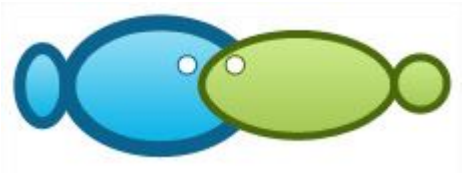

Fatty acid composition of individual polar lipids extracted from the brown seaweed *Padina australis*

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Abstract. A study on fatty acid compositions of individual polar lipids extracted from the brown seaweed *Padina australis* collected from Saugi Island of Pangkep District Indonesia has been conducted. Total lipids were extracted from the seaweed using chemical solvents of $CHCl_3$/MeOH (2:1).). The glycerolipid and phospholipid compounds were isolated from the total lipids using Thin-Layer Chromatography (TLC) with a mobile phase of $CHCl_3$/MeOH/H_2O/EtOAc/IPA (5:2:1:5:5). TLC conducted further purification of SQDG with a mobile phase of $CHCl_3$/Acetone/MeOH/H_2O/HOAc (10:6:2:1:2). The purified glycerolipids and phospholipids were then converted to methyl esters using 10% HCl in MeOH. The esterified glycerolipids and phospholipids were purified by silica column with eluted by solvents of hexane/diethyl ether (85:15 by vol). Analysis of the fatty acid methyl esters was carried out using a Shimadzu GC-14A gas chromatograph (Shimadzu) equipped with an Omegawax 320 column (30 m x 0.32 mm i.d., Supelco, PA, USA). The results showed that the dominant fatty acids found from the total lipid were the saturated fatty acids, palmitic acid (29.18 wt%), and arachidonic acid (23.10 wt%), whereas the dominant fatty acid found from free fatty acid fraction are palmitic acid (39.90 wt%) and oleic acid (21.3 wt%). The fatty acid compositions of individual polar lipids showed relatively similar except for MGDG and PC, which accounted for high amounts of oleic acid (21.62 wt%) and arachidonic acid (23.10 wt%), respectively. The seaweed could be a natural source of essential fatty acid, especially MGDG and PC extracts for food supplement for human health.

Key Words: glycerolipids, phospholipids, MGDG, GC.

Introduction. The fatty acid composition of brown seaweed is commonly rich in polyunsaturated fatty acids, such as arachidonic acid (AA) and eicosapentaenoic acid (EPA), which link to glycoglycerolipids, the major components of membrane lipids and phospholipids. Those fatty acids are commonly located at *sn*-1 and *sn*-2 positions of the glycoglycerolipids and phospholipids of the seaweed. The position of fatty acids in neutral lipids affects the fatty acid digestion and absorption in the metabolism of the human body (Innis 2011). *sn*-2 fatty acids are more digestible than the others, *sn*-1 and *sn*-3 positions. For example, human milk fat (HMF) is rich in palmitic acid (70%) in the *sn*-2 position of triacylglycerol structure. This unique structure gives advantages to infants, such as an increase in digestion and absorption of fatty acids (Takeuchi 2010).

The brown seaweed *Padina australis* is abundantly found attached to the sand bottom substrate of coastal areas of Indonesia. Unlike the red seaweed *Kappaphycus alvarezii*, the brown seaweed *P. australis* and other species have not been developed their cultivation, although the seaweed cultivation has successfully been tried in Seram Island, Indonesia. Several potentials of the seaweed, especially antioxidant activity, antibacterial and antiviral from polysaccharide compounds have been reported (Karmakar et al 2010; Jaswir et al 2014; Mohsin et al 2014). However, analysis of the detailed fatty acid composition of individual neutral lipids from the seaweed has not been studied well. So, in the present study, the fatty acid composition of individual glycoglycerolipids, such as monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and

sulvoquinoosyldiacylglycerol (SQDG), and phospholipids, such as phosphatidylcholine (PC) and phosphatidylglycerol (PG) were analyzed in detail and the results are discussed.

