FATTY ACID COMPOSITION AND PROSTAGLANDIN CONTENT OF THE RED SEAWEED *Gracilaria* sp. FROM INDONESIA

Muhammad Ikbal Illijas[*)#], Arifuddin[*)], Luqman Saleh[*)], and Yutaka Itabashi[**)]

[*)] Department of Aquaculture, Pangkep State Polytechnic of Agriculture, Indonesia
[**)] Graduate School of Fisheries Sciences, Hokkaido University, Japan

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ABSTRACT

High content of polyunsaturated fatty acids (PUFAs) such as arachidonic and eicosapentaenoic acids are typical for the red alga. Analysis of fatty acid composition and prostaglandin content was conducted in the red alga *Gracilaria* sp. from Indonesia. Total lipid of the alga was extracted with $CHCl_3$-MeOH (2:1, v/v). Analysis of the fatty acids composition was performed on gas chromatography (GC) equipped with omega wax column (30 m x 0,32 mm i.d., Supelco, PA, USA) and analysis of prostaglandins were carried out by HPLC on ODS column (Mightysil RP–18 GP, 250 mm x 4.6 mm, 5 µm). The content of fatty acids high for were palmitic acid (50%) and arachidonic acid (26.9%), whereas prostaglandin E_2 was identified and found lower concentration (44.2 µg/gram total lipid).

KEYWORDS: fatty acids, prostaglandins, *Gracilaria*, seaweed

INTRODUCTION

Red algae are well recognized as sources of polyunsaturated fatty acids (PUFAs) with 20 carbon atoms, mainly arachidonic acid (20:4n-6) and eicosapentaenoic acid (20:5n-3). These PUFAs have unique biological activities and are the precursors in biosynthesis of prostaglandins, leucotrienes and other eicosaenoids, which are important to the maintenance of normal mammalian physiology (Gerwick *et al*., 1993; Gerwick & Bernet, 1993). The majority of the red algal species are rich in 20:5n-3 or contain about equal amounts of 20:5n-3 and 20:4n-6. However, *G. verrucosa* (*G. vermiculophyla*) contains higher of 20:4n-6 than 20:5n-3 (Pohl *et al*., 1968; Takagi *et al*., 1985; Araki *et al*., 1986; Khotimchenko *et al*., 1991; Dawes *et al*., 1993), Khotimchenko & Levchenko, 1997).

The red alga *G. verrucosa*, which is intensively cultured in coastal areas of shrimp and fish shrimp ponds in Indonesia, has luck of information in detail regarding its lipid bioactive contents. Although there were some species of *Gracilaria* have been published in detail about their lipid bioactive contents those collected from Japanese Waters, such as *G. vermiculophylla* (Itabashi *et al*., 2006), *G. gigas* (Hsu *et al*., 2007), and *G. asiatica* (Sajiki, 1996). Another species is *G. chilensis* (Lion *et al*., 2006) collected from Chile, which contained different lipids bioactive from other *Gracilaria* species.

The aim of study was to evaluate fatty acids and eicosanoid composition of Indonesian red alga *G. verrucosa*.

Corresponding author. Department of Aquaculture, Pangkep State Polytechnic of Agriculture Jl. Poros Makassar, Pane Km 82, Pangkajene, Sulawesi Selatan, Indonesia. Tel.: 0813 4395 6621
E-mail address: muhammadikbali@yahoo.com

MATERIALS AND METHODS

Seaweed

The red seaweed *Gracilaria* sp. was collected from shrimp ponds located in Bone District, South Sulawesi. A part of the alga was extracted at Department of Aquaculture, Pangkep State Polytechnic of Agriculture. Another part of the alga was freezed for further extraction at Bioanalytical Chemistry Laboratory, Hokkaido University, Japan

Lipid Extraction

Each *Gracilaria* sp. sample was cut into pieces (3-5 mm) and homogenized for 5 min. at room temperature. Lipids was extracted by soaking the homogenate overnight in $CHCl_3$/MeOH/H_2O (2:1:0.8 by vol.). After filtration, the solvent will removed at 25°C under reduced pressure using a rotary evaporator, and then the residual lipids was made up to a known concentration with $CHCl_3$/MeOH (2:1, v/v) and stored at -30°C until use.

