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ORIGINAL ARTICLE

Effect of drying on the production of fucoxanthin isomers from brown seaweeds

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Abstract

Fucoxanthin, an algal carotenoid with bioactivity that benefits human health, is gaining popularity as a functional food. Therefore, in this study, the effect of drying methods and conditions on the production of fucoxanthin isomers in three closely related *Sargassum* species with slightly different morphological lamina were investigated. The recovery of fucoxanthin after oven-drying was 1.1–1.3-fold higher than that after sun-drying and varied 1.6-fold among the *S.* species used, indicating that each algal species has a different heat susceptibility even among closely related species, and should be optimized for each species and drying method. In *Padina australis* with homogeneous morphology, the optimal temperature to maintain all-*trans* form was 60°C with only a 5.3% loss, while other *cis*-isomer transformations were maintained at 80°C. The apparent activation energy estimated by Arrhenius plots differed for the all-*trans* form and *cis*-isomer transformations, indicating the presence of different reactions between them.

Practical applications

Drying treatment is an efficient and cost-effective technique for reducing the moisture content of post-harvest seaweed to prevent decomposition, increase shelf life, and easily extract the targeted compounds. Owing to the difference in heat susceptibility, even in closely related species, the results indicated that it should be necessary not only to select appropriate algal species and drying methods but also to optimize the thermal conditions for each target substance of the selected material. This study provided qualitative information, particularly on seaweed drying procedures, and contributed to the production of marine products in the fields of food and bioactive substances, such as fucoxanthin isomers.

1 | INTRODUCTION

Seaweeds can produce a variety of structurally and functionally unique bioactive components for human benefit. This is significant in a wide range of applications from the pharmaceutical to food industries (Bhuyar et al., 2021; Ramli et al., 2020). Some edible brown seaweeds have been used in major commercial industries as an abundant and renewable potential source of not only food, but also medicinal and chemical products, such as pigments, nutrients, cosmetics, and hydrocolloids, in Asia, particularly in Japan and China (Zhu et al., 2009). *Undaria pinnatifida* ("wakame" in Japanese), *Nemacystus decipiens* ("itomozuku"), and *Sargassum* species have already been commercially cultivated to meet the industry's growing demand (Milledge & Harvey, 2016; Nisizawa et al., 1987; Pan et al., 2019; Tako et al., 1999; Wang et al., 2018).

Sargassum species are naturally grown on almost all of Indonesia's coast, and in 2019, they had the highest production of brown seaweed, reaching 1340 tons with a value of 304,000 USD for alginate purposes (BPS–Statics Indonesia, 2020). Furthermore, it is known that *Sargassum* species contain relatively high amounts of fucoxanthin and chlorophyll (Chl) *a* averaging 0.43 to 4.11 $mg{\cdot}g^{-1}$ dry weight (d.w.) and 1.70 to 7.89 $mg{\cdot}g^{-1}$ d.w., respectively (Heriyanto et al., 2017).

Fucoxanthin is a pigment component of brown alga that is classified as a xanthophyll carotenoid possessing a distinct structure, such as allenic, epoxide, and acetyl groups. Owing to its structure, fucoxanthin is expected to provide several health benefits to humans, such as anti-obesity, anti-diabetic, anti-tumor, anti-inflammation, and antioxidant properties (Gammone & D'Orazio, 2015; Kang et al., 2014; Lee et al., 2021; Lourenço-Lopes et al., 2022; Maeda et al., 2008), and be more effective than β-carotene and lycopene in inhibiting cell growth and inducting apoptosis in human cancer cells (Hosokawa et al., 2004). The *cis*-isomers of carotenoids are suggested to enhance their incorporation into cells by increasing their solubility (Honda et al., 2017, 2018). It was demonstrated that the inhibitory effect of 13-*cis* and 13′-*cis* fucoxanthin isomers on cancer cell growth was stronger than that of the all-*trans* form (Nakazawa et al., 2009).

Recently, the effect of drying on the recovery of pigments and other functional compounds from various species of brown seaweed was reported (Badmus et al., 2019; Silva et al., 2019). They recognized the effectiveness of drying post-harvest. However, depending on the algal species and objective compounds used, the optimum temperature for drying methods involving oven, freeze, and microwave varied in the range of 25–60°C. Therefore, it was concluded that the target compound of each alga must be heated to an appropriate temperature.

