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THE SOLVENT EFFECTIVENESS ON EXTRACTION PROCESS OF SEAWEED PIGMENT

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Abstract

Eucheuma cottonii seaweed is a species of seaweed cultured in Indonesian waters, because its cultivation is relatively easy and inexpensive. It has a wide variety of colors from green to yellow green, gray, red and brown, indicating photosynthetic pigments, such as chlorophyll and carotenoids. An important factor in the effectiveness of pigment extraction is the choice of solvent. The correct type of solvent in the extraction method of specific natural materials is important so that a pigment with optimum quality that is also benefical to the society can be produced. The target of this research is to obtain a high quality solvent type of carotenoid pigment. This research was conducted using a randomized block design with three (3) replications involving two factors namely solvent type (4 levels: aceton, ethanol, petroleum benzene, hexan & petroleum benzene) and seaweed color (3 levels: brown, green and red). Research results indicated that each solvent reached a peak of maximal absorbance at λ 410-472 nm, namely carotenoids. The usage of acetone solvent gave the best pigment quality. Brown, green and red seaweed have pigment content of 1,28 mg/100 g; 0,98 mg/100 g; 1,35 mg/100 g and rendement of 6,24%; 4,85% and 6,65% respectively.

Keywords: extraction, carotenoid pigment, seaweed color, solvent type

1. Introduction

In recent time, people has become more and more suspicious of the types of artificial ingredients that are added to our everyday diet. If critical consumers do not perceive a substantial benefit from an added ingredient (e.g., an enhanced stability by the addition of antioxidants), they might not buy the products [1]. Indonesian food produces in this field still do not control the use of food additives. This includes well accuracy of measuring and also the type. It is estimated that the existence of food distribution in markets contain colorants of non-food and colorants of food which has been prohibited by the government. For that, exploration in biological potency is very needed as an effort to "return to nature". Natural colorants which are safe to be used and can be developed, for example from pigment of carotenoid to curcumin, and antocyanin, can be obtained from crop [2], and one of them was from seaweed.

In many parts of the world, seaweed is used as a source of food. Seaweeds are broken into different categories according to their colors, such as blue seaweeds, diatom, green seaweeds, brown seaweeds, and red seaweeds. Color is an important characteristic of food, and their colors could be used for food coloring [3]. According to [4], seaweed of brown, green, and red till aquamarine colour contain many pigments of carotenoid and lutein.

Recently, a pigment, such as carotenoid (fucoxanthin) could even provide a new functional food and cosmetic ingredient with anti-metabolic syndrome activity (anti-obesity, anti-diabetes) [5-6] that has important nutraceutical properties, including antioxidant besides providing anti-obesity, anti-inflammation effect [7-8], low risk for breast cancer [9-10], prostate cancer cells [11] and a diminishing risk of cardio-vascular disease [12].

Carotenoid pigment is one of fat-soluble pigmens. For that, the maximization of the extraction process must use the solvent of fat, such as acetone, alcohols, ethers and hexan preparation [13]. Each solvent gives different results of pigments depending on the suitability. The extraction process with two kinds of solvent of fat could have different results. Solvent of extraction process is effective if it is capable of producing pigments of high quality and quantity.

In order to be known, the correct solvent type in the extraction method of vitally specific natural materials can yield the quality of maximal and good pigment and also be of benefit to the society. Target of this research is to give solvent type of carotenoid with high yield and quality.

2. Methods

Raw materials used in this research were fresh seaweeds with green, red and brown colours obtained from Madura, Indonesia. Solvents used were aceton, ethanol, petroleum benzene, and hexan. Equipments used in this research were rotary vacuum evaporator, UV-vis spectrophotometer Shimadzu brands, Colour Reader CR-10, and pH meters (832 CG Gerale School).

The purpose of this study was to obtain an effective solvent in the process of carotenoid pigments extracted from the three colors of seaweed. This research was conducted using a randomized block design with three (3) replications, with two factors of solvent type which consists of 4 levels (aceton, ethanol, petroleum benzene, hexan & petroleum benzene) and seaweed color which consists of 3 levels (brown, green and red seaweed).