Analysis of FFA Composition

FFAs were converted to methyl esters by heating at 95°C for 1 h in 5% HCl/MeOH (Christie, 2003). 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. The split ratio was 1:50. The column temperature was maintained at 160°C for 17 min, then elevated to 230°C at a ramp rate of 5°C/min. The final temperature was kept for 30 min. The injector and flame-ionization detector (FID) temperatures were set at 240°C. Peaks were monitored 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; Takagi *et al*., 1986).

Identification of Prostaglandins

For identification of eicosanoid compounds, LC/MS was used. Total lipids was subjected to LC/MS equipped with a Mightysil column, RP-18 GP (250 mm x 4.6 mm, 5 µm). Identification of eicosanoid compounds was conducted by comparing their mass spectra with those of authentic standard. For complete identification of the eicosanoid compounds, co-chromatography using authentic compounds was employed.

Determination of Prostaglandin Contents

The alga was finely sliced and placed in 100 mL-bottle. To which 30 mL of ethyl acetate and 150 µL of 1 M HCl in methanol was added. The mixtures were shaken for 5 min. and then centrifuged at 2,500 rpm for 5 min. at 20°C. The supernatants were pipetted and evaporated under reduced pressure using a rotary evaporator at 25°C. This procedure was repeated once more. The residue was dissolved in 200 µL of methanol as test solution. For determination of PGs, the test solution (5 µL) was subjected to HPLC (Hitachi Ltd, Tokyo, Japan) on a Mightysil column, RP-18 GP (250 mm x 4.6 mm, 5 µm). Determination of PGs was carried out by comparing the peak of PGs extracted from samples and authentic standards of PG. The contents of PGs in the samples was calculated from standard curve of the PG standards.

HPLC and LC Conditions

HPLC was performed at 40°C using a gradient elution from acetonitrile/water (40:60, v/v) containing 0.02% acetic acid (solvent A) to 100% acetonitrile (solvent B). The mobile phase system is as follows: 0-20 min. (solvent A), 20-60 min. (gradient of solvent A and B) and 60-80 min. (solvent B). The flow rate is 0.5 mL/min. The PG peaks were monitored by a diode array detector (Model L-7455 LaChrom, Hitachi Ltd, Tokyo, Japan) set at 196 nm.

RESULTS AND DISCUSSION

Fatty Acid Composition

Analysis of fatty acid compositions of the red alga by GC was shown in Table 1. The result showed that the dominant fatty acids were palmitic acid (C16:0, 50%) and arachidonic acid (C20:4n-6, 29.6%). There were significant amounts of the fatty acids, namely stearic acid (C14:0) dan oleic acid (C18:1n-9).

C16:0 is saturated fatty acid, which is abundantly found in many kind of seaweed (Stefanov *et al*., 1988; Aknin *et al*., 1990; Khotimchenko, 1998). Whereas, the polyunsaturated acid, C20:4n-6 is a typical fatty acid for the red algae (Khotimchenko *et al*., 1990; Araki *et al*., 1990; Illijas *et al*., 2009). This fatty acid is synthesized from hydrolysis of lipid membrane, glycoglycerolipids, such as

Table 1. Fatty acid composition of the red alga *Gracilaria* sp.

Fatty acid	Composition
14:0	3.0±0.3
iso 15:0	0.2±0.0
anteiso 15:0	0.1±0.1
15:0	05±0.2
15:1	nd
iso 16:0	0.1±0.0
anteiso 16:0	0.1±0.0
16:0	50.0±4.4
iso 17:0	0.4±0.0
17:0	0.2±0.1
18:0	1.1±0.3
20:0	0.1±0.0
22:0	0.2±0.0
24:0	nd
Saturates	**56.0**
14:1n-9	nd
16:1n-7.9	1.8±0.2
16:1n-5	nd
18:1n-9	3.3±0.4
18:1n-7	1.3±0.1
18:1n-5	nd
24:1n-9	nd
Monoenes	**6.4**
16:2n-4	nd
16:3n-4	0.2±0.2
18:2n-6	0.7±0.0
18:3n-6	0.4±0.1
18:3n-3	0.1±0.0
18:4n-3	0.2±0.3
20:2n-6	0.2±0.1
20:3n-6	2.4±0.1
20:4n-6	29.6±2.6
20:3n-3	nd
20:4n-3	nd
20:5n-3	0.1±0.0
22:6n-3	nd
Polyenes	**34.0**
Others	3.6±0.9