Material and Method. The brown seaweed *P. australis* was collected from coastal areas of Saugi Island Indonesia in June 2017. The seaweed was brought to the Laboratory of Bio-analytical Chemistry, Faculty of Fisheries Sciences, Hokkaido University Japan for lipid analysis.

Chemical materials. Standard samples of MGDG, DGDG, and SQDG from plant leaves were obtained from Lipid Products (Redhill, UK). Silica Gel 60 (70-230 mesh) for column chromatography, Silica Gel 60 F254 aluminium sheets for analytical TLC, and Silica Gel 60 F254 plates for preparative TLC (20 x 20 cm, 0.25-mm thick) were obtained from Merck (Darmstadt, Germany). 1–oleoyl–2-palmitoyl-*sn*-glycero–3–phosphocholine was obtained from Sigma (St. Louis, MO, USA). HPLC-grade solvents, CH_3CN and *iso*-PrOH, were obtained from Kanto Chemicals (Tokyo, Japan). All other chemicals and solvents were of reagent grade or better quality and were obtained from Wako Pure Chemicals (Osaka, Japan).

Total lipid extraction. The seaweed sample was freeze-dried using liquid nitrogen and crushed by using mortar and pestle. The powder was then homogenized with a solvent of $CHCl_3$/MeOH/H_2O (2:1:0.8 by vol.) for 1 h at room temperature. A lipid extract was separated by putting the mixture into a conical flask overnight. After filtration, the solvent was removed at 25ºC under reduced pressure using a rotary evaporator, and then the residual lipids were made up to a known concentration with $CHCl_3$/MeOH (2:1, v/v) and stored at –30ºC until use.

Isolation of glycoglycerolipids and phospholipids. The glycoglycerolipids (MGDG, DGDG, and SQDG) and phospholipids (PG and PC) were isolated from the total lipids of the *P. australis* samples using a TLC plate (20 cm x 20 cm) according to the procedures described previously (Takahashi et al 2001). Briefly, the algal lipids (250 mg) were firstly subjected to silica gel column chromatography. A fraction containing MGDG, which eluted with 300 mL of chloroform/methanol (9:1, v/v), was further purified by TLC on silica gel with a solvent system of hexane/EtOAc/MeOH/H_2O (65:35:8:1, by vol) and with a solvent system $CHCl_3$/MeOH/H2O/EtOAc/*i*-PrOH (5:2:1:5:5, by vol). The Rf values of the MGDG, DGDG, and SQDG on TLC using the latter solvent system were 0.35, 0.27, and 0.16, respectively, which were in good agreement with those of authentic standards. After spraying 2',7'-dichlorofluorescein reagent, bands were visualized under a UV lamp. The MGDG was extracted from the adsorbent with chloroform/methanol (2:1, v/v), while DGDG and SQDG were extracted with chloroform/methanol/water (6:3:1, by vol). On analytical TLC, purified glycoglycerolipids showed characteristic dark red-colored spots when sprayed with the orcinol-sulfonic acid reagent followed by heating (Svennerholm 1956). Structures of these glycoglycerolipids, if necessary, were confirmed by 1H and 13C nuclear magnetic resonance (NMR) spectrometry, as described elsewhere (Takahashi et al 2002).

Fatty acid analysis. The fatty acid analysis was performed by converting lipids to methyl esters by heating at 95ºC for 1 h in 5% HCl/MeOH (Christie 1982). Analysis of the fatty acid methyl esters was carried out using a Shimadzu GC-14A gas chromatograph (Shimadzu) equipped with an Omegawax 320 column (30 m x 0.32 mm i.d., Supelco, PA, USA). Helium was used as the carrier gas at a constant flow rate of 1 mL min^{-1}. The split ratio was 1:50. The column temperature was maintained at 160ºC for 17 min, then elevated to 230ºC at a ramp rate of 5ºC/min. The final temperature was kept for 30 min. The injector and flame-ionization detector (FID) temperatures were set at 240ºC. The fatty acid peaks were monitored and quantified on a Chromatopac C-R6A (Shimadzu) and identified by comparing retention data of the known fatty acids from some marine organisms, including seaweeds (Takagi et al 1985, 1986). Atherogenic (AI) and thrombogenic indexes (TI) were calculated (Chan & Matanjun 2017). The fatty acid composition was quantified by