The carotenoid pigment extraction process begins by weighing 30 g. They were destroyed by organic solvents (according to treatment) with a ratio of 1 : 5 (sample: solvent), then stirred with a magnetic mixer for 3 hours, and to continued the extraction had filtered using cotton. This pigment filtrate results from concentrated filtration using a rotary vacuum evaporator at a temperature of 60-70 °C so that its volume be 1/8 of the initial volume. Then the concentrated pigment was Rf analyzed using paper chromatography (KKt) with a mobile phase of petroleum ether: benzene (1 : 1). The concentrated pigment was observed in terms of its absorbance at λ according to characteristics of carotenoid pigments (400-490nm) [14], pH, color intensity (L, ab) its.

Observation parameters for detecting the effectiveness of the extraction process with various solvents including absorbance peak (spectrophotometric method), the lightness value (chromametry tography method), pigment content and rendement [15].

3. Results and Discussion

In our present study, the pigments of the three algae species were determined using various solvents for extracting various pigments and were examined comparatively.

Lightness value. Lightness value of a concentrated solution indicated of solute (in this case, pigment) in the solution. The more bright a solution (high lightness value), the lower the solute content; the lower the lightness degree of a solution, the higher the concentration of dissolved substances.

Solvent ethanol produces pigments with the lowest brightness level for the green seaweed, and then followed by acetone solvent. Pigment filtrate and concentrate were brownish yellow colour, the relatively dark (Table 1). This means that the ethanol solvent capable to extracting of pigment more than compared third to other solvents, and the possibility of ethanol has the highest fitness level compared with other solvents.

Meanwhile, for the red and brown seaweeds, the lowest of lightness degree of pigments produced by the solvent acetone was followed by ethanol with a rather high lightness degree (Table 1). The acetone solvent has a higher level of compatibility with pigments of red and brown seaweeds from the ethanol solvent of higher polarity in the extraction process especially with two other solvent polarities (petroleum benzene and hexan solvents) being on very low levels. As noted by [16], there is a strong tendency for non-polar compounds to dissolve into the non-polar solvent and the polar covalent compound or compounds to dissolve into ions in polar solvents.

Absorbency peak. The acetone solvent tends to produce pigments with the highest absorbency peak for the green and brown seaweed than solvents ethanol, petroleum benzene and hexan preparation. Meanwhile, for the red seaweed, both solvents, acetone and ethanol, are produced relatively at the same absorbency peak height (Table 2). The absorbency peak value of the observation, which was higher, indicated an increase in concentrations of compounds that was also indicated by

Table 1. Lightness Value of Pigment Filtrate and Concentrate of Seaweeds

Solvent	Green seaweed		Red seaweed		Brown seaweed	
	Filtrate	Concentrate	Filtrate	Concentrate	Filtrate	Concentrate
Aceton	49.0 b	34.3 b	48.1 a	34.4 a	46.6 a	28.3 a
Ethanol	43.9 a	30.4 a	51.3 b	33.0 a	51.0 b	28.4 a
Petroleum benzene	58.9 c	54.9 c	58.4 c	49.8 b	57.6 c	45.1 b
Hexan : Petroleum benzene	58.5 c	55.3 c	57.2 c	53.7 c	58.1 c	55.1 b

a-b Means with different letters within the same column differ significantly ($p<0.05$)

Table 2. Absorbency Peak of Pigment Seaweeds (at λ 410-472 nm)

Solvent	Seaweeds		
	Green	Red	Brown
Aceton	0.454 d	0.676 c	0.640 c
Ethanol	0.370 c	0.707 c	0.518 b
Petroleum benzene	0.181 b	0.208 b	0.286 a
Hexan: Petroleum benzene	0.027 a	0.057 a	0.013 *

Note: * invalid
a-d Means with different letters within the same column differ significantly ($p<0.05$)

the higher content of solute. This can occur presumably because of the polarity suitability or closeness of the pigment as the solute with solvent polarity. High pigment content, leading to a dark color or black material, absorbs more light. As a result, the absorbance peak is higher, in accordance with [1] β-carotene that exhibits in hexane a λ max of 452 nm. Solvents play a major role in the process of extracting the pigments. The spectrophotometric absorbance properties of pigments facilitate the qualitative and quantitative analysis of them using different solvents [17-18] and the contribution of these solvents to the extraction in various species were comparatively studied.

