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Application of chemometric techniques: An innovative approach to discriminate two seaweed cultivars by physico-functional properties

Albert Linton Charles[a,*], Mochammad Amin Alamsjah[b,*]

[a] Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, 1 Shuefu Road, Neipu, Pingtung 912 01, Taiwan

[b] Faculty of Fisheries and Marine, Universitas Airlangga Campus C Universitas Airlangga, Mulyorejo, Surabaya 601 15, East Java, Indonesia

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ABSTRACT

Chemometric techniques were employed to discriminate two Indonesian seaweed cultivars (*Kappaphycus alvarezii* and *Sargassum duplicatum*) based on their physico-functional properties. Data of physico-functional properties, including emulsifying activity and bulk density, were processed using multivariate, discriminant analysis (DA), principal component analysis (PCA), and cluster analysis (CA). Significant differences ($p < 0.05$) and discrimination (multivariate analysis ($F_{9,14} = 57.85$; Wilks's lambda = 0.02, $p < 0.0001$)) were determined among physico-functional properties of seaweed powders. In addition, DA method clarified ($\Lambda = 0.02$ and $\chi^2 = 63.74$) variations between samples along with two discriminate functions. Moreover, PCA (49.63% with Eigenvalues > 1) and CA (clusters 1 and 2) methods confirmed that the seaweed samples possessed differing physico-functional properties, which implied their distinctive use in food product development. Overall, our results demonstrated strong variation in seaweed powers using chemometrics, which might contribute to the rapid applicability of chemometrics in evaluating novel food material.

1. Introduction

Historically, the consumption of marine-based food products has been progressively gaining consumer attention over the last few years, which is now being promoted into commercial food applications. Among the cultivated marine organisms, marine macroalgae or seaweed, plant-like organisms (Kılınç, Cirik, Turan, Tekogul, & Koru, 2013) that grow in seas and oceans, are gaining access to the consumer market due to their promotion as rich sources of natural antioxidants (Kadam & Prabhasankar, 2010; Roohinejad et al., 2017). A survey conducted by Global Market Insights (Global Market Insights Inc., 2018) reported that the value of the commercial seaweed market is anticipated to be over USD 87 billion by 2024. The high consumption of seaweed in Asian countries, particularly Japan, China, Korea, Vietnam, Indonesia, and Taiwan (Benjama & Masniyom, 2012) is also projected to contribute to the global seaweed market. High consumption and availability of seaweed in Asian countries might be attributed to different sources (farmed and wild). There are four different species of seaweed cultivars that are classified based on color: blue – green, red, brown, and green (Kılınç et al., 2013).

Seaweed powder is used as a food supplement to replace many functional ingredients in the food processing industry. For instance, a survey conducted by Kadam and Prabhasankar (2010) reported that seaweed powder could substitute pasta ingredients in pasta, which resulted in improved protein and fat contents. Therefore, seaweed powder is potentially rich source of functional ingredients and of good alternate source for a food supplement. For the successful application of seaweed powder in the functional food industry, it is necessary to understand the physical properties of seaweed powder or flours that may have influence on functional property of seaweed powder. Several studies reported that physical properties of food materials are essential considerations to design food product and to understand the processing behavior of foods (Berk, 2018; Masood & Trujillo, 2016). For instance, Mamat, Akanda, Zainol, and Ling (2018) examined the effect of seaweed powder (*Kappaphycus alvarezii*) on the physico-chemical properties (texture profile, proximate analyses, and sensory evaluation) of muffins and concluded that the physical properties (density and color) of seaweed powder significantly influenced the final textural properties, proximate analyses, and organoleptic properties of muffins. A study on functional properties of *K. alvarezii* and its incorporation in fish cutlet and fried snacks suggested that the high-water holding capacity and solubility index of *K. alvarezii* effected the textural quality of the developed products (Senthil, Mamatha, & Mahadevaswamy, 2005). These studies clearly indicated that the physico-functional properties of

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* Corresponding authors.
E-mail addresses: albert.lintoncharles@mail.npust.edu.tw (A.L. Charles), alamsjah@fpk.unair.ac.id (M.A. Alamsjah).

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seaweed powders of different cultivars exert pronounced effect on quality parameters of the food product. Thus, there is a need to focus on evaluating physico-functional properties of seaweed powders of different cultivars.

Chemometrics is the scientific application of mathematical and statistical methods designed to measure the magnitude of the correlation between quality or physical factors and analytical instrumental data, which is used to interpret chemical information. These techniques are commonly employed tools in the collection (Sridhar & Charles, 2018) and evaluation of food data (Barbosa et al., 2019) in the field of food science and technology. Recent evidence suggests that these methods can be applied to determine the contribution of random variables in distinguishing the cultivars based on antioxidant activity (Sridhar & Charles, 2018). More recently, VIII Brazilian Chemometrics Workshop was held exclusively to discuss the application of chemometric tools for the evaluation and calibration of food-related studies using multivariate analysis (Ferreira et al., 2019). This approach corroborates the central idea of our study using chemometrics to discriminate two seaweed cultivars based on their physico-functional properties. Indonesia is a well-known archipelago contributing 2/3 of its territory to the ocean with the longest coastline in the world (Nurjanah, Nurilmala, Anwar, Luthfiyana, & Hidayat, 2017). *Kappaphycus alvarezii* (red algae) and *Sargassum duplicatum* (brown algae) are popular (farmed and wild) seaweed species in Indonesia. *K. alvarezii* belongs to Rhodophyceae, whereas *S. duplicatum* belongs to Phaeophyceae (Nurjanah et al., 2017). To date, few studies have investigated the physical and functional properties of seaweed; however, none has reported on the relative contribution of each seaweed cultivar based on physico-functional properties of seaweed powder. Recently, many researchers (Belchior, Botelho, Oliveira, & Franca, 2019; Efenberger-Szmechtyk, Nowak, & Kregiel, 2018; Melucci et al., 2019) have successfully applied chemometrics in quality evaluation of food and beverages, including to confirm food authenticity (e.g. natural and adulterated honey), to identify food adulterations or mislabeling, to determine food safety, to discriminate espresso coffees with different sensory characteristics, and to analyze food data. Hence, we were interested to apply chemometric analytical tools to evaluate the physico-functional properties that could discriminate the two seaweed cultivars used in this study.