In this study, we investigated the effect of drying on the recovery of fucoxanthin and its isomers, which have potential bioactivity and health benefits for humans. Three closely related *Sargassum* with slightly different morphological structures were used to clarify the relationship between morphological structure and heat susceptibility after drying with a fixed moisture content of 10%. After drying, an optimum temperature for fucoxanthin isomer production was determined in *Padina australis* with a simple fan-shaped structure. The use of the intensity ratio of mass spectrometry (MS) fragment ions for the differentiation of fucoxanthin isomers with geometric fine structures is also discussed as a useful alternative method.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Analytical grade methanol (MeOH), acetone, methyl tert-butyl ether (MTBE), and ammonium acetate were the solvents used for pigment extraction and high-performance liquid chromatography (HPLC), while liquid chromatography grade water (H_2O), MeOH, and formic acid were used for MS analysis and were obtained from Merck (Darmstadt, Germany). Standard pigments, Chl c_1, Chl c_2, all-*trans* fucoxanthin, Chl *a*, and pheophytin (pheo) *a*, were obtained from NATChrom (Malang, East Java, Indonesia).

2.2 | Seaweed samples

Three closely related but morphologically distinct *S.* species and *P. australis* were used as algal materials. *S. polycystum* with large laminae, *S.* species with wide and long laminae, *Sargassum filipendula* with small laminae, and *P. australis* with fan-shaped homogenous laminae were collected from certified seaweed farms in Teluk Awur beach, Jepara, Central Java, Indonesia (6°36′46.8′′S 110°38′29.1′′E). Photos of *S.* species are presented in Figure S1 (for a photo of *P. australis*, refer to Heriyanto et al., 2017). The seaweed was cleaned of any associated debris by rinsing with clean seawater and then tap water. During transportation to the laboratory, samples were placed in black plastic bags and placed in a cooling box.

2.3 | Pigment extraction

Fresh algal thalli were frozen with liquid N_2 before being ground into fine particles with a blender. To extract the pigment, 0.1 g of sample was homogenized in a vortex for 1 min with 1 ml of acetone:MeOH (3:7, v/v) and then sonicated for 1 min to break the cells. The crude pigment extract was separated from its residue by centrifugation at 10,000*g* for 2 min at ambient temperature. The residue was continuously extracted using the same procedure until the residue appeared colorless. The pigment extracts were mixed and dried in an N_2 gas stream. The dry powders from *Sargassum* and *Padina* species were extracted using a method proposed by Ishihara et al. (2008) with a slight modification. The pigments from 0.1 g of dry powder were extracted using the same methods as the fresh sample and finally dissolved in acetone.

2.4 | Pigment determination

The pigments were identified using absorption spectrometry, HPLC, and MS analysis based on the spectral shape, maximal absorption wavelength (λ_{max}), and Q-ratio (spectrometry); retention time (t_R) (HPLC); and precursor and fragment ions and intensity ratio of fragment ions (MS). The following sections describe the specific techniques performed for pigment identification. The modified standard curve of the pigments from the linear equations was used to calculate pigment concentrations in $mg{\cdot}g^{-1}$ d.w. (Heriyanto et al., 2017). Similarly, fucoxanthin isomer concentrations were calculated using a linear equation based on the all-*trans* fucoxanthin standard curve ($y = 198.89x - 335.62$; $R^2 = 0.9994$, where y is the peak area detected at 450 nm ($\times 10^{-3}$) and x is fucoxanthin isomer concentration ($\mu g{\cdot}ml^{-1}$)).

2.4.1 | Absorption spectrometry

The UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan) was used to record the absorption spectra of the pigment extracts in acetone from 300 to 800 nm. To identify the types of *cis*-isomers, an empirical absorption ratio, Q-ratio ($A_{\lambda max\ cis\ peak}/A_{\lambda max\ main\ peak}$), was used.

2.4.2 | HPLC analysis

HPLC analysis was performed using reversed-phase (RP)-HPLC with a photodiode array detector (Shimadzu). The pigment was separated using a Shim-pack VP-ODS C_{18} column (250×4.6 mm i.d.) (Shimadzu), and the fucoxanthin isomers were separated using a C_{30} column (150×4.6 mm i.d., 3 μm particle size) (YMC, Tokyo, Japan) according to Wibowo et al., 2022. In brief, the separation was carried out using a gradient elution program of water, MeOH, and MTBE mixture as the mobile phase at a column oven temperature of 30°C and a flow rate of 1.0 ml·min^{-1}.

