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Dietary fiber and fatty acids in the Thallus of brown alga (*Sargassum duplicatum* J.G. Agardh)

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Abstract

Brown alga (Phaeophyta) is often used as food resources due to its dietary fiber content. *Sargassum duplicatum* is a brown algae which has high dietary fiber and fatty acids. However, the use of brown algae has not been developed for agar and carrageenan. Hence, the nutritional composition of brown algae needs more assessment to optimize its uses. This study was aimed to investigate the dietary fiber and fatty acids of *Sargassum duplicatum*. Explorative method was used for investigating insoluble dietary fiber, soluble dietary fiber, and total dietary fiber using enzymatic methods, while normal phase gas chromatography was used for fatty acid analysis. Results showed that insoluble dietary fiber and total dietary fiber were found mostly in the shaft whereas soluble dietary fiber was found mostly in the shaft and whole Thallus. There were 17 types of fatty acids in the Thallus of the brown algae *(S. duplicatum)*, i.e. nine types Saturated Fatty Acid (SAFA) made up 64.32%, three types of Monounsaturated Fatty Acids (MUFA) for 29.16%, and five types of Polyunsaturated Fatty Acid (PUFA) for 5.80%.

Introduction

Seaweeds have nutrition compositions, which are; water 27.8%, protein 5.4%, carbohydrate 33.3%, fat 8.6%, crude fiber 3%, and ashes 22.25% (Wirjatmadi *et al.*, 2002). Seaweeds also contain high vitamins and minerals which are useful for human health. The mineral content of seaweeds is 7-38% (dw). Seaweeds can be classified based on its pigment into red algae (Rhodophyta), brown algae (Phaeophyta), and green algae (Chlorophyta); based on its nutrition and chemical composition. Red algae and brown algae are often used as food resources for humans (Dawczynski *et al*., 2007). The structure of algae is strongly influenced by the season, age, species, and geographical location (Rioux *et al*., 2007). The typical carbohydrates in brown algae varieties consist of fucoidan, laminaran (b-1.3-glucan), cellulose, alginates, and mannitol. The amorphous, slimy fraction of brown algae fiber is mainly consists of water-soluble alginates and/or fucoidan and main reserved are laminaran (b-1.3-glucan) and mannitol (Dawczynski *et al*., 2007). Dietary fiber consists of soluble and insoluble fiber. Soluble dietary fiber has function in to prevent diseases such as colon cancer, cardiovascular disease, and obesity (Ortiz *et al*., 2006). Whereas insoluble dietary fiber has the ability to decrease intestinal transit time (Burtin, 2003).

Fatty acids are the main constituent of fat and raw material for all lipids in living organisms. Fatty acids are divided into saturated fatty acids (SAFA) and unsaturated fatty acids. Unsaturated fatty acids can be divided into two major groups, namely monounsaturated fatty acids (MUFA) and polysaturated fatty acids (PUFA) (Kulimkova and Khotimchenko, 2000).

Sargassum sp. belongs to Phaeophyceae containing high dietary fiber and fatty acids with 20 carbon atoms, such as eicosapentaenoic acid (EPA, ω3 C20: 5) and arachidonic acid (AA, ω6 C20: 4) (Burtin, 2003). Unsaturated essential fatty acids, i.e. omega-3 (EPA, ω3 C20: 5) may reduce the risk of heart disease, thrombosis and arterioclerosis (Ortiz *et al*., 2006). Type of brown algae which different from others based on its fatty acid composition are *Sargasssum kjellmanianum* with rich PUFA (n-6) and *Sargassum thunbergii* with rich PUFA (n-3) (Li *et al*., 2002).The body of macro algae still has not been able to be distinguished between the leaves, stems and roots. But the morphology and structure of the alga body has shown tremendous variation. Part of the thallus in *S. duplicatum* have different functions, thus it has different characteristics on nutritional composition. Nutritional composition such as fatty acids and fiber in the parts of thallus produced from photosynthesis process, which is required for the growth of *S. duplicatum*.

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Compared to red and green seaweed, brown seaweed contains higher antioxidant and several compounds that are not found in other sources. Some of the main elements contained in brown seaweed, among others are polysaccharides, phenolic compounds, terpenoids, sterols, proteins, peptides, unsaturated fatty acids, vitamins and pigments (Balboa *et al.*, 2012). Therefore, potential *S. duplicatum* is expected to be developed into functional food ingredients. Therefore, we have to know the details on nutritional composition of *S. duplicatum* related on morphological differences, specifically in function and location of thallus parts, so it can applied to the further processing to generates refined products in good quality for the kind of food preferably by the people The purpose of the research is to assess the composition of dietary fiber and fatty acids in the thallus of brown algae *(S. duplicatum)*.