Therefore, the main objectives of this study were to investigate the physico-functional properties in *K. alvarezii* and *S. duplicatum* seaweed powders of Indonesia. Moreover, we further investigated the relative contribution of physico-functional properties (yield, color, pH, moisture content, water holding capacity (WHC), oil holding capacity (OHC), water swelling capacity (SC), solubility index (SI), emulsifying activity (EA), density (loose-packed bulk density), and particle shape (aspect ratio)) using chemometrics to distinguish *K. alvarezii* and *S. duplicatum* seaweed powders based on the contributions of each physico-functional property. The contributions of the study could advance the practicability of chemometrics in the optimization of food processing technologies and to promote the application of seaweed in food product development.

2. Materials and methods

2.1. Reagents and instrument

Corn oil was purchased from Yuanshun Factory, Taiwan. Colorimetric characteristics were recorded using a colorimeter (ZE 2000, Nippon Denshoku Industries Co., Ltd) and moisture analyzer (MX – 50, A & D Company Ltd, Japan) was used to estimate moisture content. Microscope (Olympus Image Corporation, Japan) equipped with a digital camera (Model E – 330) was used to measure particle shape. All reagents in experiment were of analytical grade and were used to prepare solutions with deionized water (diH$_2$O).

2.2. Study area and sample description

All seaweeds samples used in the study were given as a gift from the Department of Marine and Fisheries of Universitas Airlangga, Surabaya, Indonesia. Seaweeds cultivars of farmed *Kappaphycus alvarezii* (red algae) and wild *Sargassum duplicatum* (brown algae) were collected from the coastal area of Talango Islands – Madura (7° 0′ 0″ S and 113° 20′ 0″ E), East Java province, Indonesia, during the month of August 2018 and were identified at the Research Center of Oceanography, Jakarta, Indonesia. Samples were fully sun dried and were transported to National Pingtung University of Science and Technology (NPUST), Taiwan in LDPE ziplock pouch (280 × 400 mm). The seaweed samples were stored in a humidity-controller until experimental analysis (usually within 5 h).

2.3. Sample preparation

The seaweed samples were thoroughly cleaned with running and deionized water to remove surface adhering salts and other foreign residues (epiphytes, shells and sand). The samples were then air dried at room temperature and placed in a freezer (−20 °C) for 12 h. All seaweed samples (600 g) were dried using a freeze dryer (Kansas, USA) at the condenser temperature of −50 °C for 3 days and then ground to a fine powder using a laboratory grinder (Yu Chi Machinery Co., Ltd., Taiwan), sieved by certified standard sieves (US Standard Sieve Series, ASTME No. 20 and Tyler Standard Sieve Series – 20 Mesh). The dried samples (Supplementary Fig. S1) were stored in airtight plastic ziplock reclosable packing bags (120 × 170 mm) and placed in a digital dry cabinet (RH: 50%) at room temperature until further analysis for no longer than 7 days.

2.4. Physico-functional properties of seaweed powder

All seaweed samples were subjected to various physical and functional analyses, such as yield, color, pH, moisture content, water holding capacity (WHC), oil holding capacity (OHC), water swelling capacity (SC), solubility index (SI), emulsifying activity (EA), density (loose-packed bulk density), and particle shape (aspect ratio). All physico-functional properties were carried out in 12 replications to perform chemometric analysis. We prepared 12 sample powders from each seaweed cultivars according to a study conducted by Alkarkhi, Ramli, Yong, and Easa (2011) and Sridhar and Charles (2018).

2.4.1. Yield

The yield of seaweed powder was estimated by the quantity of powder obtained divided by the quantity of fresh seaweed used and expressed in g/g (g of seaweed powder/g of seaweed used).

2.4.2. Colorimetric characterization

The surface color of seaweed powder was measured using a colorimeter (ZE 2000, Nippon Denshoku Industries Co., Ltd), which was calibrated by a standardized white glossy ceramic tile (Arufe et al., 2018). Color of seaweed powders were evaluated by means of CIELAB coordinates: L (black to white), a (red to green), and b (blue to yellow). Total color difference (ΔE) was calculated by taking the ground seaweed powder without sieving (mixture of all fractions) as a reference (Arufe et al., 2018) according to the following Eq. (1).

$$\Delta E = [\Delta L^2 + \Delta a^2 + \Delta b^2]^{\frac{1}{2}} \quad (1)$$

where, ΔL = difference in lightness, Δa = difference in intensity (red color), and Δb = difference in intensity (yellow color).

2.4.3. pH measurement

The pH of seaweed powder was measured using a pH meter (Denver Instruments, UB – 10) according to the procedure of Choi, Kum, Jeon, Park, Choi, Hwang, et al. (2015). Seaweed powder samples (5 g) were homogenized with 20 mL deionized water for 5 min. The homogenized suspension was used for pH measurement.

2.4.4. *Moisture content*

The moisture content of seaweed powder was estimated using the moisture analyzer (MX – 50, A & D Company Ltd, Japan). The Moisture analyzer is designed based on heating method (halogen lamp: straight type, 400-Watt, 5000 h) and measured according to automatic increase of temperature every 5 min by 20 °C. RsTemp (heating temperature determination software) was used for the measurement and expressed as % moisture content.

2.4.5. *Water holding capacity (WHC)*

Water holding capacity of samples were estimated based on the conceptual framework proposed by Chan and Matanjun (2017) with some modifications. Briefly, seaweed sample (0.50 g) was mixed in a centrifuge tube with 25 mL distilled water and kept at room temperature for 1 h. The sample mixtures were centrifuged (Universal 32 R centrifuge, Germany) at room temperature (3000 × g) for 25 min. The supernatant was decanted and the samples were allowed to drain for 10 min at a 45° angle. WHC was calculated as shown in Eq. (2).

$$\text{WHC (g/g)} = \left[\frac{\text{fresh weight of residue (g)} - \text{dry weight of residue (g)}}{\text{dry weight of residue (g)}}\right] \tag{2}$$

2.4.6. *Oil holding capacity (OHC)*

The oil holding capacity (OHC) of samples were estimated according to Chan and Matanjun (2017) with some modifications. Briefly, 0.50 g of seaweed samples were mixed with corn oil (20 mL) in a centrifuge tube (pre-weighed) and allowed to stand for 1 h at room temperature. After incubation, the reaction mixtures were centrifuged (Universal 32 R centrifuge, Germany) at 3000 × g for 20 min. The corn oil supernatant was decanted and the samples were allowed to drain for 10 min at a 45° angle. The density of corn oil was recorded as 0.92 g/mL according to supplier information. OHC was calculated with the Eq. (3).