2.4.3 | MS analysis

The fucoxanthin isomers were analyzed using electrospray ionization (ESI)–LC triple quadrupole MS, LCMS-8030 (Shimadzu), on a Chromolith Performance RP-18e column (100×4.6 mm i.d.) (Merck KGaA) with an isocratic elution program of 0.1% formic acid in water (A solvent, 10%) and 0.1% formic acid in MeOH (B solvent, 90%) at a column oven temperature of 30°C and a flow rate of 1.5 ml·min^{-1}. The standard pigments were analyzed using an isocratic elution program of 50% A solvent and 50% B solvent without a column at a flow rate of 0.4 ml·min^{-1}. As previously described, MS analysis was performed in the mass range from 200 to 1000 *m/z* (Brotosudarmo et al., 2018). The spectral data obtained by a full Q1 scan and product–ion scan at the collision energy (CE) = −10 V from the fucoxanthin standard and its isomeric forms were saved in the LabSolution MS Library (Shimadzu) and then analyzed using the previously described method (Heriyanto et al., 2021).

2.4.4 | Fragment-ion ratio for fucoxanthin isomer identification

A novel ratio obtained from the MS analysis was used to identify the fucoxanthin isomers. Carotenoids with very similar structures can be distinguished by comparing the intensities of their fragments, which are commonly the base peaks produced by the product–ion scan in the ESI–MS/MS analysis (Rivera et al., 2013; Wibowo et al., 2022). Fucoxanthin isomers ($C_{42}H_{58}O_6$) had the same precursor ion at *m/z* 659.5 $[M+H]^+$ and product ions at *m/z* 641.5 $[M+H-H_2O]^+$ and *m/z* 581.4 $[M+H-H_2O-HOCOCH_3]^+$ which were formed by loss of water molecule, and water and acetic acid molecules, respectively (cf. Table S2). Their ratios are defined as follows: ratio of loss of water molecule (%) = $I_{fragment\ ion}$ [*m/z* 641.5]/$I_{fragment\ ions}$ [*m/z* 641.5 + 581.4] × 100 and ratio of loss of water and acetic acid molecules (%) = $I_{fragment\ ion}$ [*m/z* 581.4]/$I_{fragment\ ions}$ [*m/z* 641.5 + 581.4] × 100, where $I_{fragment\ ion}$ [*m/z* 641.5], $I_{fragment\ ion}$ [*m/z* 581.4], and $I_{fragment\ ions}$ [*m/z* 641.5 + 581.4] represent the intensity of loss of water molecule, intensities of loss of water and acetic acid molecules, and sum of the intensities of loss of water molecule and water and acetic acid molecules, respectively.

2.5 | Drying methods

To evaluate the efficiency of drying methods, three *S.* species with various lamina sizes and shapes were dried using two different methods: sun-drying and oven-drying. Seaweeds (1500 g fresh weight) were oven-dried at 50°C in the dark, and sun-dried at an ambient temperature of 35–41°C and light intensity (2060 μmol·m^{-2}·s^{-1} maximum). Both drying processes were continued until the final moisture content reached 10%. *P. australis* was used to determine the optimal temperature for *cis*-isomer formation during the oven-drying treatment. As a sample suitable for minimizing errors between samples, *P. australis* has a fan-shaped homogeneous lamina and high contents of fucoxanthin (Indrawati et al., 2010). To obtain consistent dried samples, the seaweed was dried in the range 40–100°C at 20°C intervals until their moisture content was 10% under dark conditions by changing the duration of drying time at each temperature as: 45.5 h at 40°C, 25.5 h at 60°C, 17.0 h at 80°C, and 5.5 h at 100°C. This treatment reduced the weight of the samples by approximately 10%, making it easier to handle, particularly the extraction of the pigments and extension of shelf life.

2.6 | Statistical analysis

All the experiments were carried out in triplicate with at least three samples. The average and standard error were calculated with a 95% confidence level. Fucoxanthin isomer concentrations were calculated using the same extinction coefficient as the all-*trans* form.

