Influence of harvest time on carotenoids content of brown seaweed (*Padina* sp. and *Sargassum* sp.) infused oil

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Abstract. Brown seaweeds (*Padina* sp. and *Sargassum* sp.) - infused oil is a seaweed extract product, that uses an edible oil (virgin coconut oil) as a solvent using the maceration method. The objective of this study was to determine the effect of harvest time of brown seaweed in the rainy and dry seasons on the carotenoid content of the seaweed-infused oil. The measurement of carotenoid content as β-carotenes in seaweed-infused oil has been done using the UV-visible spectrophotometer. *Padina* sp. and *Sargassum* sp. that grow in the dry season produced seaweed-infused oil with β-carotenes of 8.54±1.20 µg/ml and 96.48±26.90 µg/ml, and those vegetate in the rainy season 163.79±37.56 µg/ml and 350.88±32.89 µg/ml, respectively. Seaweed-infused oil contains higher carotenoid pigments from brown seaweed that harvested in the rainy than those in the dry season which they grow naturally throughout the years.

1 Introduction

The extraction of carotenoid pigments from brown seaweed using solvents has been developed. Carotenoids can be extracted using solvents such as ethanol, acetone, chloroform, ethyl acetate, hexane, and diethyl ether [1, 2]. In addition to using chemical solvents, carotenoids can be extracted from vegetable oil [3, 4, 5]. Maceration of seaweed using a vegetable oil produces active ingredients such as carotenoids. This process produces a product generally known as seaweed-infused oil or seaweed-oil infusion [6]. Thus, seaweed-infused oil can be made by the maceration method, which uses vegetable oil as a solvent and enriches its content with the metabolite of brown seaweed. Maceration is the conventional extraction method that, until now, has been used especially in the home industry, because this method is simple [7] and its operation is cheap.

Carotenoids obtained from brown seaweed are secondary metabolites, that is active compounds that function as self-protection against the surrounding environment. The role of secondary metabolites in brown seaweed such as carotenoid are: to protect themselves from the effects of extreme sunlight and to absorb sunlight as energy and send it to chlorophyll to

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help the photosynthesis process. The amount of carotenoid pigment compounds produced by seaweed extract is influenced by the harvest time of seaweed [6]. Harvest time during the rainy and dry seasons is influenced by surrounding environmental conditions such as the age of seaweed [8], tidal conditions [9], and length of sunlight [10]. Brown seaweed in this study was obtained from the harvest of naturally growing seaweed (not the result of their cultivation), so the harvest time is influenced by the environmental conditions and the age of brown seaweed, which can be reviewed in terms of its size. If it is young, then the thallus is smaller than the adult brown seaweed. Thus, the aims of this study was to determine the effect of harvest time for brown seaweed (Padina sp. and Sargassum sp.) on the carotenoid content in brown seaweed-infused oil. This will become a concern for researchers and entrepreneurs who focus on agro-maritime in the seaweed sector, especially for industries that utilize secondary metabolites of seaweed as raw materials. Moreover, seaweed has opportunities as nutraceuticals and cosmeceuticals for the next generation, which cares for environmental sustainability [11, 12].

2 Methods

2.1 Materials

Brown seaweeds Padina sp. and Sargassum sp. were harvested from Binuangeun Beach, Banten, Indonesia. They were harvested at different times: October 2019 (dry season) and February 2023 (rainy season). The sampling period had a gap of up to three years because it was hampered by the COVID-19 pandemic. Their thallus was taken whole and washed with seawater to remove sand and coral impurities. Next, the seaweeds were dried in the sun for three days at the location (Binuangeun). After drying, the seaweeds were taken to the laboratory and stored at room temperature.

Other ingredients were virgin coconut oil (MIKADA, Tasikmalaya, Indonesia) that was made by cold processing, n-hexane for analysis (EMSURE, Merck, Germany), and β-carotene standard for analysis (Sigma-Aldrich, USA).

2.2 Preparation of brown seaweed

Seaweed harvested during the dry season and rainy season at the same location, respectively, was prepared as follows: a) the seaweed was soaked in water for 1 hour and rinsed with water so that it was clean from epiphytes, salt, and sand impurities; b) the seaweed was dried using grid tray at ambient temperature 18 °C for five days; c) dried seaweed was made into flour using a grinder mill and filter size of 80 mesh; and d) brown seaweed powder of 80 mesh was ready to be processed for seaweed-infused oil.