$$\text{OHC (g/g)} = \left[\frac{\text{fresh weight of residue (g)} - \text{dry weight of residue (g)}}{\text{dry weight of residue (g)}}\right] \tag{3}$$

2.4.7. *Water swelling capacity (SC)*

The water swelling capacity (SC) of the seaweed powders was estimated by the method explained by Chan and Matanjun (2017). Briefly, 0.50 g of sample was mixed with 10 mL of distilled water and the mixture was vigorously stirred in a graduated cylinder (10 mL) and left at room temperature for 18 h. SC of seaweed powder was measured using Eq. (4) and expressed as mL of sample volume per gram of sample.

$$\text{SC (mL/g)} = \left[\frac{\text{Volume occupied by sample (mL)}}{\text{dry weight of sample (g)}}\right] \tag{4}$$

2.4.8. *Solubility index (SI)*

Solubility index (SI) of sample was determined by adapting the procedure used by Bashir, Swer, Prakash, and Aggarwal (20[illegible] Sample (2.50 g; m_0) was taken in pre-weighed centrifuge tube and mixed with 30 mL of deionized water. The mixture was vortexed (Vortex mixer-VM-2000, Digisystem Laboratory Instruments Inc., Taiwan) for 1 min and placed in an isothermal reciprocal shaking bath (SB 302, Double Eagle Enterprises Ltd, Taiwan) with an accuracy of ± 0.02 °C – ± 0.05 °C at 50 °C and with regular vortex for every 5 min to 30 min. Isothermal conditions were maintained with a microcomputer proportional–integral–derivative (PID) digital temperature control equipped with the water shaker bath and the standard uncertainty for the temperatures u (T) was found to be ± 0.20 K. The samples were then cooled to room temperature and centrifuged (Universal 32 R centrifuge, Germany) at 4500 × g for 15 min. Then the supernatant was immediately dried in moisture dishes (weight recorded) at 110 °C for 12 h until constant value (m_1). The SI of seaweed powder was expressed as g/100 g as shown in Eq. (5).

$$\text{SI (g/100 g)} = \left[\frac{m_1\ \text{(g)}}{m_0\ \text{(g)}}\right] \times 100 \tag{5}$$

2.4.9. *Emulsifying activity (EA)*

Emulsifying activity (EA) of powder samples were determined based on the method reported by Muraguri, Wakibia, and Kinyuru (2016). Powder samples (2 g) were dissolved in distilled water (20 mL), then the mixture was vortexed (Vortex mixer-VM-2000, Digisystem Laboratory Instruments Inc., Taiwan) for 5 min. After 5 min, 20 mL of corn oil was added then vortexed for 5 min. The emulsion suspension was then centrifuged (Universal 32 R centrifuge, Germany) at 2100 × g for 10 min (25 °C). EA of sample was calculated according to Eq. (6).

$$\text{EA (\%)} = \left[\left(\frac{\text{Volume of emulsified layer}}{\text{Volume of suspension}}\right)\text{x100}\right] \tag{6}$$

2.4.10. *Bulk density (loose-packed bulk density)*

The bulk density of seaweed powder was measured according to ISO standard protocol (3923/1) described by Chi-Ying[illegible]ng (2000). The method involved plugging the opening of a conical funnel by a stopper before the conical funnel was charged with seaweed powder, and a measuring cylinder was placed directly underneath the opening of the conical funnel. The plug was carefully removed from the conical funnel to fill the measuring cylinder with seaweed powder. Extreme care was taken and excess powder was removed from the rim of the measuring cylinder. The loose-packed bulk density was calculated from the total weight of the powder (kg) in the measuring cylinder to the total volume of the measuring cylinder that was occupied with seaweed powder (cm^3).

2.4.11. *Particle shape (aspect ratio)*

Particle shape (aspect ratio) of seaweed powder was measured according to the stereomicroscopy method. Briefly, samples were taken randomly from the sample container and set on the FEA microscope slide (size: 1″ × 3″ and thickness: 1–1.20 mm) to avoid overlapping of powder particles. The microscope slide was then placed under a microscope (Olympus Image Corporation, Japan) equipped with a digital camera (Model E-330). Images were analyzed for particle shape parameters (the major axis (l) and the minor axis (b) of particle) using Image-Pro® 10 (available for commercial use – http://www.mediacy.com/imageproplus). Images were calibrated (for conversion to any unit of measurement) by applying the following steps: Capture – Create – Auto Calibration. After auto calibration, Count/Size – > Count tool was employed to initiate a counting and measuring operation on the active seaweed powder images. A study conducted by Bouwman, Bosma, Vonk, Wesselingh, and Frijlink (2004) defined the major axis (l) as a straight line that connects the two most distant points of the projection area, whereas the minor axis (b) is a straight line perpendicular to the major axis, which connects the two points with the largest distance between them. The major and minor axes were identified by the Measurement tool (Measure – > Measurements). Aspect ratio, or common shape factor (φ_{AR}), was calculated as the ratio of the length of the minor and the major axis of particle as shown in Eq. (7).

$$\text{Aspect ratio } (\varphi_{AR}) = \left[\frac{\text{b}}{\text{l}}\right] \tag{7}$$

Table 1
Descriptive statistics for physico-functional properties of seaweed powders.[1]

Parameter	Seaweed powder							
	Kappaphycus alvarezii				*Sargassum duplicatum*			
	Min	Mean	Max	SD^	Min	Mean	Max	SD^
Yield (g/g)	0.427	0.428'	0.428	0.01×10^{-3}	0.442	0.442'	0.442	1.29×10^{-4}
Color	11.44	12.82^{a}	15.03	1.04	8.35	10.05^{b}	14.48	1.52
pH	7.38	7.39^{a}	7.42	0.01	7.24	7.28^{b}	7.34	0.03
Moisture content (%)	7.43	8.18^{b}	8.95	0.41	7.12	8.60^{a}	8.89	0.48
Water holding capacity (g/g)	9.56	10.28^{a}	10.68	0.32	5.96	7.09^{b}	7.56	0.41
Oil holding capacity (g/g)	2.07	2.18^{a}	2.30	0.07	1.39	1.92^{b}	2.23	0.32
Water swelling capacity (mL/g)	4	4'	4	0	4	4'	4	0
Solubility index (g/100 g)	9.84	12.88^{a}	19.60	2.86	8	9.98^{b}	10.96	0.94
Emulsifying activity (%)	10.50	27.70^{a}	57.10	15.50	10.50	14.90^{b}	22.20	4.20
Bulk density (kg/cm^{3})	794	858^{a}	899	30	686	725^{b}	754	20.20
Particle shape (aspect ratio)	1	2.06^{a}	5.16	1.12	1.06	1.84^{a}	3.71	0.85

[1] Mean (n = 12) values followed by different superscripts (a–b) within the same row are significantly different ($p < 0.05$) based on paired *t* test. ^standard deviation and 'paired *t* test cannot computed due to the standard error of the mean difference is zero.