3 | RESULTS AND DISCUSSION

3.1 | Effect of drying on the fucoxanthin and Chl concentrations

The pigment concentrations were revealed using HPLC with a photodiode array detector before (fresh) and after drying treatments at 10% moisture content, as described in Section 2.5. The concentrations of fucoxanthin in fresh seaweeds ranged from 0.88 to 0.93 mg·g^{-1} d.w. among the three *S.* species, with *P. australis* having a 1.7-fold higher concentration (1.56 mg·g^{-1} d.w.) than the other *S.* species (Table 1). These values were consistent with previously reported values of 0.75 and 1.64 mg·g^{-1} d.w. in *S. crassifolium* and *P. australis*,

respectively (Heriyanto et al., 2017). *S. filipendula* with small laminae and long stipe had a high recovery of fucoxanthin at 97.7% and 92.0% in the oven- and sun-drying methods, respectively, whereas *S.* species with wide and long laminae had the lowest recovery of 62.4% and 57.0% in the oven- and sun-drying methods, respectively. Fucoxanthin recovery after oven-drying was up to 99.9% in *P. australis*, while sun-drying had a low recovery of 36.0%. In terms of fucoxanthin recovery, these results indicated that oven-drying is more effective than sun-drying. Furthermore, despite having nearly comparable concentrations in fresh materials, their recovery by both the drying treatments varied among the *Sargassum* species. These differences in fucoxanthin recovery after drying appear to be primarily due to differences in the morphological structure of the lamina in the *S.* species. This may have resulted in different heat susceptibility. As stipe in brown seaweed has more hard tissues and high endurance to maintain pigments against heat exposure than laminae, *S. filipendula* had the highest fucoxanthin recovery. As opposed to fucoxanthin recovery, the sun-drying method was more effective in recovering Chl *a* among the three *S.* species, with 54% to 40.9% compared to 37.9% to 19.4% for the oven-drying method. These findings were consistent with the recovery of Chl *a* in *P. australis* after both drying treatments. Based on the findings of Badmus et al. (2019), not only the selection of algal materials but also the drying method is critical for each target material when drying treatment is used.

3.2 | Effect of drying on the pigment composition

The absorption spectra of fresh thalli extracts, and sun- and oven-dried samples obtained from the sample algae showed nearly identical absorption spectra. Qy-band decreased by 13.1–43.1% and pheo formation were observed in the three *S.* species, while its band in *P. australis* decreased by 18.6–32.3%, and there was no significant absorption of pheo. In terms of fucoxanthin, as revealed by absorbance at 447 nm, the recovery in oven-dried samples was 50.4–84.9% in the three *S.* species and 70.6–76.5% in *P. australis*, which was higher than the recovery in sun-dried samples, as shown in Table 1. Figure S2 shows the absorption spectra of the pigments extracted from each algal species.

The pigments extracted from fresh and oven-dried seaweed were then analyzed by HPLC alongside their standard pigments to determine the pigment composition. All the brown seaweed samples were separated and identified into four main peaks. As an example, typical HPLC elution profiles of pigment extracts from *S. polycystum* are seen in Figure 1. Peak 1 corresponds to Chl *c* (mixture of c_1 and c_2), peak 2 corresponds to fucoxanthin, peak 3 corresponds to Chl *a*, and peak 4 corresponds to Pheo *a*, based on the t_R, λ_{max}, and spectral shape. Using the same identification procedures, the minor components at t_R 39 and 58 min were also determined to be Chl *a*' and β-carotene, respectively. Figure S3 shows the in-line absorption spectra of the peaks and standard pigments. In the fresh seaweed, at least three pigments were detected: Chl *c* (peak 1), fucoxanthin (peak 2), and Chl *a* (peak 3). According to the absorption spectrum, an additional pigment, Pheo *a* (t_R = 38 min), was detected in dried seaweed. Peak 2 fucoxanthin intensity varied slightly between fresh and dried seaweeds. By contrast, a significant decrease in peak 3, Chl *a*, was observed, indicating that a portion of Chl *a* was converted into Pheo *a* of peak 4. The MS analysis of their precursor and fragment ions supported the identification of the pigments from brown seaweed. Table S1 summarizes the pigment identification process.