Results and Discussion. Although seaweed is not a source of energy because they have low lipid content, their polyunsaturated fatty acid content can be as high as land vegetables (Darcy-Vrillon 1993). In this work, the fatty acid composition of the brown seaweed, which abundantly grows in Indonesian waters and waters around Indonesia, was studied. The fatty acid composition of the total lipid was dominated by C16:0 (palmitic acid) and C20:4n-6 (arachidonic acid) (Table 1).

Table 1

Fatty acid composition of total lipid and free fatty acid (FFA) of the brown seaweed *Padina australis*

Fatty acids	*Total lipid*	*FFA*
C14:0	6.15±1.46	2.58±0.45
C14:1n-9	0.74±0.20	0.38±0.01
C16:0	29.18±8.56	39.90±5.34
C16:1n-9	2.55±0.67	1.42±0.34
C16:1n-7	4.92±1.02	1.01±0.24
C18:0	0.90±0.01	17.75±3.56
C18:1n-9	11.87±3.67	21.31±4.76
C18-1n-7	0.44±0.01	1.08±0.37
C18:2n-6	5.83±1.24	1.56±0.46
C18:3n-3	0.76±0.04	0.84±0.04
C20:0	0.16±0.01	4.63±0.87
C20:1n-9	-	-
C20:2n-6	0.71±0.02	0.10±0.01
C20:3n-6	7.58±1.46	-
C20:4n-6	23.10±5.65	1.32±0.24
C20:3n-3	0.07±0.01	-
C20:5n-3	0.58±0.02	0.16±0.01
C22:0	0.11±0.01	0.27±0.01
C22:1n-9	-	-
C23:0	0.06±0.01	0.06±0.01
C24:0	0.10±0.01	0.50±0.02
C22:6n-3	0.17±0.01	0.08±0.01
Unknown	0.32±0.01	0.29±0.01
Others	3.20±0.68	3.89±0.86
SFA	36.60	65.62
PUFA	51.67	29.27
PUFA/SFA	0.85	0.06
n-6	29.63	2.98
n-3	1.51	1.08
AI	0.60	0.78
TI	1.87	11.83

The two fatty acids are commonly found abundant in the brown seaweed and red seaweed (Illijas et al 2009, 2012; Polat & Ozogul 2013; Susanto et al 2016). Another fatty acid with a relatively high content was C18:1n-9 (oleic acid; 11.87%). This fatty acid is the only unsaturated fatty acid commonly found in brown seaweed (Shameel 1990). Meanwhile, the composition of fatty acid of free fatty acids (FFA) showed a different composition. Palmitic acid was still the most abundant one, but the second abundant fatty acid was C18:1n-9 (21.31%) and C18:0 was also in relatively high amounts (17.75%) and the eicosanoid precursor, arachidonic acid (C20: 4n-6) was very low in content (1.32%). FFA is released from the hydrolysis of the lipid membrane, both glycoglycerolipids and phospholipids by action of glycerolipid acyl-hydrolase in the red seaweed *Gracilaria vermiculophylla* (Illijas et al 2008) and by phospholipase A2 in the diatom, *Thalassiosira rotula* (Pohnert 2002). The fatty acid composition of FFA is relatively different from those of total lipids. It means that free fatty acid was only mostly released from one of the two positions of *sn*-1 and *sn*-2 of the lipid classes, glycoglycerolipids and phospholipids. The substrate of eicosanoic metabolite synthesis, free arachidonic acid (AA) was only detected very low content in FFA fraction, even the

AA is one of the dominant one in the total lipid fraction. It is different from the free fatty acid composition of the rarely collected seaweed from Indonesia *Exophyllum wentii*, where the fatty acid composition of the FFA and total lipid is relatively similar (Illijas et al 2009).