2.5. *Reliability of physico-functional analysis*

Reliability is defined as an assessment of a measurement instrument that causes similar results each time for similar inputs or same type of subjects. Sometimes, reliability provides information on the relationships between individual items in the reliability scale. Generally, intraclass correlation coefficients (ICC) can be used to compute inter-rater reliability studies. Alpha (Cronbach) model commonly used model of internal consistency based on the average of inter-item correlation. We calculated ICC using IBM® SPSS® Statistics version 22.0 (Analyze > Scale > Reliability Analysis…) according to Sridhar and Charles (2018).

2.6. *Chemometric analysis (IBM® SPSS® statistics version 22.0)*

2.6.1. *Multivariate analysis of variance (MANOVA)*

Multivariate analysis of variance (MANOVA) is a generalized procedure for analysis of variance (ANOVA) and covariance with several continuous dependent variables at a time. MANOVA is a powerful tool used for both univariate and multivariate designs. There are two major situations where MANOVA can be employed, such as to understand the influence of independent variables on the patterning of response variables and to test the effect of independent variables on several continuous dependent variables at a time (Sridhar & Charles, 2018). Moreover, MANOVA further allows the performance of several tasks like specifying nesting of effects, partition and pooled effects in models, and test user-specified special contrasts with multiple factors (IBM® SPSS® Statistics version 22.0). We employed MANOVA (Analyze > General Linear Model > Multivariate…) based on the MANOVA algorithms, which performs univariate and multivariate analysis of variance and covariance for any crossed and/or nested design according to IBM® SPSS® Statistics version 22.0 (2013).

2.6.2. *Discriminant analysis (DA)*

Discriminant analysis (DA) is a predictive model used for group membership. This model is designed with discriminant function (s) (discriminant functions (DFs) or canonical vector) based on linear combinations of the predictor variable to identify the best discrimination between the groups. DFs are generated from a data points (cases) for which group membership is known. These function(s) can be employed to new data points (cases) that have measurements for the predictor variables without unknown group membership. We used DA model (Analyze > Classify > Discriminant…) based on its relationship to one or more predictors according to IBM® SPSS® Statistics version 22.0 (2013). DA estimates coefficients (a_1, a_2, a_3 ,… a_n) of the linear DFs as shown in Eq. (8).

$$DF = a_1X1 + a_2X2 + a_3X3 + \cdots + a_nXn + A \tag{8}$$

in which, DF = discriminant score, a = discriminant coefficients, X = discriminating variables, and A = constant.

2.6.3. *Principal components analysis (PCA)*

Principal components analysis (PCA) is a variable-reduction statistical method, which is analyzed to reveal major dimensions of variation. The main application of PCA is to extract maximum variance in the raw data points from the larger set of data points to a few principle components (linear combinations of the original variables) (Yong & Pearce, 2013). A factor extraction method (factor analysis – extraction: principle components) was employed to form uncorrelated linear combinations of the observed variables (Analyze > Dimension Reduction > Factor…) based on Eigenvalue > 1 according to IBM® SPSS® Statistics version 22.0 (2013).

2.6.4. *Hierarchical cluster analysis (HCA)*

Cluster analysis (CA) is a class of statistical techniques that are commonly applied to classify natural groupings or clusters of the objects. The basic criteria for any CA are based on distance between two clusters. At each stage of the cluster analysis, the criterion by which objects are separated is relaxed in order to link the two most similar clusters until all of the objects are joined in a complete classification tree. HCA is the most commonly used method of cluster analysis for small number of objects, which produces a sequence of nested partitions (Sridhar & Charles, 2018). We classified seaweed powders (cultivars) using HCA method – between-groups linkage with a measurement of distance between two points based on single linkage matrix updating algorithm method (IBM® SPSS® Statistics version 22.0). Similarities between two cluster (X_i and Y_j) is measured based on the Euclidean distance between two clusters or pair of objects (X_i and Y_j) as described by Alkarkhi et al. (2011) using the Eq. (9).

$$D(X_i, Y_j) = \text{minimum}_{x,y}\{d(x, y) | x \in X_i, y \in Y_j\} \tag{9}$$

3. Results and discussion

The physico-functional properties of two seaweed powders are presented in Table 1. Statistical analysis of physico-functional properties between the seaweed cultivars indicated significant differences ($p < 0.05$); however, there was no significant difference in particle shape (aspect ratio) mean values between *Kappaphycus alvarezii* (2.06) and *Sargassum duplicatum* (1.84). The similarity in particle aspect ratio might be due to grinding and sieving method used in sample preparation. The efficacy of yield, which ranged from 0.428 g/g (*K. alvarezii*) to