2.3 Extraction of brown seaweed

The extraction of brown seaweed was modified from Wahyuni and Murdinah [5]. Powders of brown seaweed (Padina sp. and Sargassum sp.) harvested in the dry season and rainy season, respectively, were macerated with virgin coconut oil (VCO) in a ratio of 1:10 (w/w) using a water bath at a temperature of 60 °C, shaking at 125 rpm, for 2 hours. Then it continued with maceration without shaking for 24 hours at ambient temperature and was then filtered using a 400-mesh nylon filter to separate the seaweed powder residue. Next, the filtrate was centrifuged at 3000 rpm for 20 minutes, and the supernatant, namely brown seaweed-infused oil, was stored in a dark condition at ambient temperature. The maceration process of Padina sp. and Sargassum sp. were repeated three times.
2.4 Analysis of brown seaweed-infused oil

2.4.1 Carotenoid content analysis

The carotenoid content of seaweed-infused oil was determined using a UV-visible spectrophotometer (Evolution 220, Thermo Scientific, America). *Padina* sp. seaweed-infused oil and *Sargassum*-infused oil, respectively, harvested in dry and rainy seasons, were diluted with n-hexane, and the UV-visible spectrophotometer read at \( \lambda = 450 \) nm. As a control, VCO without enrichment with *Padina* sp. and *Sargassum* sp. was also dissolved in n-hexane, and its absorbance was measured. The curve of calibration using \( \beta \)-carotene was designed (10 to 400 µg/ml). The absorbances of seaweed-infused oil and VCO as the control were plotted for the linear regression curve of the \( \beta \)-carotene standard with the result that carotenoid content is equivalent to \( \beta \)-carotene in seaweed-infused oil (µg/ml). Carotenoid content analysis on seaweed-infused oil samples was carried out three times.

2.4.2 Colour analysis

The colour analysis of seaweed-infused oil and VCO used a Chroma Meter (KONICA MINOLTA). Samples of seaweed infused oil were filled in a glass chamber, and values \( a^* \) (redness), \( b^* \) (yellowness), and \( L^* \) (lightness) were identified in triplicate.

2.4.3 Statistical analysis

The statistical data analysis aimed to determine the presence of a significant difference (at a 95% confidence level) using randomized block design in the characteristics of seaweed-infused oil from *Padina* sp. and *Sargassum* sp. harvested during both the dry and rainy season.

3 Results and Discussion

Brown seaweeds that are *Padina* sp. (Figure 1) and *Sargassum* sp. (Figure 2) have pigment compounds which are a secondary metabolites. Pigment compounds such as carotenoids are involved in harvesting sunlight energy and protecting against excess sunlight [13]. Thus, whether the harvest time of brown seaweed affects the content of pigment compounds in a product will be discussed in this section of the chapter.
Fig. 2. Brown seaweed of Sargassum sp.

Seaweed-infused oil is an oil product that is enriched with active compounds from seaweed by macerating the seaweed using an oil solvent so that the active ingredients in the seaweed are infused into the oil [5]. In this research, seaweed-infused oil was made that used VCO as a solvent which had carotenoid 0.20±0.07 µg/ml. The active ingredient infused was a carotenoid pigment compound derived from brown seaweed, namely Padina sp. and Sargassum sp. that was enriched in VCO, and as a result, the carotenoid content increased in VCO, as shown in Figure 3, Padina sp. and Sargassum sp. that grow naturally.

The influence of brown seaweed harvest time on the β-carotene content in seaweed-infused oil products is expressed in Figure 3. During harvest time in the dry and rainy seasons, Padina sp. and Sargassum sp. produce seaweed-infused oil products with significantly different β-carotene content with a confidence level of 95%.

![Carotenoid content graph](image)

VCO and brown seaweed-infused oil (BSIO)

Fig. 3. Significant differences exist in the carotenoid of the brown seaweed-infusion oil characteristics between Padina sp. and Sargassum sp. during both the dry and rainy seasons, with a confidence level of 95%.
Padina sp. and Sargassum sp. harvest times during the dry and rainy seasons provide carotenoid content in brown seaweed-infused oil of 8.54±1.20 µg/ml, 96.48±26.90 µg/ml, 163.79±37.56 µg/ml, and 350.88±32.89 µg/ml, respectively. Research by Heffermen et al. [6] revealed that the Laminaria digitata seaweed harvested in summer has abundant carotenoid content, which is due to reaction to abundant sunlight causing seaweeds to defend themselves from environmental damage that gets excessive sunlight. However, in this research on carotenoid in seaweed-infused oil, it did not happen, but rather the opposite: Padina sp. and Sargassum sp., which were harvested in the dry season when sunlight was abundant, expressed less carotenoid content in brown seaweed-infused oil. This could be due to several factors, namely the age of the seaweed [8], tidal conditions [9], or the length of exposure to sunlight [10].

Young brown seaweeds contain fewer pigment compounds than old brown seaweeds [8]. It should be noted that when harvesting seaweed in the dry season, Padina sp. and Sargassum sp. that grow naturally are still young; that is, physically, they appear to have a shorter and thinner thallus size compared to Padina sp. and Sargassum sp., which are harvested during the rainy season and are already adults. Physically, it can be seen that their thallus is bigger and thicker. Thus, the content of carotenoid pigment compounds is lower when harvested in the dry season when seaweed is young compared to when harvested in the rainy season when seaweed is mature.