nd : not detected
tr : trace (< 0.1)

monogalactosyldiasylglyserol (MGDG), digalactosyldiasylglyserol (DGDG) and sulfoquinovosyldiasylglyserol (SQDG) catalized by glycerolip acyl-hydrolase (Figure 1, Illijas *et al*., 2008). Function of the arachidonic acid is a precursor for biosynthesis of prostaglandins and other eicosanoic compounds in the red algae (Hsu *et al*., 2007; Illijas, 2008).

Prostaglandin

In the HPLC chromatograms (Figure 2), one of peaks was identified as prostaglandin E_2 (PGE_2). The identification was conducted by comparing the chromatograms with HPLC chromatogram of prostaglandin standards (Figure 3).

This prostaglandin is also found abundantly in many species of the red algae, such as *G. gigas* (Hsu *et al*., 2007), *G. asiatica* (Sajiki *et al*., 1998), *G. vermiculophylla* (Illijas, 2008).

Biosynthesis pathway of prostaglandin in seaweed is still unclear. However, it was found that prostaglandin is formed from oxidation of arachidonic acid, which is likely involve cyclooxygenase as catalyst, because prostaglandin formation decreased as addition of aspirin, an anti cyclooxygenase compound, was conducted to reaction mixtures of *G. vermiculophylla* extract and free arachidonic acid (Figure 4) (Illijas, 2008).

Prostaglandin function is also still unclear in the seaweed. However, several result of researches showed that formation of prostaglandins occurred when the seaweed was physically treated (Nakajima *et al*., 1998) so that the prostaglandins also known as secondary metabolites. The prostaglandins have also been found to be produced along with other eicosanoid compounds when the red alga *Chondrus crispus* was incubated with pathogen extract (Bouarab *et al*., 2004; Gaquerel *et al*., 2007). In mammals and human, prostaglandins play an important role as hormone, which control several kinds of metabolism (Samuelsson, 1975).

CONCLUSION

Arachidonic acid, the dominant polyunsaturated fatty acid found in the seaweed, is precursor of synthesis of prostaglandin E_2, the only eicosanoic compound could be identified in this study.

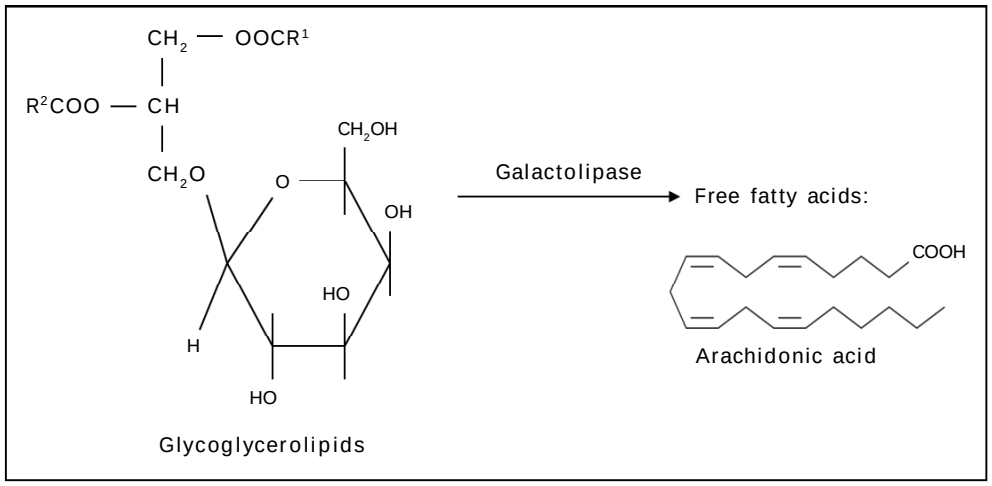

Figure 1. Biosynthesis pathway of arachidonic acid in the red alga *G. vermiculophylla* (Illijas *et al.*, 2008)

