPC were linked with findings reported by Silva et al. (2019), w[illegible]h suggested that oven drying is an efficient drying method to stabilize *Ulva rigida*, *Gracilaria* sp., and *Fucus vesiculosus* for the extraction of pigments and polysaccharides. In accordance with the present results, the previous study by Cruces et al. (2016)

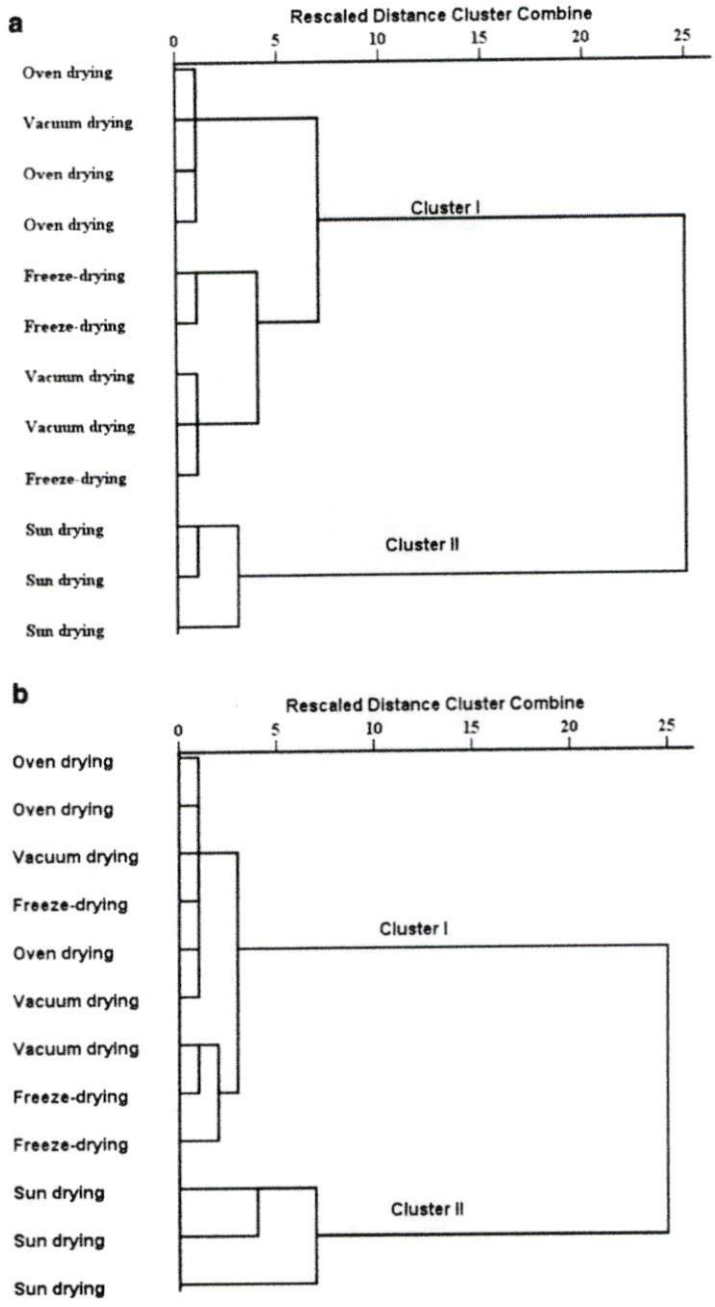

Fig. 3 Dendrogram of hierarchical cluster analysis (HCA) of two seaweed cultivar extracts as affected by drying techniques. *Kappaphycus alvarezii* (A) and *Sargassum duplicatum* (B). Each drying technique represented with three independent replicates ($n = 3$)

demonstrated that oven drying was the reliable method for the preservation of antioxidant properties of phlorotannins (phenolic compounds). Norra et al. (2016) showed significantly higher TPC for oven-dried (50 °C/24 h) Malaysian brown seaweed (*Sargassum* sp.); however, several studies indicated that freeze-drying is a suitable drying technique to preserve bioactive compounds from seaweed cultivar extracts (Neoh et al. 2016; Silva et al. 2019). Nevertheless, freeze-drying has remained expensive and unaffordable for fishing communities in low- and middle-income countries. Although losses

of phenolic compounds were minimal during oven drying, we recommended replacing sun drying with the oven-drying technique as a better alternative for industrial processing of seaweed cultivars (e.g., for the development of value-added seaweed products).