Materials and Methods

Sample

The sample of *Sargassum duplicatum* brown seaweed was obtained from the Village of Padahe Talango, Regency of Sumenep, Madura. Sample was sorted and washed, Thallus was cut into shaft, blades and whole Thallus. The cut parts were dried, and then each part was crushed. Fiber analysis on the Thallus was conducted by enzymatic method to determine insoluble dietary fiber, soluble dietary fiber, and total dietary fiber. Fatty acid was analyzed by gas chromatography with method of normal phase.

Total dietary fiber

Measurements of total dietary fiber were analyzed by enzymatic methods (Ortiz *et al.*, 2006). Total of 1g sample mixed with 25 mL of 0.1 M Sodium Sulfate buffer, and then added 0.1 mL of termamyl enzyme, incubated at 100°C for 15 minutes. After incubation, it was distilled by water distiller for 20 mL and the pH declined with 4 mL of HCl. Total of 100 mg pepsin enzymes was added and incubated at temperatures of 40°C for 60 minutes, distilled in water distiller for 20 mL. The pH of the solution was adjusted to 6.8 by using NaOH. Pancreatin enzyme was added for 100 mg and incubated at 40°C for 60 minutes. Then, the pH of solution decreased into 4.5 with HCl. After that, the solution filtered by filter paper and washed twice with distilled water to obtain filtrate and residue of the solution. Residues were used for determination of Insoluble Dietary Fiber (IDF), while filtrate was used for determination of Soluble Dietary Fiber (SDF).

Insoluble dietary fiber

Insoluble dietary fiber was analyzed by the AOAC method (Li *et al*., 2002). The residue of Total Dietary Fiber was washed with 2x10 mL of 95% ethanol and 2x10 mL acetone. Weight of filter paper named with KS_1, was dried at temperature of 105°C for 24 hours to ensure constant weight. The filter paper + residual referred as KS_2 and weighed. Ashing was conducted at a temperature of 550°C for 5 hours with porcelain cup known weight CW_1. The porcelain cup + ash were weighed after cooling in a desiccator cabinet and the weight referred as CW_2. The amount of Insoluble Dietary Fiber calculated with the following formula.

Soluble dietary fiber

Soluble dietary fiber was analyzed by the AOAC method (Li *et al*., 2002). Filtrate was taken as many as 100 mL into the 400 mL ethanol 95% (60°C) for extract separation for an hour. Filter paper (referred as KS_3) washed with 2x10 mL methanol 78%, 2x10 mL ethanol 95%, and 2x10 mL acetone. Residues and filter paper was dried at temperature of 105°C for 24 hours to ensure constant weight. The weight of filter paper and residue referred as KS_4. Ashing residue used porcelain cup and ashing conducted at 550°C for 5 hours. Weight of porcelain cup referred as CW_3, whereas the weight of porcelain cup + ash referred as CW_4. Soluble Dietary Fibers (SDF) was obtained from the following formula.

Fatty acids (SAFA, MUFA, PUFA)

Measurements of fatty acids (SAFA, MUFA, and PUFA) were analyzed by chromatography methods (Kulimkova dan Khotimchenko, 2000). Sample was fine grinded as many as 10 g, hydrolyzed with 10 mL of HCl. It was heated with water bath at a temperature of 70°C, continued until boiling and then chilled. Extraction conducted with 15 mL diethyl ether and 15 mL petroleum ether, to take the top layer. The remaining solution was re-extracted with 15 mL of diethyl ether and 15 mL of petroleum ether, and the top layer was collected, mixed with previous top layer. The mix was then flushed with N_2.

Total of 0.5 mL sample was taken, added with 1.5 mL methanolic solution of sodium, covered, and heated at 70°C for 5-10 minutes and shaken, and then chilled. The solution was added with 2 mL methanolic boron tri fluoride, heated at of 70°C for 5-10 minutes, and then chilled. It is followed by extraction with 1 mL of heptane and 1 mL of saturated NaCl. The top layer was collected and placed in Eppendorf tubes, which is proceeded with gas chromatography SHIMADZU

GC-A17 with the specifications of used gas Helium, with injector and detector temperature 290°C, type of column RTX 5, and type of detector FID1. Separation by chromatography based on differences in the components of the mixture equilibrium between the mobile phase and stationary phase. If it's polar stationary phase, the mobile phase used is non-polar, and vice versa.

