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Effects of pretreatment and drying methods on physical properties and bioactivity of sea lettuce (*Ulva rigida*)

Prajongwate SATMALEE[1*], Thidarat PANTOA[1], Safiah SAAH[1], Yupadee PAOPUN[2], Montakan TAMTIN[3], Prapat KOSAWATPAT[4], Busaba THONGDANG[4]

Abstract

Sea lettuce (*Ulva rigida*) is an underutilized green macroalga. Scant knowledge and understanding exist regarding the optimal processing technologies to maximize seaweed nutrition. Pretreatment of sea lettuce before drying, either by blanching or steaming, induced diverse physical and biological property changes. Greenness increased, while lightness of the-sea lettuce reduced. Both drying processes reduced textural parameters and impacted sea lettuce bioactivity, with increased antioxidant capacity and phenolic compound content. Steaming followed by oven drying at 60 °C for 2.30 h maintained bioactivities during 4 months of storage. Food processing increased the ease of consumption and also maintained the nutritional value of the sea lettuce, thereby promoting the utilization of this seaweed as a beneficial food ingredient.

Keywords: pretreatment; drying process; physical properties; bioactivity; *Ulva rigida*.

Practical Application: Pretreating sea lettuce (*Ulva rigida*), either by blanching or steaming before drying, improved the physical properties and maintained bioactivity, while oven drying was more beneficial compared to sun drying.

1 Introduction

Sea lettuce (*Ulva rigida*) is a nutritious green macroalga that is commonly used as a supplement in marine and poultry feed (Moroney et al., 2017; Onomu et al., 2020). This seaweed is not widely used in food industries but is sold in markets as a dry flake seasoning. Thunyawanichnondh et al. (2020) studied sea lettuce as an ingredient in healthy food such as low-fat snacks. Sea lettuce contains large amounts of tocopherol, carotene and phenolic compounds (Yildiz et al., 2012; Ismail et al., 2020; El Shafay et al., 2022), with low fat content and high unsaturated fatty acids. Protein content is low but easily digestible with good levels of essential amino acids (Paiva et al., 2017), high fiber and the unique polysaccharide ulvan (Neto et al., 2018; Hernández-Cruz et al., 2022). Sea lettuce shows potential for use as a food ingredient. The public now demand alternative healthy superfoods and seaweed is one ingredient in the spotlight. Processing methods have been recently studied to retain nutrient contents (Blikra et al., 2021).

A common process to preserve seaweed is drying, while brine is usually used for pickling as an easy and inexpensive procedure. Pinheiro et al. (2019) reported that hardness of sea lettuce increased after air drying at 25 °C, and was more pronounced if salt pickle was applied, while Silva et al. (2019) found that hot air oven drying at 60 °C maintained the greenness of seaweed better than drying at 25 °C. However, studies on sea lettuce pretreatment before drying on physical and bioactive properties are limited.

Here, pretreatment and drying methods on the physical properties and bioactivity of sea lettuce after rehydration were assessed. This information can promote the use of sea lettuce in the food industry to respond to future consumer demand.

2 Materials and methods

2.1 Materials

Cultivated sea lettuce was collected from the Phetchaburi Coastal Fisheries Research and Development Center between December 2021 and January 2022. Food grade and laboratory grade chemicals were purchased from local suppliers.

2.2 Sea lettuce preparation

The sea lettuce was soaked in 1% sodium bicarbonate solution for 5 min, then rinsed with tap water and drained before processing.

2.3 Pretreatment and drying methods

To prevent enzymatic discoloration, the cleaned sea lettuce was pretreated either by blanching in boiling water for 5 min or steamed for 5 min. The pretreated sea lettuce was then cooled to room temperature and spread on a stainless steel sieve. Hot air oven drying at 60 °C was compared to solar drying. The dried sea lettuce was collected in sealed metalized bags for further

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[1] *Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand*
[2] *Kasetsart University Research and Development Institute, Kasetsart University, Bangkok, Thailand*
[3] *Kung Krabaen Bay Royal Development Study Center, Chantha Buri, Thailand*
[4] *Phetchaburi Coastal Aquaculture Research and Development Center, Phetchaburi, Thailand*
*Corresponding author: ifrpws@ku.ac.th

analysis. The dried sample was rehydrated by soaking in water for 10 min before physical properties were determined.

