Effects of Different Heat Processing on Fucoxanthin, Antioxidant Activity and Colour of Indonesian Brown Seaweeds

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Abstract. Fucoxanthin (Fx) is a major carotenoids in brown algae. It showed many health beneficial effects for oxidative stress. Fucoxanthin is lower stability which may cause problem in the application for functional food. The objective of this study was to evaluate the effects of various heat processing on Fx, antioxidant activity (IC₅₀), total phenolic content, and colour stability of Sargassum ilicifolium. The various heat processing methods showed were not significantly affected to fucoxanthin and antioxidant activities however all treatments lower affected to brown seaweeds colour. Moreover, this study showed a useful proved in the design of brown seaweeds processing which minimize Fx, antioxidant activity and colour changes.

Keywords: brown seaweed, heat processing, antioxidant activity, Sargassum ilicifolium, fucoxanthin

1. Introduction

Marine resources offer important bioactive molecules that have advantages on the human body. They can be applied in many fields such as the drug, cosmetic, and food industries [1]. Seaweeds have been utilized since ancient times as foods in the far East and Asia and are the raw material for industrial production of agar, carrageenan and alginites [2]. South Asian countries were the first to introduce seaweeds for their utilization for medicinal and food purposes. Brown seaweeds represent a suitable supplement and additive for food due to their high nutritional value and the health benefits they can provide [3]. Brown seaweeds contain many bioactive compounds such as polysaccarida, phlorotannin, terpenoid, and fucoxanthin [4, 5]. Brown seaweeds contain Fucoxanthin (Fx) as a major carotenoid in brown seaweeds that is responsible for its colour [4, 6]. This carotenoid has been reported showing many health benefit effects as anti-obesity, antidiabetic [7, 8], Antioxidant [9], however Fucoxantin is unstable carotenoids due to rich electron in its structure [10]. Some factors have been responsible for its instability such as pH, light exposure, oxidator and reductar, and temperature [11, 12]). Several study reported fucoxanthin are abundantly presents in sub tropic brown algae such as Himanthalia elongata [13], Sargassum horneri [14, 15], Undaria pinnatifida [16], Cystoseira hakodatensis [15], Ecklonia kurome [14]. This carotenoids also presents in tropical brown seaweeds such as S. crassifolium, Padina australis,
Turbinaria ornata [14], Sargassum cinereum, Sargassum filipendula and Sargassum echinocarpum [17].

Processing of foods and food ingredients often exerts a major effect on their constituents, including nutraceutical products [18]. Some study reported, heat treatment in seaweeds changed their nutritional compounds and phytochemical contents [2, 19, 20]. It is known that heat treatment may also affect to fucoxanthin content, antioxidant activities and colour of brown seaweeds. For the design of an optimized processing to maximized preservation of Fx and antioxidant activity, the optimum heat treatment need to be evaluate. Therefore, the purpose of this study was to evaluate the effects of heat processing on Fx content and antioxidant activities in S. ilicifolium which is abundantly growth in Indonesia. The way to preserve and process of seaweeds are blanching, boiling, steaming and sterilizing. To our knowledge, there is no report on stability of Fx, phytochemical compound and their antioxidant activity after heat treatment, in Indonesian brown seaweeds.

2. Materials and Methods

2.1. Materials and sample preparation

DPPH, Folin Ciocalteu, Na₂CO₃, and gallic acids were purchased from Sigma Aldrich. Fucoxanthin standard, isolated from Padina australis, was purchased from MRCPP Universitas Ma Chung, Malang, Indonesia with Fx concentration >98.4%.

Fresh Sargassum ilicifolium was taken from Bandengan Beach, Jepara, Central Java, Indonesia. Immediately after cultivation, Fresh S. ilicifolium was transported to laboratory in low temperature condition. It was washed with fresh water to eliminate any presence of salt, and remove tiny particles also ephyphites. Afterward, S. ilicifolium was stored in refrigerator (-27°C) until further analysis.