Fatty acid composition of individual glycolipids and phospholipids. Fatty acid composition of individual polar lipid glycoglycerolipids and phospholipids (Table 2) showed relatively similar except for PC, which contained higher amounts of the unsaturated fatty acid, AA (23,10 %wt) and MGDG, which contained higher oleic acid (21.62 wt%). PC and MGDG are typical to contain unsaturated fatty acids at both *sn*-1 or *sn*-2 positions (Honda et al 2016, 2019). Figure 1 shows GC chromatograms of FAME of MGDG, which exhibited an unidentified chromatogram in relatively high amounts (10.38 wt%). This chromatogram was also detected in DGDG (4.26 wt%), but it was not found in other polar lipids, SQDG, PC, and PG. It suggested that the unidentified fatty acid is cyclopentyl fatty acid that was similarly found from the fatty acid composition of the red seaweed belonging to Solieriaceae (Miralles et al 1990). SQDG contained the highest palmitic acid (66.46 wt%) and was followed by PG (49.03 wt%) and DGDG (42.29 wt%). This is in line with SQDG extracted from the seaweed *Agarophyton vermiculophyllum* and *A. chilensis*, which contain more saturated fatty acid, palmitic acid both at *sn*-1 or *sn*-2 positions (Honda et al 2019).

Table 2

Fatty acid composition of individual polar lipids extracted from the brown seaweed *Padina australis*

Fatty acids	*Glycoglycerolipids*			*Phospholipids*	
	MGDG	*DGDG*	*SQDG*	*PC*	*PG*
C14:0	4.61±0.52	5.77±0.6	9.65±0.9	6.15±0.7	8.19±0.8
C14:1n-9	0.45±0.13	0.76±0.3	0.60±0.1	0.74±0.1	0.44±0.1
C16:0	32.47±6.32	42.29±8.2	66.46±9.6	29.18±6.7	49.03±8.5
C16:1n-9	2.59±0.31	4.65±0.2	1.50±0.4	2.55±0.5	4.06±0.3
C16:1n-7	2.24±0.23	4.68±0.2	5.58±0.3	4.92±0.4	5.77±0.3
C18:0	5.77±0.92	2.13±0.47	1.60±0.14	0.90±0.04	1.47±0.47
C18:1n-9	21.62±2.54	9.94±1.27	9.62±1.67	11.87±1.87	10.13±2.08
C18-1n-7	0.62±0.12	0.72±0.09	-	0.44±0.03	2.07±0.18
C18:2n-6	3.21±0.17	5.26±1.06	1.61±0.36	5.83±0.67	2.69±0.28
C18:3n-3	5.27±0.32	5.14±1.07	0.87±0.06	0.76±0.02	2.93±0.06
C20:0	0.92±0.18	0.22±0.00	-	0.16±0.00	0.19±0.00
C20:1n-9	-	-	-	-	-
C20:2n-6	0.06±0.00	0.19±0.00	0.36±0.00	0.71±0.00	0.2±0.00
C20:3n-6	-	-	-	7.58±2.02	0.31±0.00
C20:4n-6	2.74±0.06	4.30±0.13	-	23.10±2.06	4.62±1,02
C20:3n-3	-	-	-	-	-
C20:5n-3	0.74±0.03	1.14±0.06	-	0.58±0.03	0.15±0.00
C22:0	0.08±0.00	0.43±0.00	-	0.11±0.00	0.19±0.00
C22:1n-9	0.21±0.00	-	-	-	-
C23:0	-	-	-	-	-
C24:0	0.07±0.00	0.32±0.00	0.61±0.00	0.10±0.00	0.35±0.00
C22:6n-3	0.18±0.00	0.21±0.00	-	0.17±0.00	0.19±0.00
Unknown	10.38±2.03	4.26±0.78	-	-	0.44±0.02
Others	4.83±1.34	6.34±1.24	1.55±0.46	3.41±0.67	4.56±0.58
SFA	43.92	51.17	78.32	36.60	59.42
PUFA	12.19	16.25	2,83	39.18	11,09
PUFA/SFA	0.28	0.32	0.04	1.07	0.19
n-6	6.01	9.76	1.97	37.22	7.62
n-3	6.19	6.48	0.87	1.51	3.27
AI	2.03	1.30	3.90	0.60	1.73
TI	1.90	2.01	19.25	1.56	4.18

