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Encapsulation of Brown Seaweed Pigment by Freeze Drying: Characterization and Its Stability During Storage

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Abstract

The general objective of this study was to evaluate the stability of encapsulated pigment prepared from brown seaweed (*Sargassum* sp). A simple and rapid extraction procedure was employed to collect brown–algal photosynthetic pigments, followed by encapsulation using freeze drying with maltodextrin and Tween–80 as the encapsulating agents. The product showed yellowish green powdery pigments. Chromatography analysis confirmed the presence of *trans*–fucoxanthin as major carotenoid. Then, the stability of pigment in the freeze–dried powder during dark storage was studied at 28 °C, 45 °C, and 65 °C. The degradation of the surface of the encapsulated pigments was evaluated chromametrically by measuring the L*, a*, and b* values. Finally, the half–life of the encapsulated pigment was estimated.

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Keywords: Brown seaweed; encapsulation; pigment; stability; *Sargassum* sp.

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Nomenclature

sp.	species
w/v	weight per volume
rpm	revolution per minute (60 rpm equal 1 hentz)
CIELAB	Commission Internationale de l'Eclairage
L*	luminosity: black (0) – white (100)
a*	greenness (negative value) – redness (positive value)
b*	blueness (negative value) – yellowness (positive value)
HPLC	High Performance Liquid Chromatography
UV	ultra–violet
ln	natural logarithm
log	logarithm
C	concentration
C_0	initial concentration
k	degradation rate constants
t	time
E_a	activation energy
A	frequency factor
e	Euler's number
R	Boltzmann constant
r^2	goodness-of-fit of linear regression
T	temperature
Tween-80	polysorbate 80, PEG (80) sorbitan monooleate, polyoxyethylenesorbitan monooleate

1. Introduction

Brown seaweed (Phaeophyceae) is the largest and most complex type of algae, having brown, olive or yellowish–brown in color. There are about 1 800 species of brown seaweed, broadly distributed from tropical to polar zones of ocean in the world, but in fact only some species are highly exploited for industries, such as *Laminaria japonica, Undaria pinnatifida*, *Ascophyllum nodosum*, and *Hizikia fusiformis*. To date, several well–traded products are alginates, foods, animal feeds, fertilizers, and there is also a little number of some emerging nutraceutical products[1-3].

Besides its valuable polysaccharides and nutrient content, algal pigment received only small attention even it is commonly bleached and left wasted during manufactures. The biggest concern is then addressed to fucoxanthin, a typical dominant carotenoid of brown algae. Recent studies have proven the bio–functionality of fucoxanthin as antiobesity[4,5], antioxidant[6,7], chemopreventive and chemotherapeutic carotenoid[8], as well as anti–inflammation[9]. In addition, there are also chlorophyll *a* and *c* as the primary photosynthetic pigments, *β*-carotene, and other xanthophylls which have no doubt of its biological benefit as those in our daily vegetable intakes.

Behind the important functions of seaweed pigments, chlorophylls and carotenoids are easily degraded upon heat and light exposure, as well as any chemical treatment (acid-base, oxidator-reductor). Due to their instability, short and rapid extraction steps are required to collect the worthwhile seaweed pigments. This can be applied to create new products from species which are still industrially unutilized. Moreover, it would be an advantage to the food industry if the photosynthetic pigments could be recovered prior to hydrocolloid extraction process. Thus, it is possible to build an integrated manufacturing that generates both seaweed pigment and polysaccharides.

Furthermore, microencapsulation technique is known to enhance the stability of active compounds from oxidative and photochemical degradation[10]. Encapsulation procedure also modifies the solubility of targeted compound since most carotenoids are lipophilic and hence there is limitation of its application. Standardization, handling and storage setting should also be eased. Among several drying methods in encapsulation, freeze drying is preferable to

minimize pigment degradation during processing[11,12]. However, the percentage of encapsulation efficiency will influence the amount of active compound that remains unwrapped on the surface of microcapsules, and hence it determines the stability of whole active compound which exists on the product of encapsulation. The present study was aimed to attain: (i) characterization of freeze–dried encapsulated pigment prepared from brown seaweed, (ii) stability evaluation of encapsulated pigment during dark storage at 28 °C, 45 °C, and 65 °C, and (iii) shelf-life estimation based on chromametric measurements.