2.4 Morphology of rehydrated sea lettuce

The morphology of rehydrated sea lettuce was studied by a Scanning Electron Microscope SEM (SU8020, Hitachi, Japan). Rehydrated sea lettuce was cut into 0.5x0.5 cm pieces and gradually dehydrated with 60, 80 and absolute ethanol before vacuum drying at 70 °C for 3 h. The dried samples were attached to a stub by carbon tape and platinum-coated using a sputter coater (Q150R ES, Quorum, UK).

2.5 Textural analysis

The hardness and firmness of rehydrated sea lettuce were measured with a texture analyzer (TA-XTplus, Stable Micro Systems, UK), equipped with a 0.25" diameter spherical probe and crisp fracture support rig. The speed of the test probe was 1 mm/sec. The hardness of the sample was recorded as the maximum force (g) required to compress the sample until rupture, while firmness was calculated from the slope of the first peak (g/sec).

2.6 Color measurement

The color of the rehydrated sea lettuce was determined using a Datacolor Spectrophotometer (Spectraflash SF 600 plus, Datacolor International, USA).

2.7 Determination of dried sea lettuce bioactivity

One gram of dried sea lettuce was treated with 50 mL of 60% ethanol for 3 h. The clear supernatant was collected and analyzed for total phenolic content using Folin-Ciocalteu reagent (Kaur & Kapoor, 2002) and free radical scavenging activity assay using the DPPH assay (Brand-Williams et al., 1995). Gallic acid and Trolox were used as the respective standards.

2.8 Statistical analysis

All experiments were carried out in duplicate, except for morphology analysis, with results reported as average values with standard deviations. Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) at p=0.05 were used to determine the differences between treatments by SPSS version 12.0.

3 Results

3.1 Effects of drying condition on sea lettuce properties.

Blanched and steamed sea lettuces are shown in Figure 1. Treated sea lettuce was duller and greener than fresh (untreated) sea lettuce, while dried (Figure 2) and rehydrated (Figure 3) sea lettuce showed no differences between conditions. Rehydrated sea lettuce appearance was not different from fresh sea lettuce.

The morphology of fresh and rehydrated sea lettuce studied by SEM is shown in Figure 4. The SEM photograph depicts the epibionts attached to the surface of fresh sea lettuce, while treated sea lettuce had fewer epibionts, especially the steamed oven dried sea lettuce. Overall appearance of rehydrated sea lettuce was similar to fresh seaweed. Blanched sea lettuce had a smoother surface than steamed sea lettuce, while oven drying resulted in a rougher surface.

The lightness (L*) and greenness (-a*) values of rehydrated sea lettuce were significantly lower than fresh sea lettuce for every drying condition, whereas yellowness (b*) values were significantly higher (Table 1). This result agreed with the appearance of the sea lettuce, as shown in Figure 1. Enzyme inactivation by steaming resulted in higher lightness and yellowness but lower greenness value than blanching, while drying methods had less effect on color parameters.

After 4 months of storage, greenness increased while lightness significantly increased. The yellowness value of the blanched sample did not change but yellowness decreased in steamed samples, implying that the color of dried sea lettuce faded during storage.

Hardness and firmness of rehydrated sea lettuce were significantly lower than fresh sea lettuce (Table 2). Enzyme inactivation by steaming sea lettuce had lower hardness than blanching, while inactivation methods did not alter firmness values. Storage time resulted in increased hardness and firmness of rehydrated sea lettuce for all drying conditions.

Free radical scavenging activities as antioxidant capacity and phenolic content of dried sea lettuce were significantly higher than in fresh sea lettuce (Table 3). Initial storage values of blanched and oven dried sea lettuce had the highest DPPH value while blanching and sun drying gave the lowest value. The DPPH value decreased when storage time increased. Phenolic content showed a similar result to the DPPH study. Fresh sea lettuce had lower phenolic compounds compared with the dry

Figure 1. Fresh and enzyme inactivated sea lettuce.