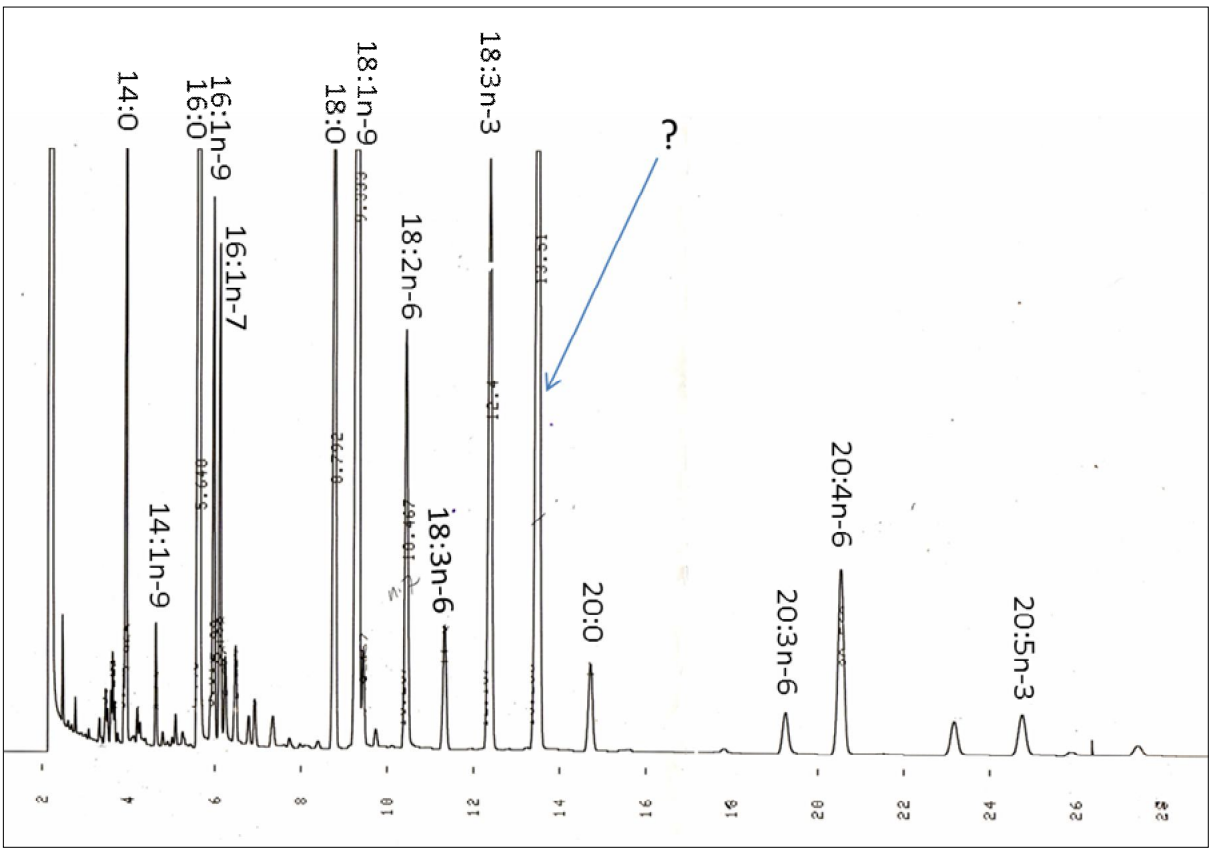

Figure 1. GC chromatogram of MGDG extracted from *Padina australis*.