2. Material and methods

2.1. Sample preparation and pigment extraction

Adequate amount of *Sargassum* sp. was harvested from Baluran National Park, East Java, Indonesia, and stored in a freezer until the moment of use. Pigment extraction was done with 100 % acetone (1 : 10 w/v) and repeated twice. The extract was filtered and concentrated using low–pressure rotary evaporator at 30 °C. The remnant water from seaweed tissues was separated from organic solvent system by adding small amount of anhydrous sodium sulfate. (Otherwise, the temperature of water bath might be elevated up to 40 °C during vacuum evaporation.) Dried crude pigment extract was collected in dark glass bottle, sealed after being filled up by inert gas (Nitrogen gas), and stored at -15 °C until subsequent treatment.

2.2. Encapsulation

The encapsulating materials, maltodextrin (70.02 g) and Tween 80 (1.07 g), were solubilized in 105 mL water at 60 °C and kept under stirring until the temperature reached 30 °C. Microcapsule was prepared by dissolving crude pigment extract collected from 60 g of fresh seaweed in 15 mL canola oil, which was added to the polysaccharide solutions achieving 40 % of soluble solids. The solution was vigorously homogenized at 11 000 rpm (IKA Turrax T–18 basic) for 3 min at room temperature, frozen overnight, and subjected to freeze drying in -45 °C under high (0.04 mbar) vacuum conditions (Labconco Co., USA). Dried material was ground and sieved to a desired particle size (250 μm) with moisture content less than 10 %. The pigment powder was directly stored at -15 °C.

2.3. Storage condition

Encapsulated pigment powder (1.5 mg) was placed in 5 mL brown gas bottles and sealed after sufficient inert gas was flowed. The series of sample were stored at 28 °C (28 d), 45 °C (9 d), and 65 °C (4 d). The data of water content, color, and surface pigment absorption spectrum were recorded periodically during storage. Experiments of storage were duplicated for each temperature.

2.4. Pigment analysis

Surface fucoxanthin (SF) was determined by direct extraction of 0.25 g of pigment microcapsule with 5 mL acetone in a Vortex for 20 s, followed by centrifugation at 5 000 rpm (10 min, at 4 °C). After phase separation, the supernatant was immediately filtered through a Millipore (0.22 μm) and subjected to HPLC analysis. Identical procedure was carried out without microfiltration for surface pigment absorption spectrum measurement using spectrophotometer (UV-1700, Shimadzu). For the total fucoxanthin (TF) determination, 1 g of encapsulated pigment powder was homogenized with 20 mL water in a Vortex for 1 min to break the capsules, followed by exhaustive extraction with acetone and diethyl ether to collect total pigment for chromatographic analysis. Encapsulation efficiency (EE) was calculated according to Barbosa et al.[10] in which % EE = [(TF-SF)/TF] × 100. Pigment identification and quantification were accomplished after separation by a Shimadzu high-performance liquid chromatograph using Shimp-pack VP–ODS C–18 column, equipped with photo–diode array detector, according to protocols previously detailed[13].

2.5. Color measurement

The encapsulated pigment color was measured instrumentally using ColorFlex® EZ (HunterLab) and the results were expressed in terms of L*–lightness, a*–redness, and b*–yellowness according to *Commission Internationale de l'Eclairage* (CIELAB system). Color reading was conducted under the same light conditions, at room temperature, repeated in triplicate and the average value was taken. The instrument was standardized using a standard white tile (L* 92.93, a* -0.92, b* 1.48).

2.6. Kinetic analysis

The data were best-fitted by a first-order-kinetic model, $\ln C = \ln C_0 - k(t)$. Degradation rate constants (k) were calculated from the slope of a plot of the natural log of the chromatic value vs. time. The activation energy (E_a) and frequency factor (A) were determined from the Arrhenius model $k = Ae^{-Ea/RT}$, in which E_a/R is the slope and $\ln A$ is the intercept of the relationship between natural log$k$ and ($1/T$) in kelvins. For a first-order reaction, the half–life was determined at a specific temperature, in which the difference of chromatic values before and after 50 % of degradation (monitored at 447 nm) is divided by degradation rate constant. The linear regression (95 % confidence limit) was used to determine the reaction order, rate constants, and activation energies.