On the other hand, the percent inhibition between the oven- and vacuum-dried extracts exhibited similar radical scavenging activities, which followed the same tendency as observed for the DPPH˙ method. Moreover, both freeze-dried extracts demonstrated insignificant differences in percent inhibition compared with oven- and vacuum-dried seaweed extracts using DPPH˙ (*K. alvarezii*) and ABTS˙ (*S. duplicatum*) methods. This confirmed the similarities in the effects of drying techniques on antioxidant activities of seaweed extracts. Oven-dried extracts statistically reported higher FRAP values than those of other three drying techniques for both seaweed cultivars. These findings agreed with the recent study by Neoh et al. (2016), who reported higher FRAP values for Malaysian red seaweed using oven drying. Moreover, these findings supported the evidence from previous observations by Ling et al. (2015), in which they concluded stronger scavenging and reducing abilities for oven-dried (40 and 80 °C) *K. alvarezii* extracts. However, the few studies that recommended a freeze-drying technique for drying of seaweed (Wong and Cheung 2001; Cruces et al. 2016; Neoh et al. 2016; Silva et al. 2019) seemed to have succeeded in creating a dispute over the use of this expensive technique in drying seaweed. Therefore, evaluation of drying effect on bioactive compounds without proper statistical studies could be considered as purely theoretical, and hence, such studies have failed to address the more serious need to recommend cheaper and affordable drying techniques to low- and middle-income fishing communities.

The results obtained from principal component analysis (PCA) were comparable with color characteristics of seaweed cultivars (Table 1), where drying techniques (oven, vacuum, and freeze-drying) statistically reported similar findings in color change over the sun-drying technique. Groups I and II highlighted the discrimination behavior of drying techniques that could be contributed to the novelty of our hypothesis using PCA. More recently, a study investigated by Hamid et al. (2018) performed the PCA for two drying techniques (oven drying: 40 and 80 °C and freeze-drying) using brown seaweeds and concluded clear separation between the oven- and freeze-dried samples based on metabolite concentration. In our study, the findings from PCA showed that the drying techniques (oven, vacuum, and freeze) had similar color characteristics, and thus, either drying techniques could be used to preserve the color of seaweed cultivars for the formulation of seaweed enriched functional foods. However, as vacuum- and freeze-drying techniques are more expensive for use in low- and middle-income countries, oven drying could be a cheaper alternative among these three drying techniques.

In the hierarchical cluster analysis (HCA), observed homo- and/or heterogeneities in cluster analysis were attributed to the different drying techniques used. Moreover, these results agreed with the PCA results and thus are compatible with the findings reported on the effects of drying and extraction of edible brown seaweed, in which one study highlighted the influence of drying techniques on the formation of clear clusters (Hamid et al. 2018). A study has established the effects of drying techniques on chemical constituents of Malaysian red seaweed (Neoh et al. 2016); however, we would recommend using HCA to further investigate and clarify the relationship between the drying techniques and chemical constituents. Recently, we have investigated the differences and/or similarities in grape extracts (Sridhar and Charles 2018), seaweed cultivars (Charles and Alamsjah 2019), and solvents used for extraction (unpublished observations) using HCA. These prior findings using HCA motivated this study and were recommended to deepen understanding of the influence of drying techniques on color characteristics. Generally, color is an attractive parameter in the consumer market that influences consumer acceptance and willingness to buy. The data from HCA could be useful to seaweed-processing industries and/or fishing communities for the selection of an efficient drying technique (for example, oven drying) for the potential applications in the development of seaweed products than other drying techniques (sun, vacuum, and freeze-drying).

Conclusions

The purpose of this study was to determine the effect of drying techniques on color and antioxidant potential of *K. alvarezii* and *S. duplicatum*. Additionally, multivariate statistical methods were employed to discriminate drying techniques by instrumental color analysis. All the drying techniques reported a significant effect on phenolics and antioxidant activities of the two seaweed cultivar extracts. Moreover, PCA and HCA highlighted the similarities and/or dissimilarities among the drying techniques with two well-defined groups. Hence, from this study, we recommended employing PCA and HCA methods to screen and select a suitable drying technique. The findings suggested that oven, vacuum, and freeze-drying demonstrated high similarities in their effect on color characteristics and resulted in preserving the color of the dried products, and thus were recommended for the development of seaweed-enriched products at an industrial level. For this reason, color is the most important visual attribute of product appearance that influences the consumers' purchase intention. Therefore, color is a strategic parameter for commercialization of seaweed value-added products. Hence, among the three drying techniques, we proposed oven drying (at a recommended temperature of 50 °C), which might be easily adapted to improve

drying of seaweeds (to preserve the color attributes) in fishing communities of low- and middle-income countries.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest

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