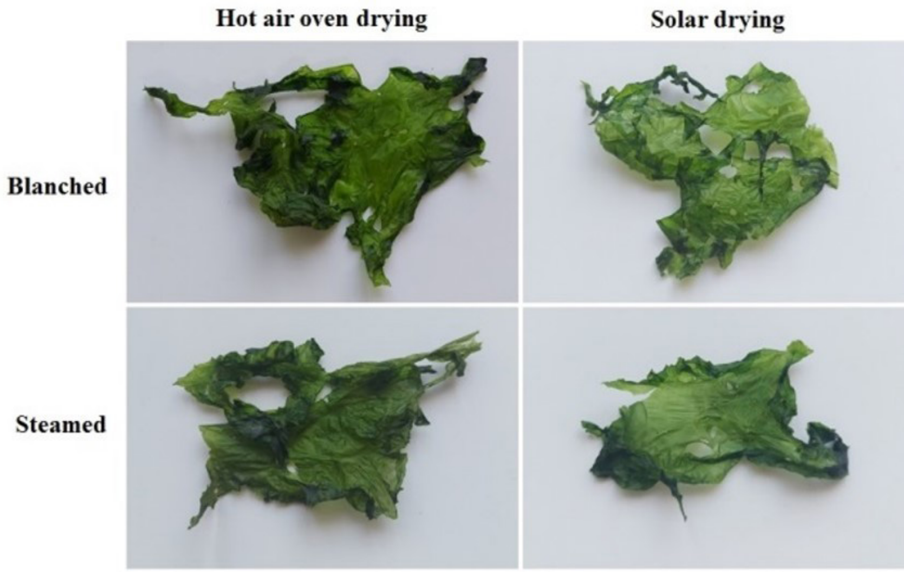

Figure 2. Sea lettuce dried under different conditions.

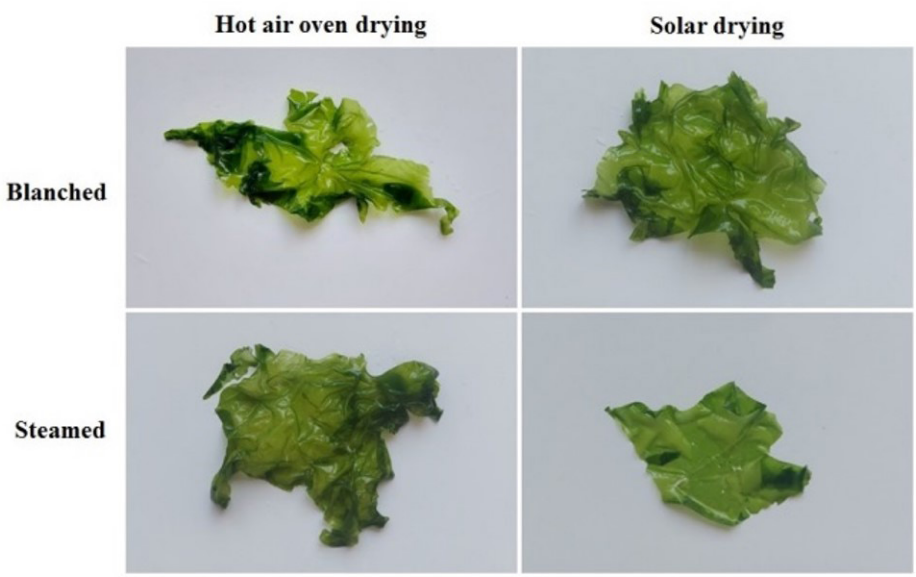

Figure 3. Dried sea lettuce after rehydration.

samples. Initial storage value of blanched sea lettuce was higher than steamed sea lettuce, while oven drying demonstrated larger amounts of phenolic compounds than sun drying. As storage time increased, free radical scavenging activity and phenolic contents of dry sea lettuce declined. Results indicated that enzyme inactivation and drying methods impacted the bioactivity of sea lettuce, especially for phenolic compounds.

4 Discussion

Bacteria and fungi are commonly found on the surface of macroalgae (Vallet et al., 2018; Mei et al., 2019). Bacteria form protein or polysaccharide networks that provide more attachment on the seaweed surface (Singh & Reddy, 2014). This contamination impacts the quality of seaweed (Subaryono & Kusumawati, 2020). Mechanical cleaning processes are time-consuming and may cause textural changes (Ruangchuay et al., 2021). Chemical cleaning methods are preferred in industrial applications that require less time and manpower. However, chemical cleaning may disrupt the algal cells (Nys et al., 1998) of sea lettuce due to the fragile structure of the monostromatic thallus (Liu et al., 2022; Hernández–Cruz et al., 2022). A 1% sodium bicarbonate solution was used as a cheap and safe chemical, widely applied as a vegetable washing agent in households and in the food industry. SEM images of blanched and steamed sea lettuce in Figure 4 show that sodium bicarbonate cleaning was an adequate treatment for preparing sea lettuce before cooking but may not be adequate for fresh consumption.