2.2. Sargassum ilicifolium heat treatment processing

2.2.1. Blanching
About 150 g of S. ilicifolium was thawed and blanched by immersion in 40°C warm water using a water bath (Wisebath, Wenk LabTec, Germany) for 15 minutes.

2.2.2. Sterilization
About 150 g of S. ilicifolium were included in the Jar (250 ml) containing saturated sugar solution. About 15 minutes, seaweed was sterilized in 121°C, 1 atm air pressure.

2.2.3. Boiling
The seaweed was thawed prior to boil. Alga sample was immersed in 96°C boiled water for 15 minutes using a water bath (Wisebath, Wenk LabTec, Germany).

2.2.4. Steaming
About 150 g seaweed sample was placed on tray in a steamer and steamed over boiling water for 15 minutes.

2.3. Sample extraction
After 15 minutes of heat treatment, S. ilicifolium were dried with tissue and crudely cut into small pieces. Seaweed extracts were prepared with methanol solution. About 50 gr of treated seaweed
were immersed in methanol solution (1:5 w/v) for overnight. After 1 night extraction, seaweeds extract were filtered and removed organic solvent under vacuum using rotary evaporator. The extract were stored with methanol in concentration (3 mg/ml) and stored at freezer (-27°C) until further analysis.

2.4. Fucoxanthin analysis

Fx concentration was determined using a HPLC Shimadzu LC-20AT, column oven CTO-20AC system, Pump FCV-11AL, Degasser: DGU-20A, UV/Vis Detector SPD-20A, and communications bus module CBM-20A. About 20 µL methanolic seaweeds extract were used in the analysis. Before analysis, all seaweeds extract were filtrated using bulk Acrodisc PSF Syringe Filter 0,45 µM GHP membrane, 25 mm. Fucoxanthin analysis were done according to Susanto et.al [14] with slightly modification. A 5 points calibration curve was constructed with HPLC Shimadzu LC 20AT dissolved in methanol and acetonitrile (7:3 v/v) and accrued out at 28°C with octadecylsilica (ODS) column (TSK-gel ODS 80-Ts, 250 × 4.6 mm id, 5 mm particle size; Tosoh, Japan) protected with a guard column (15×3.2 mm) with the same stationary phase. The calibration formula was y=2.7977e-008X -0.000269384 with R²= 0.9999744.

2.5. Total Phenolic Content Analysis

Total Phenolic Content (TPC) were analyzed according to Kuda et al. [21] with slightly modification. Each 100 µL of sample solution was added to 750 µL of 10% Folin-Ciocalteu solution and incubated at 37°C. After 5 mins, 750 µL of 6% Na₂CO₃ was added and the mixture was allowed to stand at 37°C for 30 mins in the dark. The absorbance was measured at 761 nm. Total phenolics for each seaweed lipid was then calculated on the basis of a phloroglucinol standard curve and expressed as mg of galic acids equivalent (PGE)/g extract with formula Y= 0.0088x + 0.083 R²= 0.9948.

2.6. DPPH analysis

In present study, DPPH was used to analyze antioxidant activity in treated seaweeds. The methods was carried out according to Blois [22] with slightly modification. About 0.1 ml seaweeds methanolic extracts were reacted with 3.9 ml DPPH (with variable concentration 1, 2, 3, 4, 5 ppm in ethanol), afterward homogenized using vortex for 2 minutes. After homogenized, all solutions were incubated for 30 minutes at dark room temperature. The scavenging activity of DPPH radicals was calculated as percentages by the following equation:

$$\text{DPPH radical scavenging activity} \% = \frac{(1 - A_{\text{samples}})}{A_{\text{control}}} \times 100\%$$

Where A_{\text{samples}} and A_{\text{blank}} are the sample and blank absorbance in 517 nm, respectively. The IC₅₀ was calculated by linier regression.

2.7. Colour intensity (L, a, b) analysis

Quantitative evaluation of color changes in methanolic extract was done using portable colorimeter (Apple, GM52223LSGJ). The extract samples were transferred into glass cell and measured with portable colorimeter (Apple, GM52223LSGJ).