0.442 g/g (*S. duplicatum*), indicated that seaweed cultivars yielded relatively same trends with zero mean differences. The color characteristics of the *K. alvarezii* seaweed powder exhibited more lightness (51.34 ± 0.50) and less red to green (−0.31 ± 0.04) and yellowness (6.14 ± 0.27) compared with *S. duplicatum* color characteristics. The visual appearance of seaweed powders further confirmed the differences in color characteristic of seaweed cultivars as shown in Supplementary Fig. S1. Trending related studies classify different seaweed cultivars based on their color characteristics (Kılınç et al., 2013). The pH values of *K. alvarezii* and *S. duplicatum* were determined as neutral pH of 7.39 and 7.28, respectively. The pH values of the seaweed powders were slightly higher compared to a previous study on pH of four seaweed cultivars (Choi et al., 2015) widely grown in South Korea. The slightly higher pH of our samples was attributed to environmental differences of these two regions. *S. duplicatum* reported significantly higher moisture content (8.60 ± 0.48%) than *K. alvarezii* (8.18 ± 0.41%). *K. alvarezii* exhibited higher mean values for water holding capacity (WHC) (10.28 ± 0.32 g/g) and solubility index (SI) (12.88 ± 2.86 g/100 g), whereas *S. duplicatum* showed lower mean values for WHC (7.09 ± 0.41 g/g) and SI (9.98 ± 0.94 g/100 g). These values were in conformity with the studies investigated on WHC and SI of seaweed (*Fucus vesiculosus*) powders (Arufe et al., 2018). The variations in WHC and SI were attributed to intrinsic botanical differences of the cultivars. There are, however, other possible reasons including other solubility of fibres and proteins (Yaich et al., 2011) as well as nature of the water binding sites on the protein molecules and their conformation (Chan & Matanjun, 2017), which affected the functional properties of the seaweed powders. Similarly, oil holding capacity (OHC) followed the same tendency as WHC in both seaweed powders, which strongly supported the potential application of seaweed powders in the formulation of high-fat food products and emulsions. Several studies reported that the efficacy of OHC was due to physical entrapment of oil, hydrophobicity nature of proteins, and hydrophilic nature of the seaweed powder (Yaich et al., 2011). The highest mean values of emulsifying activity (EA) were 27.70 ± 15.50% in *K. alvarezii*, whereas the lowest in *S. duplicatum* (14.90 ± 4.20). Previous studies have investigated the EA of different seaweed cultivars, particularly seaweed powders and reported good emulsification properties, a factor that is widely applicable in food processing industries (Muraguri et al., 2016), and which indicates the usefulness of both seaweed powders in emulsions such as ice cream and salad dressings. Bulk density was 858 ± 30 kg/cm^3 in *K. alvarezii* and 725 ± 20.20 kg/cm^3 in *S. duplicatum*. These differences in bulk densities of powders were partly attributed to moisture content of seaweed cultivars. A study by Chi-Ying Wong (2000) stated that bulk density is affected by the moisture content of powdered materials. In our study, *K. alvarezii* exhibited lower moisture content and is attributed to this cultivar's higher loose-packed bulk density. On the other hand, other factors including particle size and shape (coarse and fine) appeared to have little or no effect on bulk density. Characterization of particle shape was explained by aspect ratio, which was found to be 2.06 (*K. alvarezii*) and 1.84 (*S. duplicatum*). Physico-functional properties like yield, color, pH, moisture content, WHC, OHC, SI, and particle shape exhibited less deviations from mean values except for EA and bulk density in seaweed powders, which could be related to several factors including particle size (in case of bulk density) and or experimental/handling errors. The findings from this study made several contributions to the current literature by providing useful and practical information on physico-functional properties of seaweed powders to food and chemical processing industries, which requires the understanding and information of their use in emulsions.

3.1. Reliability of physico-functional analysis

The intraclass correlation coefficient of physico-functional methods used for seaweed powders is highlighted in Supplementary Table T1. The data revealed that the intraclass correlation coefficient was similar ($\alpha = 1$) in both seaweed powders, which confirmed the reliability of the physico-functional methods. A recent study defined the range of Cronbach's alpha coefficient (< 0.40, the method is unreliable; 0.40–0.59, low reliability; 0.60–0.79, quite reliable; 0.80–1.00, highly reliable) for the reliability of the method used (Yılmaz, Dişsiz, Demir, Irız, & Alacacioglu, 2017). Hence, according to the above study as well as our study, our data (Supplementary Table T1) confirmed and supported the reliability of the internal consistency of applied physico-functional methods. High reliability in our study further demonstrated that the measurement errors were negligible; however, observed deviations from mean values of physico-functional properties (Table 1) of seaweed powders was possibly due to the presence of outliers in the experimental data. High Cronbach's alpha coefficient with minor deviations were also observed in reliability studies of the antioxidant assay using grape extracts in previous work (Sridhar & Charles, 2018). All 12 replicates were considered for each physico-functional method, which if deleted, impacts Cronbach's alpha coefficient. Therefore, 12 replicated were used for the reliability analysis. These results demonstrated that the physico-functional methods for determination of physico-functional properties of seaweed powders were reliable and applicable in further use in laboratories of developing or less developed countries.

3.2. Multivariate general linear analysis

A series of multivariate general linear models were employed to perform significance tests with physico-functional properties (yield, color, pH, moisture content, water holding capacity, oil holding capacity, water swelling capacity, solubility index, emulsifying activity, bulk density, and particle shape) as dependent variables and seaweed powders as independent variables (Table 2). Multivariate significant associations were further analyzed by MANOVA significant tests (Pillai's trace, Wilks's lambda, Hotelling's trace, and Roy's largest root). The analyses revealed a significant ($p < 0.0001$) relationship between seaweed powders based on physico-functional properties as shown in Table 2. The values of the four significance tests (Pillai's trace = 0.97, Wilks's lambda = 0.02, Hotelling's trace = 37.19, and Roy's largest root = 37.19) statistically exhibited consistent significance in all multivariate general linear analyses. The reported MANOVA significant tests were all important to reject or accept the null hypothesis based on the nature of the hypothesis. Therefore, based on Levene's test of equality of error variances, Wilks's lambda significant test ($F_{9,14} = 57.85$; Wilks's lambda = 0.02, $p < 0.0001$) convincingly explained the significant associations between seaweed powders based on physico-functional properties. The findings were also in accord with our earlier observations, in which Wilk's lambda was used to interpret results of multivariate general linear analyses (Sridhar & Charles, 2018). These results also contributed to the application of multivariate general linear analyses in understanding the role of physico-functional properties in discriminating two seaweed powders.

3.3. Discriminant analysis (DA)

Discriminant analysis (DA) was applied to determine the most

Table 2
Multivariate general linear analysis of seaweed powders.[1]

Test	Value	F-value	Hypothesis df	Error df	p-value
Pillai's Trace	0.97	57.85	9	14	< 0.0001
Wilks' Lambda	0.02	57.85	9	14	< 0.0001
Hotelling's Trace	37.19	57.85	9	14	< 0.0001
Roy's Largest Root	37.19	57.85	9	14	< 0.0001

[1] df is degree of freedom.