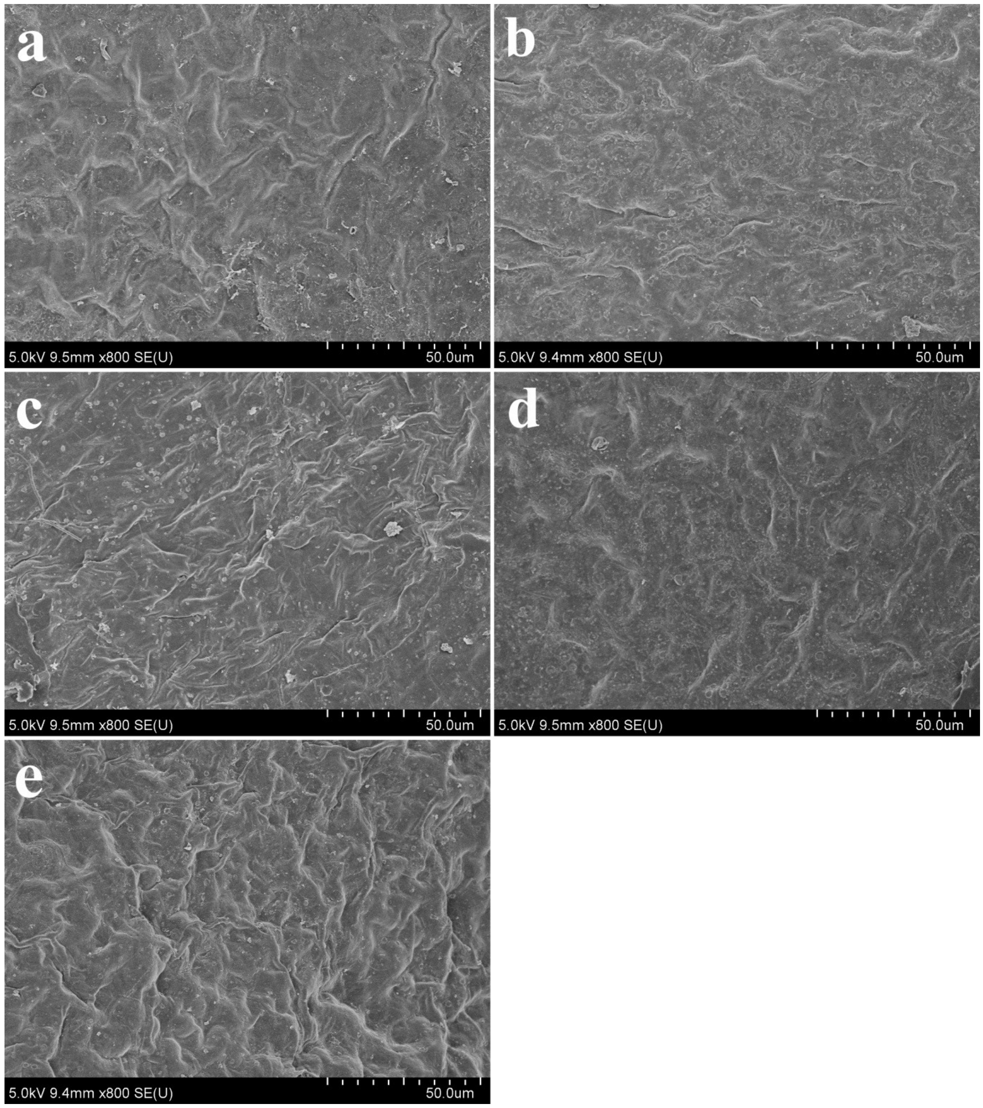

Figure 4. Morphology of rehydrated sea lettuce studied by SEM at 800x magnification; (a) fresh sea lettuce; (b) blanched and sun dried; (c) blanched and oven dried; (d) steamed and sun dried; and (e) steamed and oven dried.

Table 1. Effect of drying condition on color parameters of rehydrated sea lettuce.