The total amount of colour change to see effects of heat treatment was calculated as per equation [23].

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2}$$
Where $L_0$, $a_0$ and $b_0$ are the colour parameters or fresh seaweeds. This total change takes in to account the change in each colour describes by the $L^*$, $a^*$ and $b^*$ parameters. $a^*$ positive value reflected red colour to negative value reflected green) and $b^*$ (positive value reflected yellow colour to negative value reflected blue).

2.8. FTIR analysis

Fucoxanthin structure in each treatment were analyzed with IRPRSTIGE-21 Shimadzu. The analysis was based on the manual instruction of FTIR IRPRSTIGE-21.

2.9. Data analysis

The data obtained for all parameters excluded FTIR analysis were statistically treated to obtain 95% confidence interval. The results obtained were analyzed using one way ANOVA among treatment using SPSS 16.

3. Results and discussion

3.1. Fucoxanthin Content

Fucoxanthin is xanthophyll which is abundant in brown seaweed and shows many health benefit effects. Fx were unstable due to some factors such as temperature, light, acid and pH [12, 13]. Brown seaweeds were processed in different ways before consumption. Thermal processing will affect to Fx availability in brown seaweeds. As consequence, Fx content in brown seaweeds need to evaluate after processing. Fx in *S. ilicifolium* was investigated after four different thermal processing (Fig. 1). Fx content in brown seaweeds before processing was 39.53 ppm, after processing resulted different content of Fx. The different thermal processings were not significantly different ($p > 0.05$) to Fx content. The highest Fx content was found in blanching treatment, however sterilizing treatment showed the lowest content of Fx. These showed that Fx content in *S. ilicifolium* affected by thermal processing.

![Figure 2](image-url) **Figure 2.** Fx content in fresh and thermal processing in *S. ilicifolium*. Values are express in $\mu$g/ml.

Fx content of *S. ilicifolium* were higher in thermal processing than fresh samples. The heat treatment processing might be increasing free Fx from protein during thermal processing. In oxygen-evolving photosynthetic organism, Fx is in form of Fx-chlorophyll protein (FCP) which is responsible for absorption and conversion of light energy [24]. In case of blanching treatment showed the highest Fx content among other treatment and was negatively affected by the heating.
this might be due to blanching treatment was able to inactive enzyme that degraded Fx to other compounds during preparation and extraction. This reason was supported with blanching treatment chromatogram (Fig 2.). Carrilho et al. [19], have reported that dried seaweeds followed by blanching significantly increase Fx content in Himanthalia elongata. In addition to the result obtained Carrilho et al. [25], which observed an increasing of Fx content Undaria pinnatifida and Laminaria japonica which were boiled for 15 minutes and 1 hour respectively. Pre-treatment of L. japonica (konbu) including heating, washing, cutting and freezing was able to increasing amount of Fx [26].

Fucoxanthin which is chemically structure in all-trans isomer is unstable during heat treatment. This compound might be degraded into 9’-,13- and 13’- cis Fucoxanthin which were cis isomers of Fx [27]. This isomerization is recognized Fx degradation in heat treatment. During separation of Fx from Hijika fusimorme, there were two other peaks along with all-trans-fucoxanthin peak. The ratio of all-trans-fucoxanthin and its two cis-isomers were 100:2:3 [28].

![Figure 2. HPLC chromatogram of blanching (a), cooking (b), steaming (c), sterilizing (d) of S. ilicifolium. (1) all-trans fucoxanthin (2) cis fucoxanthin.](image)

3.2. Total Phenolic Content

The total of phenolic content (TPC) was determined using the Folin Ciocalteu Method based on Kuda et al. [21]. The total phenol content of examined S. ilicifolium ranged from 33.36 to 47.60 (µg GAE/mg extract) (Table 1.). Blanching treatment showed the highest content of TPC, however the lowest content found in steaming treatment. TPC were not significantly different (p>0.05) in all heat treatment. This result indicated heat treatments were not significantly affected to TPC. In contrast, Dang et al., 2014 [29] who studied the effect of different drying method in Hormosira banksii and found that different drying method significantly affected to TPC in sample.
The sequence of TPC content in *S. ilicifolium* tested were blanching > boiling > steaming > sterilizing. The different total phenolic content (TPC) decreasing rate might be due to the different temperature on the treatments, TPC decreased when the heat temperature increased. Carrilho *et al.* [25], reported total phenolic content on wakame and konbu were decreasing after cooking treatment. However, TPC in *H. elongata*, *L. Saccharina*, and *L. digitata* increasing until 95°C, heating and decreased continuously until 121°C heating [29].