3. Result and discussion

Among various seaweed species, most *Sargassum* spp. are still underutilized when it is compared with other species in the order Fucales. The widely consumed species among Sargassaceae family is only *Sargassum fusiforme* (*Hizikia fusiformis*), in addition to *Ascophyllum nodosum* from the family of Fucaceae that has been manufactured for use in alginates, fertilizers and animal feed[14]. Most *Sargassum* spp. are not regarded as edible marine algae because of its bitterness[15]. In several areas, *Sargassum* spp. even raise problem because of its fast growth rate[16].

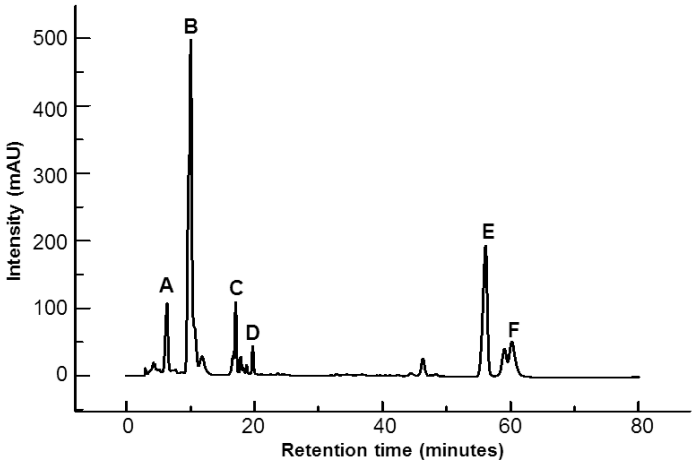

Fig. 1. Chromatographic separation of the major pigments on encapsulated *Sargassum* sp. extract, performed by gradient protocol in VP–ODS C–18 column, detected at 430 nm. (A) chlorophyll *c*; (B) *trans*-fucoxanthin; (C) *cis*-fucoxanthin; (D) zeaxanthin; (E) pheophytin *a*; (F) *β*-carotene.

Six major peaks were identified after chromatographic separation of *Sargassum* sp. pigment which was encapsulated in maltodextrin and Tween–80 system (Fig. 1). *Trans*-fucoxanthin (107.2 μg g^{-1}) was found as the

most dominant pigment, accompanied by *cis*-fucoxanthin, zeaxanthin, and *β*-carotene as minor carotenoids. Major chlorophylls observed in microcapsule were chlorophyll *c* and pheophytin *a*. This composition gave yellowish green color to the pigment powder. The high content of fucoxanthin and other pigments found in the crude extract of *Sargassum* sp. confirms that this invasive macro-alga is a good source of bioactive compounds.

The number of pigment fractions in the present study is less than that of previously published data for fresh seaweed. Fresh seaweed may contain up to 14 types of photosynthetic pigment, i.e. fucoxanthin, violaxanthin, flavoxanthin, fucoxanthol, antheraxanthin, 9-*cis*-neoxanthin, diatoxanthin, zeaxanthin, *β*-carotene, chlorophyll *a*, chlorophyll *a*', chlorophyll c_1, chlorophyll c_2, and phaeophytin *a*[13, 17]. Total losses of chlorophyll *a* and several minor pigments occurred during harvesting and long–distance transport. The spectral data of crude pigment extract isolated from unprocessed seaweed samples was similar to that of encapsulated pigment (data not shown), indicating that most of chlorophylls degradation did not take place during microcapsule preparation. The presence of pheophytin *a* was confirmed as the main derivative product of chlorophyll *a*. Fortunately, the degradation of chlorophyll *a* to become pheophytin *a* will increase antioxidant activity of the crude extract[18].

Encapsulation efficiency was calculated as 88.94 % on the base of fucoxanthin content. This value was sufficiently higher than the previous study using equal encapsulation matrix for bixin that was prepared by means of spray-dryer, giving only 75.10 % of microencapsulation efficiency[10]. Increased amount of Tween–80 as the emulsifier was supposed to perform better encapsulation, in which a ratio of 98.5 % of maltodextrin and 1.5 % of Tween–80 was applied in the present study.