Treatment		Storage time (month)	L*	a*	b*
	Fresh		24.65 ± 0.36a	-2.54 ± 0.15a	3.24 ± 0.02a
Blanched	Oven dried	0	17.29 ± 0.24b	-6.93 ± 0.05b	7.55 ± 0.10b
		2	17.54 ± 0.06b	-7.59 ± 0.12c	7.59 ± 0.05b
		4	20.47 ± 0.22c	-6.04 ± 0.46d	6.47 ± 0.36c
	Sun dried	0	17.62 ± 0.21b	-7.57 ± 0.08b	7.37 ± 0.12b
		2	18.32 ± 0.15c	-8.45 ± 0.27c	7.47 ± 0.15b
		4	19.56 ± 0.00d	-7.47 ± 0.31b	7.47 ± 0.28b
Steamed	Oven dried	0	18.55 ± 0.09b	-8.24 ± 0.10b	8.89 ± 0.21b
		2	18.60 ± 0.04b	-8.46 ± 0.32b	9.27 ± 0.11b
		4	20.49 ± 0.13c	-7.48 ± 0.12c	7.11 ± 0.35c
	Sun dried	0	18.50 ± 0.08b	-8.56 ± 0.07b	8.54 ± 0.21b
		2	18.59 ± 0.10b	-8.70 ± 0.23b	8.49 ± 0.13b
		4	20.54 ± 0.96c	-6.57 ± 0.13c	7.23 ± 0.03c

Different letters in the same column and drying condition indicate statistical differences (p<0.05)

Table 2. Effect of drying condition on hardness and firmness of rehydrated sea lettuce.

Treatment		Storage time (month)	Hardness (g force)	Firmness (g/sec)
	Fresh		175.85 ± 3.37a	47.47 ± 0.70a
Blanched	Oven dried	0	90.14 ± 6.89bA	30.02 ± 1.94bA
		2	100.89 ± 7.43cAB	34.94 ± 1.90cA
		4	141.23 ± 13.94dA	39.89 ± 3.56dA
	Sun dried	0	90.25 ± 8.40bA	29.61 ± 1.55bA
		2	100.53 ± 9.38cAB	32.87 ± 3.43cB
		4	145.36 ± 11.71dAB	38.78 ± 3.67dA
Steamed	Oven dried	0	93.83 ± 3.76bA	29.4 ± 1.67bA
		2	96.37 ± 6.10bA	32.80 ± 1.74cB
		4	152.64 ± 10.59cB	39.01 ± 3.32dA
	Sun dried	0	91.52 ± 5.75bA	29.98 ± 1.18bA
		2	105.88 ± 8.14cB	33.27 ± 1.58cB
		4	150.12 ± 17.10dAB	38.17 ± 4.68dA

Different letters indicate statistical differences ($p<0.05$); lower case alphabets indicate the storage effects and upper case alphabets indicate processing effects.

Table 3. Effect of drying condition on bioactivity (DPPH and phenolic compounds) of dried sea lettuce.

Treatment		Storage time (month)	DPPH (µg Trolox eq/g db)	Phenolic compounds (µg gallic eq/g db)
	Fresh		185.22 ± 3.47a	2,510.34 ± 11.09a
Blanched	Oven dried	0	232.12 ± 20.48bA	3,569.23 ± 44.07bA
		2	230.87 ± 10.26bAB	3,489.24 ± 16.99cA
		4	217.23 ± 3.76abB	3,149.25 ± 5.67dA
	Sun dried	0	207.37 ± 6.57bcA	3,497.29 ± 48.82bAB
		2	220.23 ± 4.70cA	3,472.92 ± 11.31bA
		4	204.88 ± 4.64bA	3,150.19 ± 5.66cA
Steamed	Oven dried	0	219.90 ± 12.11bA	3,477.24 ± 13.83bB
		2	240.54 ± 4.91cB	3,460.38 ± 22.65bA
		4	216.28 ± 2.95bB	3,151.32 ± 22.66cA
	Sun dried	0	224.58 ± 0.61bA	3,459.01 ± 0.51bB
		2	228.20 ± 5.38bAB	3,464.33 ± 11.31bA
		4	207.13 ± 4.90cAB	3,152.45 ± 11.34cA

Different letters indicate statistical differences ($p<0.05$), lower case alphabets indicate storage effects and upper case alphabets indicate processing effects.