Statistical analysis

Statistical analysis based on Completely Block Randomized Factorial Design by ANOVA. Further analysis was continued with Least Significant Difference Test (LSD) method.

Result and Discussion

Composition of fiber

Dietary fiber is the largest component of the polysaccharide (Venugopal, 2009). Based on the solubility, dietary fiber is classified into SDF and IDF. Dietary fiber is fiber that soluble in water, while insoluble dietary fiber is fiber that does not soluble in water (Gallaher and Schneeman, 1996). Dietary fiber is composed of water soluble pectin and gums, while insoluble fiber is composed of cellulose, hemicellulose, and lignin (Nassar *et al*., 2008).

Total dietary fiber

Total dietary fiber in the Thallus of brown algae *S. duplicatum* are 33.67% on the shaft, 33.52% on the blades, and 33.48% on the whole Thallus (Figure 1). Composition of total dietary fiber in *S. duplicatum* is lower than *S. chinerium* of 39.67%, but higher than *Caulerpa lentillifera* (Chlorophyta) of 32.99% and *Eucheuma cottonii* (Rhodophyta) of 25.05% (Matanjun *et al*., 2009).

Total dietary fiber content which ranged from 29.3 to 37.4% of 39.1–74.7% was soluble. For brown algae, the soluble fibre contained uronic acids from alginates and neutral sugars from sulphated fucoidan and laminarin. For red seaweeds, the main neutral sugars corresponded to sulphated galactans (carrageenan or agar). Insoluble fibres (7.4–22.7%) were essentially made of cellulose with an important contribution of Klason lignin especially in brown seaweeds (9.5–10.8%) (Ordonez *et al.*, 2010).

The average daily requirement of dietary fiber is 25 g.day^{-1} for women younger than 50, 21 g.day^{-1} for women older than 50, 38 g.day^{-1} for men younger than 50, and 30 g.day^{-1} for men older than 50 (Elleuch *et al.*, 2011). Consumed fiber such as cellulose and hemicelluloses affect the condition of obesity and diabetes. Although fiber cannot be digested in the intestinal digestion, it helps bind digestive enzymes, cholesterol, glucose, and subsequent toxin excreted through feces. By reducing the absorption of foods containing fats, soluble fiber can help people who are obese by reducing the digestion of starch and glucose uptake in the diet can help in controlling the sugar levels in blood for diabetic patient (Venugopal, 2009).

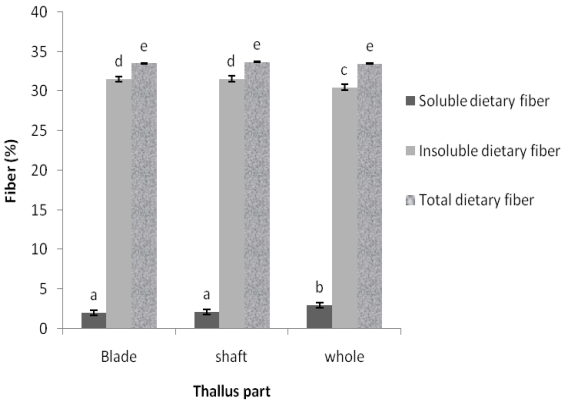

Figure 1. Composition of dietary fiber in *S. duplicatum*

Insoluble dietary fiber

The insoluble dietary fibers from Thallus of *S. duplicatum* are 31.57% on the shaft, 31.54% on the blade, and 30.49% for whole thallus (Figure 1). The composition of insoluble dietary fiber in *S. duplicatum* is lower than *S. chinerium* of 34.10%, but higher than *Caulerpa lentillifera* (Chlorophyta) of 15.78% and *Eucheuma cottonii* (Rhodophyta) of 6.8% (Matanjun *et al.*, 2009).

The importance of other dietary approaches, such as increasing the intake of water-soluble dietary fibers is increasingly recognized. Controlled intervention studies have now shown four major water-soluble fiber types—β-glucan, psyllium, pectin and guar gum (Theuwissen and Mensink, 2008). Cellulose is the core elements which form the skeleton of plants. Gummy cellulose helps to maintain the intestinal peristalsis which serves to excrete the normal feces. It is shown that dietary fiber not only could evade the hydrolysis, digestion and absorption in the human small intestine, but also at least one of these functions: increases the faecal bulk, stimulates colonic fermentation, reduces the postprandial blood of glucose (reduces insulin responses) and reduces the pre-prandial levels of cholesterol (Elleuch *et al*., 2011).