Brown seaweeds contain phloroglucinol phenolics, phlorotannin [31, 32, 33]. These compounds exhibit many beneficial biological activities such as antioxidant, anticancer, anti-diabetic, anti-human immunodeficiency virus, antihypertensive, matrix metalloproteinase enzyme inhibition, radioprotective, and antiallergic activities [34]. The TPC results of this study are promising on the application of food processing based algae as source of phenolic content.

### 3.3. DPPH radical scavenging activity of methanolic extract

DPPH reagent has been used extensively for investigating the free radical scavenging activities of compounds [35]. DPPH is primarily evaluates proton radical-scavenging ability [36]. One of parameter that has been introduced recently for the interpretation of the results from the DPPH method is efficient concentration EC$_{50}$ or IC$_{50}$. EC$_{50}$ or IC$_{50}$ is defined as the concentration of substrate that causes 50% loss of the DPPH activity (colour) [37].

All treatments were measured for DPPH radical scavenging activity. As shown in Fig 2. All heat treatments show radical scavenging activity in *S. ilicifolium* methanolic extracts. DPPH radical scavenging activity shows various degree of antioxidant activity IC$_{50}$ (Fig. 2.). DPPH (IC$_{50}$) of the methanolic extract from fresh *S. ilicifolium* was 59.14 µg/ml. Of the tested in heat treatments *S. ilicifolium* showed that, steaming treatment showed the highest antioxidant activity. In contrast, boiling and sterilizing showed the lowest antioxidant activity among treatments tested. These results indicated different heat treatment influencing their extractability. The phenolic compounds usually present in bound states of conjugates with sugar, fatty acids or protein. Thermal processing might be disrupt of cell wall and cell membrane which may produce different extractability resulting different availability of antioxidant compounds [30, 38]). In addition, heat treatment could deactivate endogenous oxidative enzymes that are responsible for the destruction of antioxidants. Therefore, its absence could increase antioxidant [30].

#### Table 1. TPC (µg GAE/mg extract) content in fresh and thermal processing in *S. ilicifolium*

| Treatments    | TPC (µg GAE/mg extract) |
|---------------|-------------------------|
| Fresh         | 47.60±15.05$^{a}$       |
| Blanching     | 42.90± 2.63$^{a}$       |
| Boiling       | 37.00± 8.46$^{a}$       |
| Steaming      | 34.08± 1.12$^{a}$       |
| Sterilizing   | 33.36± 0.11$^{a}$       |

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The different antioxidant activity related with presence of Fx than phenolic compounds. This analysis was supported by Pearson correlation test. Fucoxanthin was found positively correlated with 0.282 besides that, TPC less correlated with 0.013. Different results found by Wang et al. [39], the extracts containing high levels of TPC were also potent DPPH radical scavenger, suggesting that algal polyphenols may be the principal constituents responsible for antioxidants. Fx was revealed as high antioxidant activity [9], however this compounds is unstable due to increasing temperature (Zhao et al., 2014) [12]. The ability of phenolic compounds as antioxidant in consequence of these compounds can donate hydrogen ions or electrons and their stable radical intermediates, which prevents the oxidation of various food ingredients [40]. In addition, as antioxidant, carotenoids are able to quench singlet oxygen without changing its structure and scavenge of free radical [41]. Another antioxidant compound in brown algae is alpha tocopherol [42]. This compound is highly stable to heat [25].