The fucoxanthin component was then analyzed to determine its isomers using HPLC and MS analysis with pigment extracts from

TABLE 1 Concentrations of the pigments were quantified by HPLC with a photodiode array detector before (fresh) and after drying treatments

		Fucoxanthin		Chl *a*	
Brown seaweed	Drying treatment	Concentration ± SE ($mg \cdot g^{-1}$)	Recovery (%)	Concentration ± SE ($mg \cdot g^{-1}$)	Recovery (%)
Sargassum polycystum	Fresh	0.89 ± 0.03	100	2.61 ± 0.18	100
	Oven	0.75 ± 0.01	84.3	0.99 ± 0.07	37.9
	Sun	0.58 ± 0.02	65.2	1.17 ± 0.05	44.8
Sargassum sp.	Fresh	0.93 ± 0.07	100	2.47 ± 0.19	100
	Oven	0.58 ± 0.02	62.4	0.64 ± 0.03	25.9
	Sun	0.53 ± 0.02	57.0	1.01 ± 0.01	40.9
Sargassum filipendula	Fresh	0.88 ± 0.05	100	2.37 ± 0.33	100
	Oven	0.86 ± 0.07	97.7	0.46 ± 0.02	19.4
	Sun	0.81 ± 0.11	92.0	1.28 ± 0.05	54.0
Padina australis	Fresh	1.56 ± 0.05	100	3.81 ± 0.30	100
	Oven	1.56 ± 0.11	99.9	0.80 ± 0.07	21.0
	Sun	0.53 ± 0.01	36.0	0.95 ± 0.02	24.9

Note: Oven-drying treatment was performed at 50°C for 30 h in the dark, while sun-drying was performed at approximately 40°C for 36 h with light intensity of 2060 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (maximum) to reach 10% of moisture content. The results are averages of three experiments, and SE is shown.

oven-dried *P. australis* as a sample. At least four peaks belonging to fucoxanthin isomers were well separated on a C_{30} column within 20 min at elution times of 13.72 (peak 1), 14.88 (peak 2), 15.55 (peak 3), and 17.44 min (peak 4) (Figure S4). These fucoxanthin isomers were initially identified spectrophotometrically by comparing their λ_{max} and Q-ratio ($A_{\lambda max\ cis\ peak}/A_{\lambda max\ main\ peak}$) from their in-line absorption spectra of the HPLC (Haugan & Liaaen-Jensen, 1994), namely all-*trans* (peak 1; Q-ratio = 0.08), 13′-*cis* (peak 2; Q-ratio = 0.48), 13-*cis* (peak 3; Q-ratio = 0.43), and 9′-*cis* (peak 4; Q-ratio = 0.12) as summarized in Table 2. Furthermore, the purified fucoxanthin isomers from *P. australis* were analyzed by LCMS/MS using the modes of Q1 (+) and product ion scans (CE = −10 V) at the described analytical conditions. Table S2 shows a typical ESI–LCMS/MS spectral data.