Insoluble dietary fiber is a bulking agent that contributes to the volume of feces and intestinal transit time, thus prevent colon cancer and diverticulosis. With high fiber consumed, the water is easily absorbed, and then the fiber becomes softer,

Table 1. Identified Fatty Acids in *S. duplicatum*

Type of Fatty Acids	Identified Fatty Acids	Carbon chain – Numbers
SAFA	Capric acid	(C10: 0)
	Lauric acid	(C12: 0)
	Myristic acid	(C14: 0)
	Palmitic acid	(C16: 0)
	Margariat acid	(C17: 0)
	Stearic acid	(C18 : 0)
	Eicosanoic acid	(C20: 0)
	Behenic acid	(C22: 0)
	Tetracosanoic acid	(C24: 0)
MUFA	Palmitoleic acid	(C16: 1)
	Oleic acid	(C18: 1, n-9)
	Eicosenoic acid	(C20: 1, n-9)
PUFA	Linoleic acid	(C18: 2)
	Linolenic acid	(C18: 3)
	Eicosadienoic acid	(C20: 2)
	Arachidic acid	(C20: 4)
	Eicosapentaenoic acid/EPA	(C20: 5)

thereby reducing pain in patients with diverticulosis.

Soluble dietary fiber

Soluble dietary fiber in the Thallus of *S. duplicatum* is 2.99% in overall Thallus, 2.1% on the shaft, and 1.98% on the blade (Figure 1). The content of soluble fiber of *S. duplicatum* is lower than *Sargassum chinerium* of 5.57%, *Caulerpa lentillifera* (Chlorophyta) of 17.21% and *Eucheuma cottonii* (Rhodophyta) of 18.25% (Matanjun *et al*., 2009). However, soluble dietary fiber on *S. duplicatum* is higher than the content of soluble dietary fiber in cinnamon leaf by 1.71% (Johnson and Southgate, 1994).

Composition of soluble dietary fiber in *S. duplicatum* as whole is greater than the Thallus on blades. Based on Ruperez *et al*. (2001), the content of soluble fiber in brown algae is very small, i.e. only 9.8% of the whole plant, and the rest consists of insoluble fiber. It is causing the leaves having a lower soluble fiber.

Dietary fiber may also use several functional traits for food, e.g. increases the water capacity, oil capacity, emulsification and/or gel formation. Indeed, we shall illustrate that dietary fiber which was inserted into food products (bakery products, milk, jams, meats, soups) could modified the textural traits, avoid synaeresis (the separation of liquid from a gel caused by contraction), stabilize the high fat of food and emulsions, and improve the shelf-life (Elleuch *et al*., 2011).

Structurally cellulose, hemicellulose and pectin are polymeric sugar straight or branched chain with a varying number of molecules. Hemicellulose is a dietary fiber consisting of xylose, galactose, glucose and other monosaccharides compounds. The function of the hemicellulose is to reduce the transit time of food in the intestine (Wardlaw *et al*., 2004).

Composition of fatty acids

Based on qualitative analysis, the diversity of fatty acids identified into 17 types, which is consists of 9 types of saturated fatty acids (SAFA), 3 types of monounsaturated fatty acids (MUFA) and 5 types of polyunsaturated fatty acids (PUFA). The types of fatty acids were shown in Table 1. In parts of the brown algae Sargassum duplicatum thallus identified dominant palmitic acid namely (C16: 0), which is saturated fatty acid, whereas oleic acid (C18: 1) which is monounsaturated fatty acid and eicosapentaenoic acid / EPA (C20: 5) which is the plural of unsaturated fatty acids.

The lipid composition and metabolism of plants is influenced by several environmental factors as follows: light, temperature, atmospheric pollutants, salt availability in the soil and xenobiotics such as a pesticide. The effect of light can be seen through the stimulation of photosynthetic membrane function. Light is required for chloroplast development because it can stimulate photosynthesis (related to ATP and NADPH production) and the synthesis of fatty acid. The measurement of fatty acid synthesis indicated that the formation of lipid is about 20 times faster on bladess in the light than in the dark (Dey and Harborne. 1997).

Saturated fatty acids (SAFA)

SAFA in the *S. duplicatum* of thallus which identified is palmitic acid, myristic acid and stearic acid. The highest content of each SAFA in the thallus parts of *S. duplicatum* are 51.19% palmitic acid on blades, 15.82% myristic acid on blades; and 5.11% stearic acid on shaft (Table 2).