Table 3A

Eigenvalue and canonical correlation of discriminant function for the two seaweed powders.

Function	Eigenvalue[1]	% of variance	Cumulative %	Canonical correlation
1	37.19	100	100	0.987

[1] Canonial discriminate functions were used in the analysis.

Table 3B

Wilks' Λ and χ^2 value of discriminant test function(s) for the two seaweed powders.[1]

Test of Function (s)	Wilks' Λ	χ^2 value	df	*p*-value
1	0.02	63.74	9	< 0.001

[1] df is degree of freedom.

useful and significant variables in the differentiation of seaweed powders based on physico-functional properties. The DA employed using data obtained from 11 physico-functional parameters of each seaweed powders to determine the discrimination function(s) for the new classification of seaweed powders. Table 3A presented the first canonical linear discriminant function to discriminate the two seaweed powders with the proportion of variance (Eigenvalue – the ratio between the between-groups and within-groups sums of squares) and discriminating ability of the continuous variables (physico-functional properties) associated with a discriminant function. The findings revealed that the higher Eigenvalue (37.19) and 100% recognition variance explained the proportion of discriminating ability of a test function (Table 3A). Several reports suggest that larger Eigenvalues are associated with strong discriminant function. Moreover, an association between the discriminant score of seaweed powders and groups in the dependent variable were reported as canonical correlation (Table 3A). High canonical correlation of 0.987 indicated a high level of association between the groups. The significance of the discriminant function(s) was selected based on Wilk's Lambda (Λ) and Chi-square (χ^2) as shown in Table 3B. Wilk's Λ is one of the multivariate selection algorithms calculated by the product of values, 1 – canonical correlation^2, (Wilk's Λ = 1–0.987^2) for the measurement of discrimination between groups. The lower value for Wilk's Lambda (Λ = 0.02) and higher χ^2 value (63.74) indicated significantly greater discriminatory ability of the function for the classification method at the probability level of < 0.001.

Canonical discriminant function coefficients were calculated to discriminate the two groups of seaweed powders, and the discriminant score for each physico-functional property in each DF was determined using Fisher's linear discriminant functions according to following Eqs. (10) and (11).

$$\begin{aligned} \mathrm{DF}_1 = {} & \mathrm{Yield}^{\dagger} - 157.85\,\mathrm{color} + 21078.50\,\mathrm{pH} - 329.64\,\mathrm{moisture} \\ & + 409.93\,\mathrm{WHC} - 1670.73\,\mathrm{OHC} + \mathrm{SC}^{\dagger} + 1.91\,\mathrm{SI} - 2.40\,\mathrm{EA} \\ & - 7.68\,\mathrm{bulk\ density} + 4.87\,\mathrm{particle\ shape} - 72586.22 \end{aligned} \tag{10}$$

$$\begin{aligned} \mathrm{DF}_2 = {} & \mathrm{Yield}^{\dagger} - 159.79\,\mathrm{color} + 20871.19\,\mathrm{pH} - 320.59\,\mathrm{moisture} \\ & + 383.46\,\mathrm{WHC} - 1659.71\,\mathrm{OHC} + \mathrm{SC}^{\dagger} + 2.31\,\mathrm{SI} - 2.47\,\mathrm{EA} \\ & - 7.85\,\mathrm{bulk\ density} + 6.23\,\mathrm{particle\ shape} - 70777.50 \end{aligned} \tag{11}$$

where, WHC = water holding capacity, OHC = oil holding capacity, SC = water swelling capacity, SI = solubility index, EA = emulsifying activity, and †variables failed in tolerance test.

Two statistically significant discriminant functions (DF_1 and DF_2) were used to explain 100% of the variability in discriminating the seaweed powders as shown in Eqs. (10) and (11). It was apparent from the Eqs. (10) and (11) that few physico-functional properties (color, moisture content, OHC, EA, and bulk density) reported negative magnitudes, whereas pH, WHC, SI, and particle shape exhibited positive magnitudes or were best at discriminating both seaweed powders. The magnitudes of the parameters (physico-functional properties) indicated the power of discriminating variables effect in differentiating the seaweed powders. It was interesting to note that the SI and EA influenced less, whilst pH and OHC strongly impacted the discrepancies between the two seaweed powders. However, yield and SC of the two seaweed powders were not computed in Eqs. (10) and (11), which could be possible due to failure in tolerance test (yield and water swelling capacity reported zero variance within-groups) and or the standard error of the difference, which was zero. A key study by Wong and Cheung (2000) stated that variations in contributions were explained in terms of the chemical composition of seaweed cultivars, habitats, and environmental conditions. These variations in seaweed powders might be due to different physico-functional methods that were used to determine physico-functional properties. A recent systematic study investigated by Muraguri et al. (2016) on the functional properties of seaweeds highlighted the differences in the functional properties of selected seaweed cultivars from the Kenya coast. Therefore, based on their findings and those of our study, we confirmed that the physico-functional properties of each seaweed powder played a vital role in discriminating the two seaweed powders.

The correlation analysis between physico-functional properties and standardized canonical discriminant function coefficients for the two seaweed powders based on the Eqs. (10) and (11) are highlighted in Table 3C. The results of the analyses demonstrated that color, pH, WHC, EA, and bulk density positively correlated with standardized canonical discriminant function coefficients, whereas other physico-functional parameters, including moisture content, OHC, solubility index, and particle shape, were negatively correlated, which was attributed to discriminant functions. On the other hand, we predicted the frequencies of the groups using predicted group membership and are summarized in Table 3D. The results were correctly classified according to the samples used for analysis. Moreover, original and cross-validated groups were recorded as 100% in each seaweed cultivar. Hence, these results confirmed that the two cultivars shared no membership based on their physico-functional properties. These findings contributed to understanding the application of DA in the classification of plant and animal products.

3.4. Association between seaweed powders and DFs score

Association between seaweed powders and DFs score were calculated and are illustrated in Fig. 1. The association between these two variables were used to represent discrimination between the seaweed cultivars. The seaweed sample nos. 1–12 represented the seaweed powders (n = 12 for each seaweed powder). It can be noted from Fig. 1, *K. alvarezii* powder samples contributed greatly and positively to discriminant function score, which ranged from 4.23 to 6.96. The highest

Table 3C

Correlation analysis between physico-functional properties and standardized canonical discriminant function coefficients for two seaweed powders.