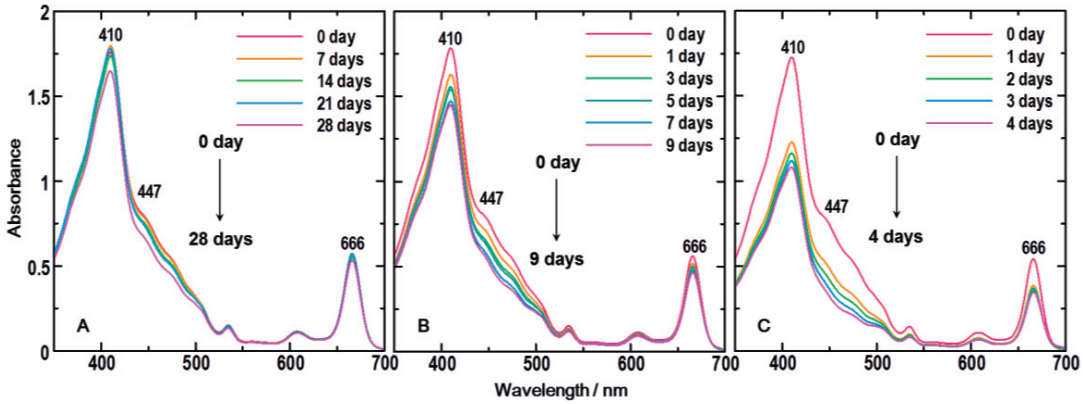

Fig. 2. Spectral evolution of surface pigment on encapsulated *Sargassum* sp. extract which is stored at 28 °C (A), 45 °C (B), and 65 (C) °C.

Fig. 2 depicts spectral evolution of surface pigment of the pigment powder during storage at 28 °C, 45 °C, and 65 °C. The initial absorption spectrum revealed the presence of pheophytin *a* as the most dominant pigment, having Soret and Qy band at 410 nm and 666 nm, respectively. The presence of fucoxanthin was represented as a shoulder at 447 nm. Generally, pigment degradation was indicated as the reduction of its absorption spectrum, and this was hastened at higher temperature of storage. During storage at 28 °C and 45 °C, thermal degradation appeared significant at the Soret band of pheophytin *a* (410 nm) as well as at maximum absorption of fucoxanthin (447 nm). The reduction of absorption spectrum is also obvious for Qy band (666 nm) when the encapsulated pigment was stored at 65 °C. In the study of Happy et al., the mixture of chlorophyll *a* and pheophytin *a* in organic solvent showed higher stability upon heat exposure at 90 °C for 24 h, compared to the solution of fucoxanthin only[19]. This finding is in line with the data of the present study, in which the shoulder peak of fucoxanthin at 447 nm exhibited faster depletion than the Soret and Qy band of pheophytin *a*.

Furthermore, pigment degradation was also monitored as color change by means of ColorFlex. During storage, the lightness (L*) value was increased, whereas the redness (a*) and yellowness (b*) value were reduced (Figure 3). The evolution of chromatic values followed the first–order kinetic with the increased slope at higher temperature of storage. Table 1 shows the Arrhenius parameters and half-lives for the encapsulated pigment of *Sargassum* sp. The

activation energy calculated for b* value was higher than that for a* value. It was suggested that b* value is representative of pheophytin *a* as well as chlorophyll *c*, while a* value represents the thermo-labile carotenoids. Higher activation energy is important to retard pigment degradation, and hence prolong the half-life. Stephane et al., reported activation energy of 12.64 kcal · mol^{-1} for freeze–dried encapsulated *β*-carotene[11]. Higher activation energy was reported by Robert et al. that is 14.2 kcal · mol^{-1} to 19.2 kcal mol^{-1} for encapsulation of carotenoids from *Rosa rubiginosa*[20]. The reasonable half–life value estimated at 28 °C was 63 d, and it could be prolonged up to 118 d when the pigment powder is stored at 21 °C.