Sun drying took 1.30 h, while oven drying required 2.30 h to completely dry the sea lettuce. The rate of dehydration differed from Uribe et al. (2019) who reported that oven drying at 70 °C was faster than solar drying at 50 °C in a closed solar dryer due to the higher effective water diffusion coefficient value (Ataudes et al., 2022). In this research, solar drying was performed in an open-air environment with higher air ventilation than in a hot air oven. Higher airflow caused greater water vapor diffusion of the sample (Walker et al., 2020) which reduced drying time (Lei et al., 2022). Sun drying involves many parameters that cannot be controlled, while oven drying allows modifications to the temperature, air ventilation and rehydration rate.

Color parameters identified by the International Commission on Illumination are L* a* and b*. L* is a measurement of luminosity, a* with a negative value indicates greenish color of the sample, whereas a positive value of b* refers to yellowish color (Silva et al., 2019). The sea lettuce turned dull green after both food processing methods. Change in color resulted from chlorophyll alteration (Funamoto et al., 2002). Green chlorophyll commonly degrades to gray-brown phosphytin or pheophorbide (Gunawan & Barringer, 2007; Pinheiro et al., 2019). Turkmen et al. (2006) showed that boiling and steaming reduced chlorophyll a and chlorophyll b contents, while inducing the appearance of pheophytin a and pheophytin b in cooked vegetables. Guo et al. (2023) reported that steaming increased the yellowish color of dried Flos Sophorae. Cooking methods had different effects on color or chlorophyll content due to energy transfer (Yang et al, 2022). The influence of drying processes on the pigments of sea lettuce was previously reported. Uribe et al. (2019) informed that hot air drying retained 70% of the carotenoid content while sun drying retained only 50%. Drying conditions had no effect on chlorophyll retention, while boiling and microwave cooking gave the same chlorophyll retention percentage (Chen & Roca, 2018). Pigment compound content was affected but the hue value that referred to greenness was not different between the cooking methods (Turkmen et al., 2006). Steam pretreatment showed better color stability during 4 months of storage than blanching.

Softening of the texture after processing, as shown in Table 2, was also reported by Mateluna et al. (2020). They suggested that microstructural damage resulted in a decline in hardness. Pretreatments had no significant effects on softening due to the fragile structure of the sea lettuce tallus, while storage time had a more pronounced impact on textural changes. Textural changes occurred during drying as a result of matrix collapse (Pantoja Espinosa et al., 2022). Hardness increase in stored dry sea lettuce was also reported by Pinheiro et al. (2019). At 4 months of storage, hardness of all the samples significantly increased, especially after steam pretreatment.

Apart from the physical alteration, processing also induced changes in sea lettuce bioactivity. Results in Table 3 show increased antioxidant capacity and phenolic compound content after processing as a result of internal disruption which enhanced biomass dissolution (Pezoa-Conte et al., 2015; Lima et al., 2022), while enzyme deactivity before drying increased phenolic compound content (Ho & Redan, 2022). Antioxidant capacity, as the DPPH value, positively related to total phenolic compounds (Gawron-Gzella et al., 2012; Ismail et al., 2020). The antioxidant capacity of blanching was not clearly different from steaming, while phenolic content of blanching was higher than steaming. Drying methods had an unclear effect on the bioactivity of dried sea lettuce. Uribe et al. (2019) found that larger amounts of phenolic compounds were retained after sun drying than hot air drying, while Silva et al. (2019) found that hot air drying temperatures of 25 to 60 °C retained the same amounts of phenolic compounds.

Longer storage time showed a decline in bioactivity, with no difference recorded between the pretreatment drying processes. Decreasing bioactivity values during storage time were caused by the interaction of polyphenols and other compounds (Mrad et al., 2012).

5 Conclusions

The appearance of sea lettuce after processing was not different from fresh sea lettuce, but color, texture and bioactivity properties changed. Processed sea lettuce had higher antioxidant capacity and phenolic compound content than fresh sea lettuce. Steaming before drying yielded a greener color than blanching. Rehydrated sun dried sea lettuce was also greener than rehydrated oven dried sea lettuce. Each drying process reduced the hardness and firmness of the sea lettuce, while bioactivity was slightly different. Results showed that the food processes softened and improved the bioactivity of sea lettuce, making it more suitable for consumption and utilization by food industries.

Conflict of interest

The authors declare no conflicts of interest.

Availability of data and material

Research data were not shared.

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