In according with the opinion of [19], stating that the carotenoid pigments is a fatty substance that can dissolve in fat, such as acetone, alcohol, diethyl ether and chloroform as well as non-polar solvents, for example, petroleum ether and hexan preparation. The effectiveness of extraction or solubility of a substance is largely determined by the match between the natural properties of the solute with similar solvents that dissolve like them because of such polarity [16], in addition to the extraction conditions. Different conditions indicated by [20] the methanol gave the best result of fucoxanthin extracted. However, because ethanol is the best solvent for processing food from the viewpoint of safety, ethanol as an alternative to methanol would be recommended, although it is slightly more expensive.

Carotenoid content. Carotenoids extracted from seaweeds of different colors in fact contain different types and content of pigments. The red seaweed type produces relatively more carotenoid than the two other kinds of green and brown seaweeds. The acetone solvent tends to produce the highest content of carotenoid for the green and brown seaweeds compared to the other three solvents (ethanol, petroleum benzene and hexan solvents). Meanwhile, for the red seaweed, the solvents acetone and ethanol produce the same high pigment content (Table 3).

Carotenoid rendement. The acetone solvent tends to produce the highest yield for the green and brown seaweeds compared to the other three solvents (ethanol, petroleum benzene and hexan solvents). Whereas for the red seaweed, the solvents acetone and ethanol both yield high results compared to petroleum benzene and hexan preparation (Table 4). The yield of carotenoids in addition was affected by the solvent that was treated. It was also influenced by the amount of carotenoid pigments extracted and contained in each ingredient. The acetone solvent that produces an amount of carotenoids from the green seaweed 0.98 mg/100g, and the brown seaweed 1.28 mg/100 g is the largest compared to the solvent ethanol, petroleum benzene and hexan preparation. Different conditions indicated by [21] observed ethyl acetate showed higher significance during the extraction process when compared to ethanol and acetone.

As for the red seaweed, the highest yield produced by the solvent acetone and ethanol was namely 6.65 and 6.90% (Table 4). This can happen because the highest absorbance peak is also produced by the two solvents. Both solvents have similar relatively high polarity, while the petroleum benzene and hexan are less polar solvents. The effectiveness of extraction of a substance is largely determined by the match between the natural properties of the solute with a similar solvent that dissolves like them because of their polarity [16].

Table 3. Carotenoid Content (mg/100 g) of Pigment of Three Colour Seaweeds

Solvent	Seaweeds		
	Green	Red	Brown
Aceton	0.980 d	1.352 c	1.280 c
Ethanol	0.740 c	1.412 c	1.036 b
Petroleum benzene	0.362 b	0.416 b	0.572 a
Hexan: Petroleum benzene	0.054 a	0.114 a	*

Note : * Not detect
a-b Means with different letters with in the same column differ significantly ($p<0.05$)

Table 4. Rendement (%) of Pigment of Three Colour Seaweeds

Solvent	Seaweeds		
	Green	Red	Brown
Aceton	4.850 d	6.650 c	6.24 c
Ethanol	3.630 c	6.900 c	5.05 b
Petroleum benzene	1.770 b	2.033 b	2.75 a
Hexan: Petroleum benzene	0.264 a	0.561 a	*

Note : * Not detect
a-d Means with different letters with in the same column differ significantly ($p<0.05$)

4. Conclusion

Research result indicated that each solvent reached a peak of maximal absorbance λ 410-472 nm in accordance with the character of carotenoids. Each pigment type is β-caroten, ε-carotena and khlorofil (green seaweed), xanthofil i.e lutein and fucoxanthin (brown seaweed). However, for red seaweed. xathofil of type zeaxanthin and fucoxanthin was only shown. The overall usage of acetone solvent gave a better pigment quality. Green, brown and red seaweed had pigment content of 0.98 mg/100 g, 1.28 mg/100 g, 1.35 mg/100 g and rendement of pigment each of 4.85%, 6.24% and 6.65% respectively. Futher research is needed to evaluate the nutritional value of seaweeds that can be regarded as an under-exploited source of health benefit molecules for food processing and neutraceutic industry.

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