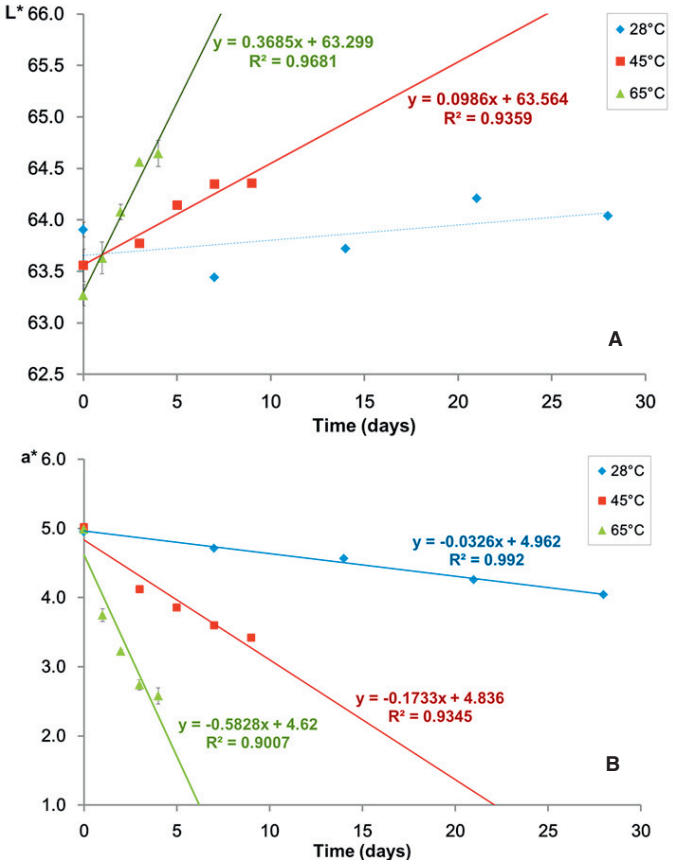

Fig. 3. First–order degradation plots for L* (luminosity) (A) and a* (redness) (B) on encapsulated *Sargassum* sp. pigment. Each point represents an average of duplicate experiments at each temperature.

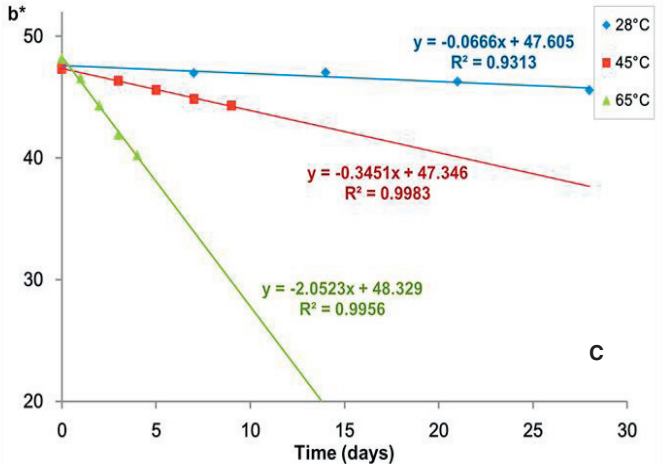

Fig. 3. (continued) First–order degradation plots for b* (yellowness) (C) on encapsulated *Sargassum* sp. pigment. Each point represents an average of duplicate experiments at each temperature.

Table 1. Degradation and rate constants (k), Arrhenius parameter, and half-life values for encapsulated *Sargassum* sp. pigment based on red and yellow hue color parameter

Color parameter	Temperature (°C)	Degradation rate constant (K)	Activation energy (kcal mol^{-1})	Intercept ($ln\ K_0$)	r^2 (Arrhenius plot)	Calculated half–life (days)
a*	28	0.03 56	15.73	22.98	0.98 90	63
	45	0.14 54				15
	65	0.63 49				4
b*	28	0.06 59	18.72	28.60	0.99 99	88
	45	0.35 18				16
	65	2.03 31				3

4. Conclusion

Rapid extraction procedure and high efficiency encapsulation of seaweed pigment have been performed in maltodextrin and Tween-80 matrices by means of freeze drying method. The dominant pigments were pheophytin *a* and *trans*-fucoxanthin which have potency as bioactive compounds. The pigment powder could be stored at 28 °C under inert atmosphere at least 63 d prior to significant pigment degradation. This study can be a part of promoting underutilized seaweed species as well as marine by-products to be functional food ingredient.

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