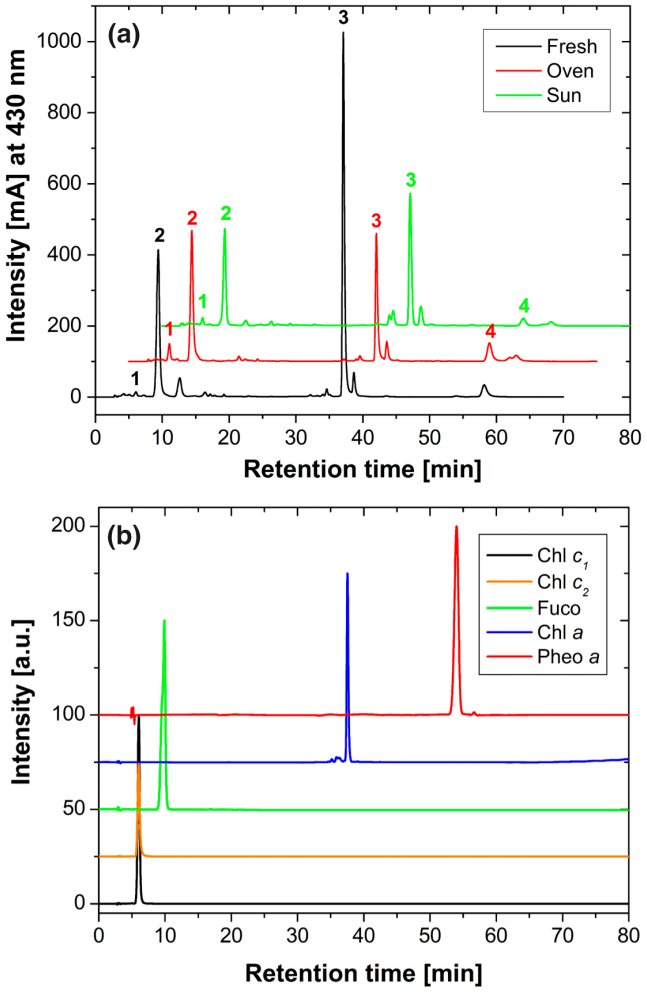

FIGURE 1 Typical HPLC elution profiles of the pigment extracts from *Sargassum polycystum* detected at 430 nm (a) and the standard pigments at their λ_{max} (b). (a): fresh (black), oven (red), and sun (green). (b): Chl c_1 (black), Chl c_2 (orange), fucoxanthin (green), Chl *a* (blue), and Pheo *a* (red).

Another newly discovered ratio through MS analysis was also used to identify the fucoxanthin isomers. As seen in Table 2, the ratio of water molecule loss (I) was highest in 13′-*cis* (70%) followed by all-*trans* (66%), 13-*cis* (56%), and 9′-*cis* (17%), while the ratio of water and acetic acid molecule loss (II) was highest in 9′-*cis* isomer of fucoxanthin (83%). The same results were obtained previously using high purity isomers for (I) (Wibowo et al., 2022). Therefore, either ratio, in addition to another absorption ratio, the Q-ratio, which has been frequently used to identify *cis*-isomers as mentioned above, is well applicable to different types of fucoxanthin isomers. Therefore, it is reasonable to conclude that this ratio is a useful alternative for identifying different types of fucoxanthin isomers, although further research into the correlation between these fragment-ion ratios and geometrical fine structures is required.

3.3 | Effect of drying on the formation of fucoxanthin isomers

Based on the findings of the preceding study, the optimal conditions for fucoxanthin and its isomers were determined using the oven-drying method. Due to its simple morphological structure and to obtain homogeneous samples, *P. australis* was selected as the source of fucoxanthin rather than *S.* species. To determine the optimum concentrations of fucoxanthin isomers, oven drying temperatures varied from 40 to 100°C at 20°C intervals. Each sample was dried to 10% of its moisture content to achieve constant drying.

As seen in Figure 2a, relatively high concentrations of all-*trans* fucoxanthin of 1244.1 and 1176.0 $\mu g \cdot g^{-1}$ d.w. were obtained after 45.5 h at 40°C and 25.5 h at 60°C. This indicated that at 60°C, 94.5% of the all-*trans* fucoxanthin was recovered compared to 40°C

TABLE 2 Simultaneous separation and identification of fucoxanthin isomers by HPLC with a C_{30} column and MS analysis

No peak	t_R (min)	λ_{max} (nm)	Fucoxanthin isomer	Q-ratio		Intensity		Intensity ratio (%)	
				This study	Reference[a]	*m/z* 641.5	*m/z* 581.4	(I)[b]	(II)[c]
1	13.72	334, –, 451, –	All-*trans*	0.08	0.07	8382	4350	66	34
2	14.88	333, –, 445, –	13′-*cis*	0.48	0.52	10,007	4346	70	30
3	15.55	332, –, 440, –	13-*cis*	0.43	0.45	13,886	10,678	56	44
4	17.44	332, –, 448, –	9′-*cis*	0.12	0.12	3435	16,215	17	83

[a]Haugan and Liaaen-Jensen (1994).

[b]$I_{\text{fragment ratio}}\ (\text{I})\ (\%) = I_{[m/z\ 641.5]}/I_{([m/z\ 641.5]\ +\ [m/z\ 581.4])} \times 100$.

[c]$I_{\text{fragment ratio}}\ (\text{II})\ (\%) = I_{[m/z\ 581.4]}/I_{([m/z\ 641.5]\ +\ [m/z\ 581.4])} \times 100$.