3.4. Colour change

The color parameter and the change in color of methanol extract of all seaweeds treated were evaluated and calculated. The change in Hunter colour of the heating treated S. ilicifolium methanolic extracts are shown in Table 4. Parameter L*,a*,b showed significantly different between freshand blanching treatment. Differences of L,a*,b value affected to ΔE*, the highest and the lowest ΔE* value showed in blanching and steaming treatment, respectively. This value visually meant dark and brighter color, respectively. In brown seaweeds, fucoxanthin, β carotene and chlorophyll were responsible for its colour [43].

Result showed that treatment showed low effect in the colour change among treatments, as the temperature increase the ΔE value. These results were different from Rajauria et al. [29], heat treatment strongly effect on change in color as the temperature increase. The differences on the heat treatment significantly affected (p<0.05) to L,a, b value on each extract.

The colour change in steaming treatment showed the lowest among other treatment. The smaller ΔE value, the closer the samples are in colour close to fresh seaweeds. In contrast, the blanching treatment revealed highest ΔE, dark green colour on this treatment due to much content of Fx.
Table 4. Color extract in fresh and heat treatment processing of *S. ilicifolium*

| Treatment    | L     | a     | B     | ΔE    |
|--------------|-------|-------|-------|-------|
| Fresh        | 27.700<sup>a</sup> | 2.208<sup>a</sup> | 57.533<sup>a</sup> | -     |
| Blanching    | -8.857<sup>b</sup>  | -31.498<sup>b</sup> | 24.288<sup>b</sup> | 77.643<sup>a</sup> |
| Boiling      | 33.539<sup>bc</sup> | -54.871<sup>bc</sup> | 64.585<sup>ab</sup> | 86.508<sup>a</sup> |
| Steaming     | 17.84<sup>bd</sup>  | -42.183<sup>bd</sup> | 50.070<sup>ab</sup> | 70.906<sup>a</sup> |
| Sterilizing  | 23.620<sup>ab</sup> | -39.687<sup>be</sup> | 37.304<sup>ab</sup> | 76.618<sup>a</sup> |

ΔE in heat treated seaweeds was ranged from 70.906 to 86.508, which is indicated a huge different with fresh seaweeds. Blanching colour revealed great different (GD) to boiling and sterilizing treatment respectively; this is related with Fx content in treated seaweeds. According to Silva and Silva [24], stated that value ΔE > 12 for very great difference (VGD). Colour can be used to design adequate thermal processing conditions for maximizing final product quality. In this study, the increased ΔE value was reflected in high antioxidant activity.

3.5. FTIR

FTIR analysis was used for the characterization of the methanolic extract of fresh and treated seaweeds. The FTIR spectrum of Fx tested in seaweeds is shown in Fig 4, and it was found that different treatment have different absorption band with fresh seaweeds. From FTIR spectra we confirmed that the functional group that describe Fx is allenic bond (C=C=C) were very low in all samples. This might be due to the higher content of moisture which interfere with the infrared absorbance [44].

![FTIR spectra](image)

Figure 4. FTIR spectra of fresh seaweeds (a), blanching seaweeds (b), cooking seaweeds (c), steaming seaweeds (d), sterilizing seaweeds (e).

These results showed that the presence of allenic bound, which is presence in fucoxanthin is very low. This phenomenon might be due to high amount of methanol(O-H) in samples which is interfere infrared
The presence of alcohol (methanol) is attributed in peak 3425.28 to 3394.73. Carboxylic acids (C=O) was occurred in absorption 2924.09. Esters with -C=O bonds showed presence in absorption 1735.93. In addition, absorption of 1635.64 showed presence of alkenes (C=C) bonds. Therefore, the presence of allenic functional group in samples contain fucoxanthin as major carotenoids.

4. Conclusion

In this study, blanching treatment was efficiently inhibited degradation of fucoxanthin phenolic content compared to other treatments. The antioxidant activity showed higher content in boiling treatment. Steaming process was able to minimize colour change. This report provides useful data in the design of brown seaweeds processing which minimize Fx, antioxidant activity and colour changes.

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