Aside from the age factor of seaweed, tidal conditions influence the pigment content of seaweed [9, 14, 15]. Tide conditions higher than 1 m cause seaweed to be protected from excessive sunlight so that carotenoid pigment compounds absorb sunlight more effectively, whereas carotenoids absorb sunlight at a wavelength of 450–550 nm and transfer it to chlorophyll pigment [16, 17]. When harvesting seaweed in the dry season, sea conditions are receding. Padina sp. and Sargassum sp. seaweeds are less than 1 meter below the water surface (low tide conditions), so they are exposed directly to sunlight. Exposure to direct sunlight increase sea water temperature, which causes bleaching pigment [18] that results in less carotenoid pigment content compared to when harvested in the rainy season where the seaweed is more than 1 m below sea level, which is high tide conditions.

The factor of the length of exposure to sunlight affects the production of carotenoid pigments in seaweed, where carotenoid pigments function as protection from exposure to sunlight and absorb sunlight to transfer it to chlorophyll so that it indirectly helps in the photosynthesis process [16] but if the exposure to sunlight is extreme, it will cause bleaching of the pigment [10]. According to the Meteorology, Climatology, and Geophysical Agency, Indonesia, through its data service system, it is informed that in Lebak Regency during the rainy season (December 2022 to February 2023), the duration of sunlight is shorter than during the dry season (August 2019 to October 2019). This results in seaweed being exposed to extreme sunlight so that the seawater temperature is higher and the carotenoid pigment content experiences bleaching, which causes the carotenoid content to decrease during the dry season compared to the rainy season [18].

Thus, the discussion above explains that the influence of harvest time during the dry and rainy seasons results in differences in the carotenoid content of brown seaweed-infused oil. It also affects the colour difference in brown seaweed-infused oil. Analyses L* a* b* of VCO colour had been done that was 50.24±0.13, 0.00±0.02, -0.31±0.02, respectively. The L* a* b* of VCO color showed clarity before VCO was enriched with Padina sp. and Sargassum sp. (Figure 4 and Figure 5). The VCO color had changed after VCO was used as a solvent for Padina sp. and Sargassum sp., so the metabolite of Padina sp. and Sargassum sp. was infused in VCO, a so called seaweed-infused oil (Figure 4 and Figure 5).
VCO has been influenced by the carotenoid content of seaweed, whose carotenoids have yellow, orange, and reddish colors [13, 19]. Table 1 shows the results of the colour analysis of brown seaweed-infused oil, which states that *Padina* sp. and *Sargassum* sp. harvested during the rainy and dry seasons have significant color differences with a confidence level of 95%.

**Table 1.** Significant differences exist in the brown seaweed-infusion oil characteristics between *Padina* sp. and *Sargassum* sp. during both the dry and rainy seasons, with a confidence level of 95%

| Brown seaweed-infused oil | Dry season | | | Rainy season | |
|---------------------------|------------|-----------------|-----------------|
|                           | L*         | a*              | b*              | L*              | a*              | b*              |
| *Padina*-BSIO             | 46.36±0.64 | -0.58±0.10      | 4.81±0.16       | 40.78±0.80      | 0.45±0.11       | 1.84±0.23       |
| *Sargassum*-BSIO          | 41.88±2.95 | 1.47±0.08       | 1.31±0.31       | 39.49±0.23      | 1.70±0.04       | 0.62±0.07       |

Table 1 reveals that the carotenoid contents of *Padina* and *Sargassum* harvested during the rainy season give a reddish yellow of brown seaweed-infused oil color that is more intense than *Padina* sp. and *Sargassum* sp. harvested during the dry season, namely the brown seaweed-infused oil colour looks brighter reddish yellow. It happens because brown seaweeds harvested during the rainy season have a higher carotenoid content, so the color is
a deep reddish yellow compared to those harvested during the dry season, which has a low carotenoid content, so the color is a very bright reddish yellow.

4 Conclusion

Brown seaweed-infused oil contains carotenoid as active ingredients. The bioactive ingredient content in brown seaweed-infused oil products has been influenced by the harvest time of brown seaweed. Brown seaweed-infused oil contains carotenoid as active ingredients. The carotenoid content for Padina sp. was higher than for Sargassum sp. Harvesting seaweed, in rainy season resulted in carotenoid content higher than in dry season. Thus, this information can be used as a reference for researchers and entrepreneurs in the seaweed industries. The harvest time of seaweed that grows naturally under the influence of seaweed life and environmental conditions and the age of seaweed, affects the metabolite of seaweed, which affects the quality of the raw material and its product.

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