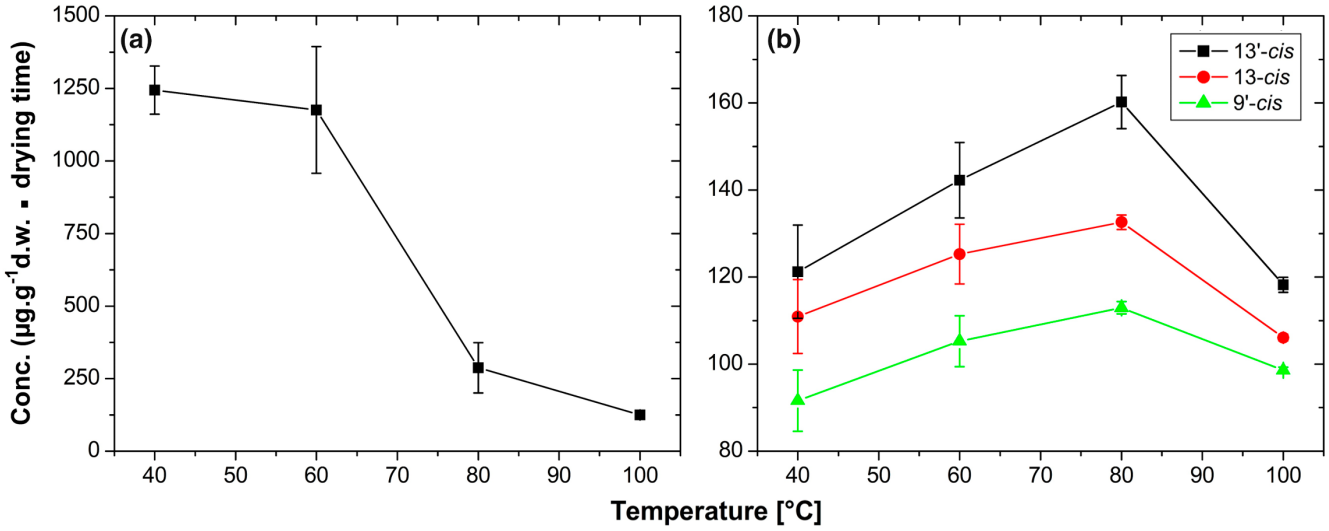

FIGURE 2 Concentrations of the fucoxanthin isomers extracted from oven-dried *Padina australis* at the indicated temperatures for drying time required to reach 10% moisture content. The results are averages of three experiments with SE. (a) All-*trans* isomer, and (b) 13′-*cis*, 13-*cis*, and 9′-*cis* isomers.

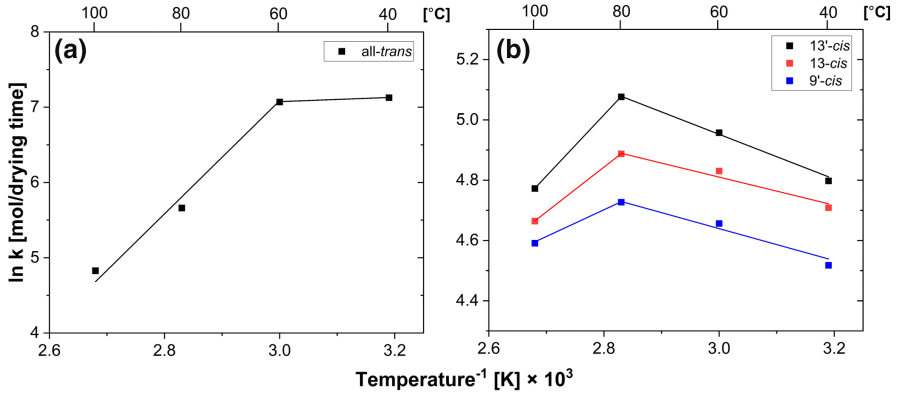

FIGURE 3 Arrhenius plots of the concentrations of all-*trans* fucoxanthin (a) and *cis*-isomers of fucoxanthin (b) versus drying temperature and time for reaching 10% moisture content of the oven-dried *Padina australis*. The results are averages of three experiments.

and nearly half the drying time. However, with increasing temperatures, the recovery of all-*trans* fucoxanthin decreased significantly, reaching 23.1% at 80°C for 17 h and nearly 10.0% at 100°C for 5.5 h of drying with the same moisture content.

Arrhenius plots are frequently used to investigate the effect of temperature on the rates of chemical reactions. This method was also used to examine the temperature-dependent results in greater detail (Figure 3). The Arrhenius plots obtained from all-*trans* fucoxanthin concentration measurements were biphasic with a break at 60°C, indicating the existence of two reaction processes with different straight lines (40–60°C and 60–100°C) for which apparent activation energies of −2.46 kJ/mol (40–60°C) and − 58.2 kJ/mol (60–100°C) can be calculated (Figure 3a). These negative values indicated the spontaneous degradation of all-*trans* isomers by heating, although, in a low-temperature phase (40–60°C), some amounts of the isomer appear not to be degraded, but transformed to *cis*-isomers, since their amounts are almost equivalent and the low-temperature phase (40–80°C) of *cis*-isomers increase with positive activation energy due to unspontaneous degradation.