The fatty acid compositions of the dietary fats, particularly of some individual fatty acid, are essential in human nutrition and health concern. Low intake of saturated fat and increased PUFAs to SFAs ratio are associated with a lower risk of human coronary heart diseases (Kumar et al 2011). Thus, the PUFAs /SFAs ratio is one of the parameters used to assess the nutritional quality of the lipid fraction of foods. In the present study, the PUFAs /SAFs ratio was 0.85 in the total lipid fatty acids, which is within the nutritional guidelines that recommended a PUFAs/SFAs ratio above 0.4 (Kumar et al 2011). The highest ratio of PUFA/SFA was detected in PC (1.07). This is due to high amounts of AA found from the PC. In addition to PUFAs/SFAs ratio, the AI and TI are also relating to nutritional factors linked with coronary diseases, which are also used to assess the fatty acid nutritional quality. The AI indicates the relationship between the sum of the main saturated fatty acids and that of the main class of unsaturated fatty acids, while TI showing the relationship between the pro-thrombogenic and the anti-thrombogenic of fatty acids as thrombosis is a central event in atherosclerosis (Ghaeni et al 2013). Therefore, lower AI and TI maintain better nutritional quality of the fatty acids. The AI and TI of *P. australis* TL, in the current study was 0.60 and 1.87, respectively. Whereas in the polar lipids, the lowest values of AI and TI was obtained from PC (0.60 and 1.56). These values were relatively lower than several Rhodophyta species (AI: 0.45-2.87, TI: 0.52-5.75) previously reported (Kumar et al 2011) but they were significantly higher than the red seaweed *G. changii* (AI: 0.03 and TI: 0.04) has been reported (Chan & Matanjun 2017). However, this TI value was still lower than milk-based products (2.1). In view of this, the addition of seaweeds to milk products, may not only be useful for the technological reason (gel-forming) but also could be of a more satisfactory strategy for the development of healthier lipid formulation.

The fatty acid compositions of MGDG and PC extracted from brown seaweed *P. australis* could be a natural source of essential fatty acids that could be added to food ingredients or could be used as a nutraceutical agent for human health, especially for PC, which rich in palmitic acid and arachidonic acid that required for infant growth (Carlson et

al 1993). Although one of the fatty acid peaks in MGDG was unable to identify, it is under identifying it using GC/MS.

Applications of seaweed in aquaculture have been reported in some studies. The beneficial effect of the use of seaweed meal in the diet of fish has been reported (Mustafa 1995; Mustafa et al 1995). The addition of seaweed increases the growth rate of fish. Another advantage of using seaweed in the fish diet is an improvement in the stress response and in disease resistance (Satoh et al 1987; Gabrielsen & Austreng 1998). Seaweed lipid, which is rich in polyunsaturated fatty acids (PUFAs) has been reported to improve performance of abalone juveniles. The best growth of juveniles of abalone is observed in feeding on the brown seaweed, *Undaria pinnatifida*, which is rich in PUFAs, such as C18:3n-3, C18:4n-3, C20:4n-6 and C20:5n-3 (Floreto et al 1996). Those fatty acids are relatively similar to fatty acid composition of the brown seaweed, *Padina australis.* The PUFAs of C18:3n-3, C20:4n-6 and C20:5n-3 was also found in *Padina australis.*

Conclusions. The polyunsaturated fatty acid, arachidonic acid was accumulated in PC, and the monounsaturated fatty acid, oleic acid was in MGDG. Whereas the saturated fatty acid, palmitic acid was dominant fatty acid in all polar lipids. Based on the lipid quality, the phospholipid, PC isolated from the brown seaweed *P. australis* could be the source of dietary lipid for infant growth.

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