Parameter	Correlation (r)
Yield†	–
Color	0.22
pH	0.52
Moisture content	−0.35
Water holding capacity	0.84
Oil holding capacity	−0.22
Water swelling capacity†	–
Solubility index	−0.71
Emulsifying activity	0.07
Bulk density	0.38
Particle shape (aspect ratio)	−0.12

† Variables failed in tolerance test (zero variance within-group).

Table 3D
Predicted group membership classification processing for discriminant analysis of the two seaweed powders.

Sample	% correct[1]	Predicted group membership		
		Kappaphycus alvarezii	*Sargassum duplicatum*	Total
Kappaphycus alvarezii	100	12		12
Sargassum duplicatum	100		12	12

[1] 100% of original and cross-validated grouped cases correctly classified.

and the lowest discriminant function scores were observed in sample nos. 1 and 2 (*K. alvarezii*). On the other hand, *S. duplicatum* powder samples exhibited relative negative contribution to discriminant scores (Fig. 1) that ranged from −8.48 (sample no. 9) to −4.16 (sample no.4). These results further supported the idea of variance between the seaweed samples from the origin point of a discriminant functional score (0). It seemed likely that these observed variations were due to different cultivars and physico-functional properties. A study on nutritional evaluation and physico-chemical properties of seaweeds showed that the chemical composition, habitats, and environmental conditions of seaweed cultivars influence variations in discrimination of seaweed cultivars (Wong & Cheung, 2000). Our findings, however from a statistical or chemometric perspective, broadly supported the work of other related studies linking to variations in functional properties among different seaweed cultivars grown in Kenya coast (Muraguri et al., 2016). In conclusion, this is an important finding in understanding the role of seaweed cultivars by discriminating each other (*K. alvarezii* and *S. duplicatum*) based on physico-functional parameters.

3.5. Principal component analysis (PCA)

PCA was performed using the raw data obtained from physico-functional properties investigations of seaweed powders in order to identify the individual characteristics of the seaweed cultivar. Physico-functional properties of seaweed powders were used as active variables, whereas seaweed powders (cultivars) were used as active cases to reduce the measurements of the raw data based on the correlation matrix or variance-covariance of the physico-functional properties. Before conducting PCA, we conducted the series of necessary analysis for original data including correlations between the variables and sampling adequacy (recorded as 0.92). Therefore, we confirmed that the raw data passed the minimum standards for conducting PCA to avoid computational difficulties. Major successive components were extracted with Eigenvalues greater than unity (Eigenvalue > 1), which were explained by each PC. A study conducted by Gniazdowski (2017) concluded that higher Eigenvalues of component accounted for most of the variations for analyzed variables. Similarly, components with Eigenvalue lower than unity accounted for less variance (usual variance of 1). Hence, six components were selected (PC1 – 35.05%, PC2 – 14.58% PC3 – 13.06, PC4 – 10.95%, PC5 – 10.29%, and PC6 – 1.73%), which accounted for almost 85.66% for the cumulative variance of the seaweed powders (Fig. 2). The first two PCs accounted for 49.63% of the total cumulative variance. The component plot rotated space generated from the first two PCs explained the variations in the seaweed cultivars, which reported the differences in correlation with their variables. The projections of the seaweed powders along with the direction on components axis are presented in Fig. 2. In brief, *K. alvarezii* and *S. duplicatum* powder samples were separated along PCs 1 and 2, respectively. Clear separations of the seaweed samples were observed from the centroid, which consequently concluded that the physico-functional profiles were extremely different between the seaweed cultivars. The significant findings from PCA further stated that the seaweed cultivars had different physico-functional properties, which led samples to discriminate each other in PCA and DA. Overall, these findings are in accordance with findings reported by Date, Sakata, and Kikuchi (2012), who investigated biochemical profiling of various seaweeds using PCA and concluded that biochemical profiling was influenced by taxonomical differences between the cultivars. Therefore, based on the above findings, we concluded that PCA successfully highlighted the variances between seaweed powder samples.

3.6. Cluster analysis (CA)

Hierarchical CA was performed using squared between-groups linkage and squared Euclidean distances as a measure of homogeneity of groups between the seaweed powders based on physico-functional properties as shown in Fig. 3. The result of CA demonstrated that the discrimination of seaweed cultivars was grouped into different clusters based on physico- functional parameter, in which 24 seaweed powders

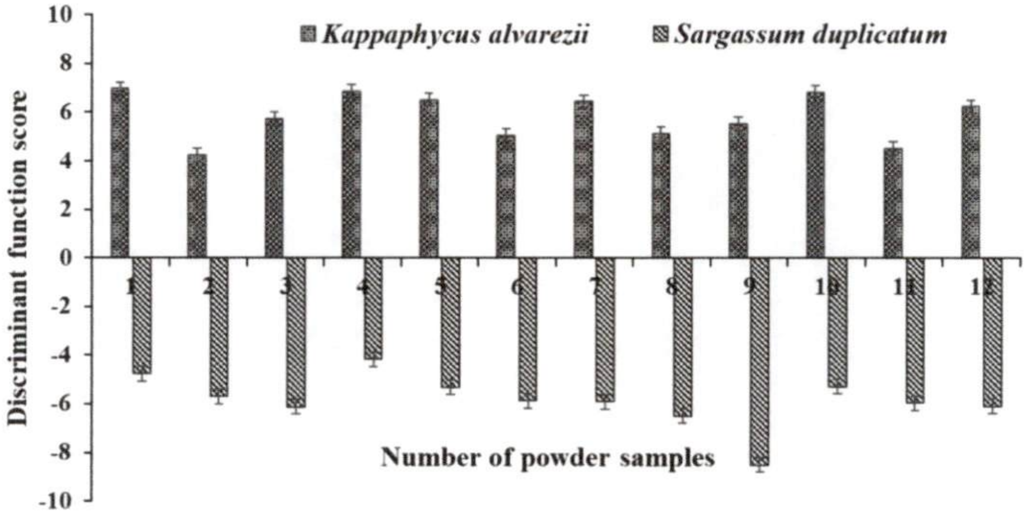

Fig. 1. Discriminant function score for seaweed powders.