In the case of *cis*-isomers, such as 13′-, 13-, and 9′-*cis* fucoxanthin, their concentrations increased with increasing temperature up to 80°C, whereas all-*trans* fucoxanthin concentrations decreased (Figure 3b), indicating the occurrence of the transformation of these *cis*-isomers. The concentrations of all *cis*-isomers, including 9′-*cis*, increased by 16.7–35% at 80°C and reached a maximum, then dropped sharply to those found at 40°C or less at 100°C. The Arrhenius plots obtained from the measurements of the three fucoxanthin isomers were mostly parallel with a similar slope of lines and biphasic with a break at 80°C, switching from the formation (positive activation energy) to the degradation (negative activation energy) process. The activation energy represents the minimum amount of energy required to undergo stereomutation. The apparent activation energies of 13′-*cis*, 13-*cis*, and 9′-*cis* were calculated to be 6.44 kJ/mol (40–80°C) and − 16.8 kJ/mol (80–100°C), 4.12 kJ/mol (40–80°C) and − 12.4 kJ/mol (80–100°C), and 4.84 kJ/mol (40–80°C) and − 7.54 kJ/mol (80–100°C), respectively. The Arrhenius plot was used to transform fucoxanthin isomers for the first time, and no comparable data exist. These findings imply that optimal conditions for fucoxanthin isomer formation differ between all-*trans* and

other *cis*-isomer transformations, implying the presence of different reactions between them. As demonstrated here, heat treatment improved the stereomutation of fucoxanthin isomers in thalli. This is the first study that reported in vivo stereomutation of fucoxanthin in algal samples by heat, although there were several reports on in vitro stereomutation in canola oil (Zhao et al., 2014) and iodine-catalyzed stereomutation in solvents (Haugan et al., 1992; He et al., 2002).

According to the findings of this study, when using drying for post-harvested dehydration, it is necessary to select and optimize the appropriate drying methods and conditions for each target material, taking into account those with different susceptibilities. Heat susceptibility varied among the three closely related *S.* species with slightly different morphological structures. Therefore, this study demonstrated that an appropriate temperature must be used for each target compound in each alga used (Badmus et al., 2019; Silva et al., 2019).

4 | CONCLUSION

Drying treatment is an effective and essential technique for reducing the moisture content of post-harvest seaweeds in the food and pharmaceutical industries. This study indicated that when using the drying treatment, selecting the right algal materials and drying method is crucial. This is due to differences in heat susceptibility even among closely related species. The optimal temperature to maintain all-*trans* form in *P. australis* with homogeneous morphology was 60°C with only a 5.3% loss, while other *cis*-isomer transformations were at 80°C. The apparent activation energy estimated by Arrhenius plots differed for the all-*trans* form and *cis*-isomer transformations, indicating the presence of different reactions. In this study, the use of the intensity ratio of the MS fragment-ions was demonstrated to be a potential alternative method for identifying the types of fucoxanthin isomers with geometric fine structures. Therefore, the findings of this study provided useful information on the production of fucoxanthin isomers from brown seaweed, which has health benefits for humans.

AUTHOR CONTRIBUTIONS

Arif Agung Wibowo: Investigation; Validation; Visualization; Writing - original draft; Software; Formal analysis; Data curation. **Philip Estera Elim**: Investigation; Formal analysis; Software. **Heriyanto**: Investigation; Writing - original draft; Software; Formal analysis; Data curation; Validation. **Monika Nur Utami Prihastyanti**: Validation; Writing - original draft; Formal analysis. **Jessica Renata Yoewono**: Writing - review & editing; Validation; Data curation. **Yuzo Shioi**: Writing - original draft; Methodology; Validation; Writing - review & editing; Supervision; Data curation; Formal analysis. **Leenawaty Limantara**: Funding acquisition; Writing - review & editing; Project administration. **Tatas Hardo Panintingjati Brotosudarmo**: Conceptualization; Funding acquisition; Validation; Methodology; Writing - review & editing; Supervision; Data curation; Resources; Formal analysis.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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