In the analysis of variety of seaweed, palmitic acid is found deeper than saturated fatty acids. Palmitic acid contents of *S. duplicatum* is higher than

Table 2. Composition of Dominant SAFA in *S. duplicatum*

Dominant Fatty Acids	Percentage in Thallus Part (%)		
	blade	shaft	whole
Palmitic acid	**51.19**	42.06	47.15
Myristic acid	**15.82**	10.53	12.67
Stearic acid	1.48	**5.11**	2.27

Porphyra sp. from China that is 37% and Undaria pinnatifida that is 14% (Ortiz *et al*., 2005). The content of myristic acid in *S. duplicatum* is also upper than *U. pinnatifida* (2.25%), *Hizikia fusiforme* (0.30%), and *Laminaria* sp. (2.88%). The content of stearic acid in *S. duplicatum* is also bigger than *U. pinnatifida* (0.86%), *H. fusiforme* (0.76%), and *Laminaria* sp. (1.49%) (Dawczynski *et al*., 2006).

Palmitic acid has the highest value in the composition of fatty acids in *S. duplicatum*. Palmitic acid is a saturated fatty acid that composed the most synthesized lipids in plants rather than stearic acid (Dey and Harborne, 1997). According to Burtin (2003), The red and brown algae are particularly rich in fatty acids with 20 carbon atoms: eicosapentanoïc acid (EPA, ω3 C20 :5) and arachidonic acid (AA , ω6 C20 :4). Spirulin provides an interesting source of gamma linolenic acid (GLA) (20 to 25% of the total lipidic fraction), which is a precursor of prostaglandins, leucotriens and thromboxans involved in the modulation of immunological, inflammatory and cardio-vascular responses.

Monounsaturated fatty acids (MUFA)

The dominant MUFA on the thallus parts of *S. duplicatum* are oleic acid and palmitoleic acid. The highest content of MUFA in the thallus of *S. duplicatum* found that 7.31% palmitoleic acid on shaft and 18.81% oleic acid in the entire of thallus. MUFA found in overall thallus part because *S. duplicatum* obtain fatty acids which is derived from the mechanism of photosynthesis.

Oleic acid content in *S. duplicatum* is higher than *Porphyra* sp. from Japan and Korea, *U. pinnatifida,* and *H. fusiform* as much as 2.6% to 9.3% (Ortiz *et al*., 2005). The content of Palmitoleic acid in *S. duplicatum* is also bigger than *U. pinnatifida* (0.44%), *H. fusiform* (0.15%), and *Laminaria* sp. (1.71%) (Dawczynski *et al.*, 2006).

Polyunsaturated fatty acid (PUFA)

The dominant PUFA found in *S. duplicatum* is eicosapentaenoic acid / EPA, with the highest content in the blades that is 1.54%, followed by 1.42% in overall thallus and 1.26% on shaft. *Sarggasum* sp. is seaweed that lives in marine waters, thus in general, the plants live in conditions that relatively

Table 3. Composition of Dominant MUFA in *S. duplicatum*

Dominant Fatty Acids	Percentage in Thallus Part (%)		
	blade	shaft	overall
Oleic acid	15.99	18.04	**18.81**
Palmitoleic acid	3.74	**7.31**	5.06

has lower temperatures. *Sargassum* is a plant poikilothermic (organisms that cannot regulate their own temperature). On this effect means that the lipid will contain large amounts of unsaturated fatty acids due to the presence of the double bond, which has a dramatic effect on the transition temperature (T_c) as acids (e.g. stearic acid T_c= 70°C, oleic acid T_c=16°C). Oleic acid is a monounsaturated fatty acid which is most commonly found, while the fatty acids that commonly found in PUFA are linoleic acid and α-linolenic acid, especially as a component of the lipid membrane plant, (Dey and Harborne. 1997).

The content of PUFA in S. duplicatum which live in waters with depths of 0.5 -10 m is affected by currents and waves. The body is strong but flexible, which can be used to against a large wave. This activity requires considerable energy from the fat reserves. Each branch contained of rounded air bubbles namely "bladder", it is useful to support the thalli branch for floating toward the surface to get the sunlight for photosynthesis. The green color in the whole section of thallus and on leaves (the blade on algae) has a role in the process of photosynthesis; because the blades have more plastids. Plastids are chief organelles found only in plants and algae. The function of plastid is for photosynthesis, and also for the synthesis of fatty acids and trepans which is necessary for the growth of plant cells.

Conclusion

Insoluble dietary fiber and total dietary fiber were found most abundant on shaft, whereas soluble dietary fiber was found most abundant on shaft and whole part of thallus. We found 17 types of fatty acids in the thallus of the brown algae *(S. duplicatum)* i.e., nine types saturated fatty acid (64.32%), three types of monounsaturated fatty acids (29.16%), and five types of polyunsaturated fatty acid (5.80%).

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