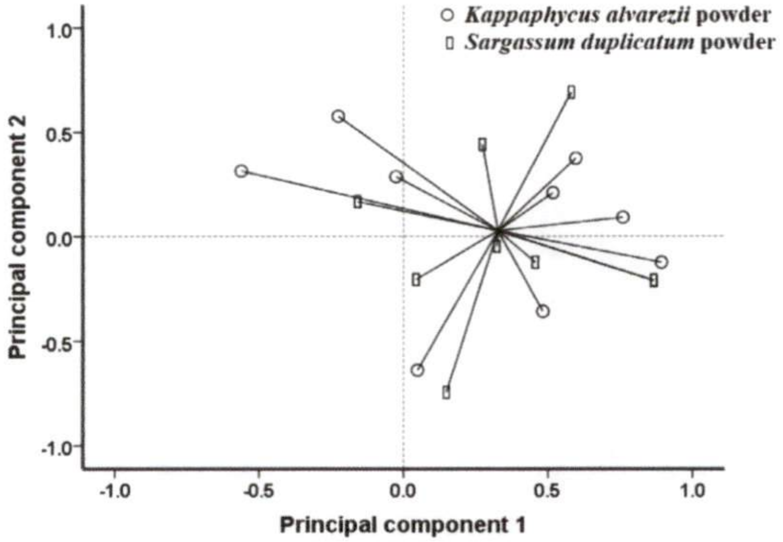

Fig. 2. Principal components plot in rotated space of variances of seaweed powders based on physico-functional properties.

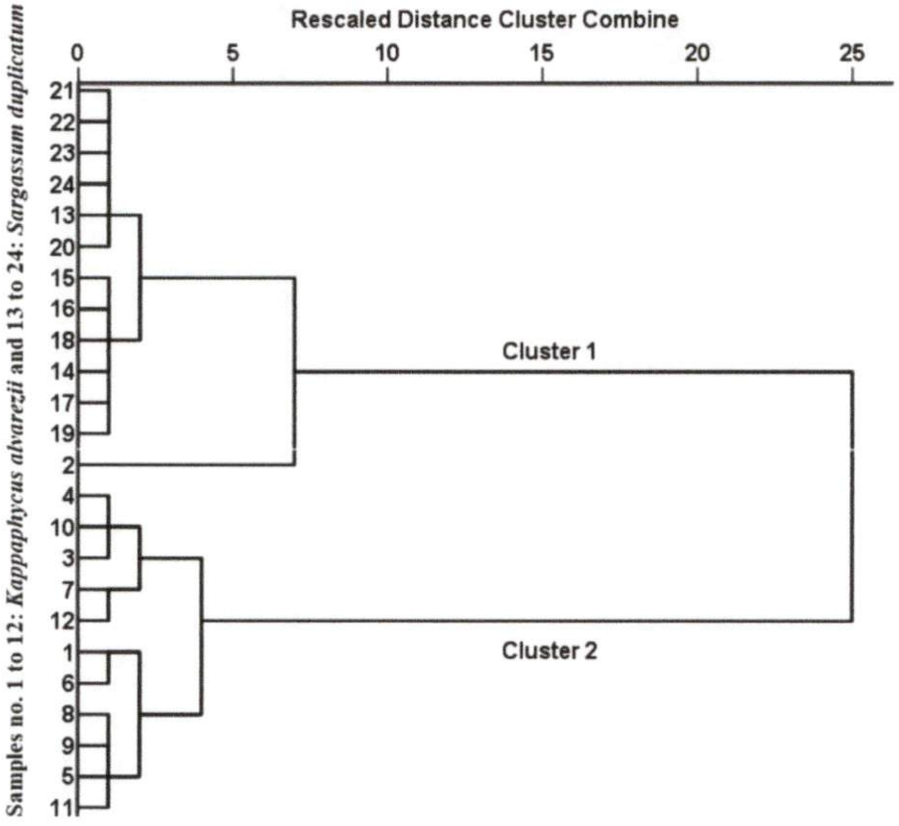

Fig. 3. Dendrogram of hierarchical cluster analysis of seaweed powders.

were well-defined into two main clusters (clusters 1 and 2). Cluster 1 was clearly discernible, which mostly consisted of *S. duplicatum* powder associated with physico-functional properties as measured by physico-functional methods; whereas, a second cluster (cluster 2) mainly consisted of *K. alvarezii* powder. These differences in clusters formation were fairly explained by heterogeneity between the physico-functional parameters of seaweed cultivars. Moreover, it is possible that clusters 1 and 2 were well separated due to variations in physico-functional properties (Table 1). The findings further reported that *K. alvarezii* powder samples formed two small groups (samples no 1 to 12) except for sample number 2, which was formed from the group with *S. duplicatum* powder. However, sample number 2 showed that the large Euclidean distance indicated dissimilarity between the cases. Similarly, a study conducted by Thareja and Trivedi (2010) concluded that short and long Euclidean distance represents the homo- and hetero-geneous clusters, respectively, depending on the average distance. These results were also supported by a recent study on fatty acid and biochemical constitutes of seaweeds by Verma, Kumar, Mishra, and Sahoo (2017), who reported different hierarchical clusters based on the different seaweed cultivars. The variation in the clusters was in agreement with the results of the reported analyses (multivariate general linear, discriminant, and principal component analyses). The present study confirmed the application grouping of seaweed powders based on their physico-functional properties. Further investigation and experimentation are strongly recommended to expand the application of cluster analysis in discrimination of cultivars based on specific criteria.

4. Conclusions

The study was designed to differentiate two Indonesian seaweed powders based on physico-functional properties using chemometric techniques. Wilks's lambda significant test ($F_{9,14} = 57.85$; Wilks's lambda = 0.02, $p < 0.0001$) conclusively reported the significant association between the two seaweed cultivars. In addition, discrimination between the two seaweed cultivars was well defined by Low Wilk's Lamda (0.02) and high Chi-square (63.74) value with two DFs in the discrimination analysis. Moreover, PCA (PCs 1 and 2) and CA methods (clusters 1 and 2) discriminated the two seaweed cultivars according to the evaluation of their physico-functional properties. The study represented one of the first attempts to thoroughly examine the discrimination between these two Indonesian seaweed cultivars in relation to their physico-functional properties. Therefore, the findings of this study could help the seaweed industry to establish a more productive classification of seaweeds using advanced chemometric techniques. Further studies are recommended to broaden the applicability of chemometric techniques on seaweed cultivars for a more innovative approach towards sustainable cultivation, postharvest technology, and food application of seaweed.

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Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.03.051.

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