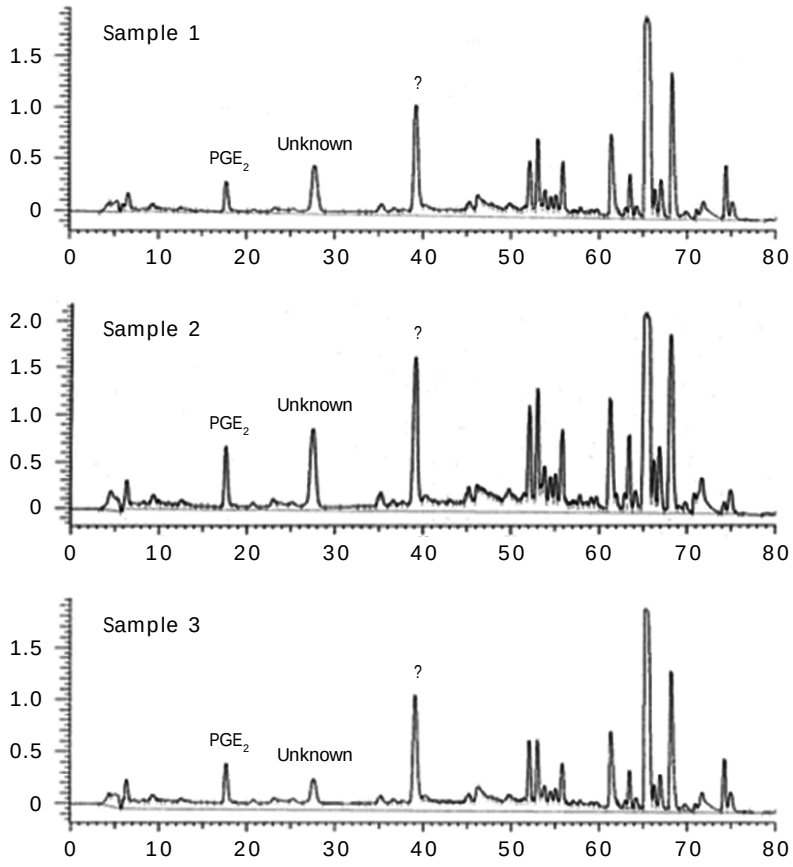

Figure 2. HPLC chromatograms of the red alga *Gracilaria* sp. extracts ($CHCl_3$-MeOH extracts)

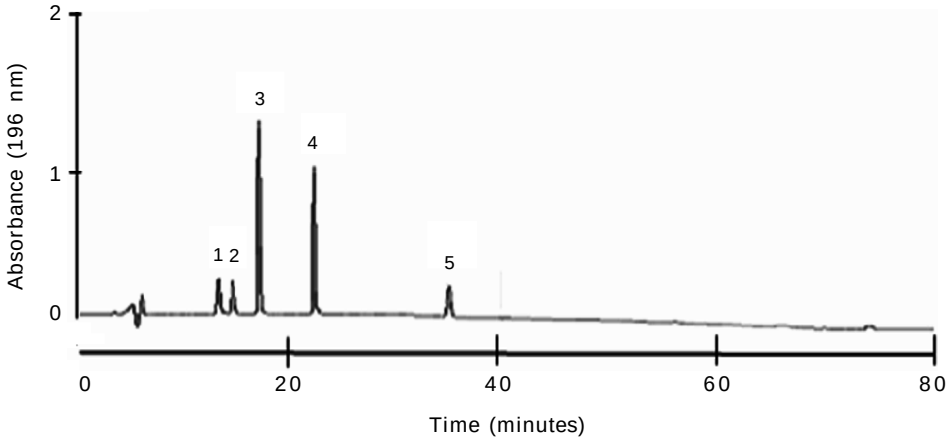

Figure 3. HPLC chromatogram of prostaglandin standards. Peak 1: PGE_3; Peak 2: $PGF_{2\alpha}$; Peak 3: PGE_2; Peak 4: 15-keto-PGE_2; Peak 5: PGA_2

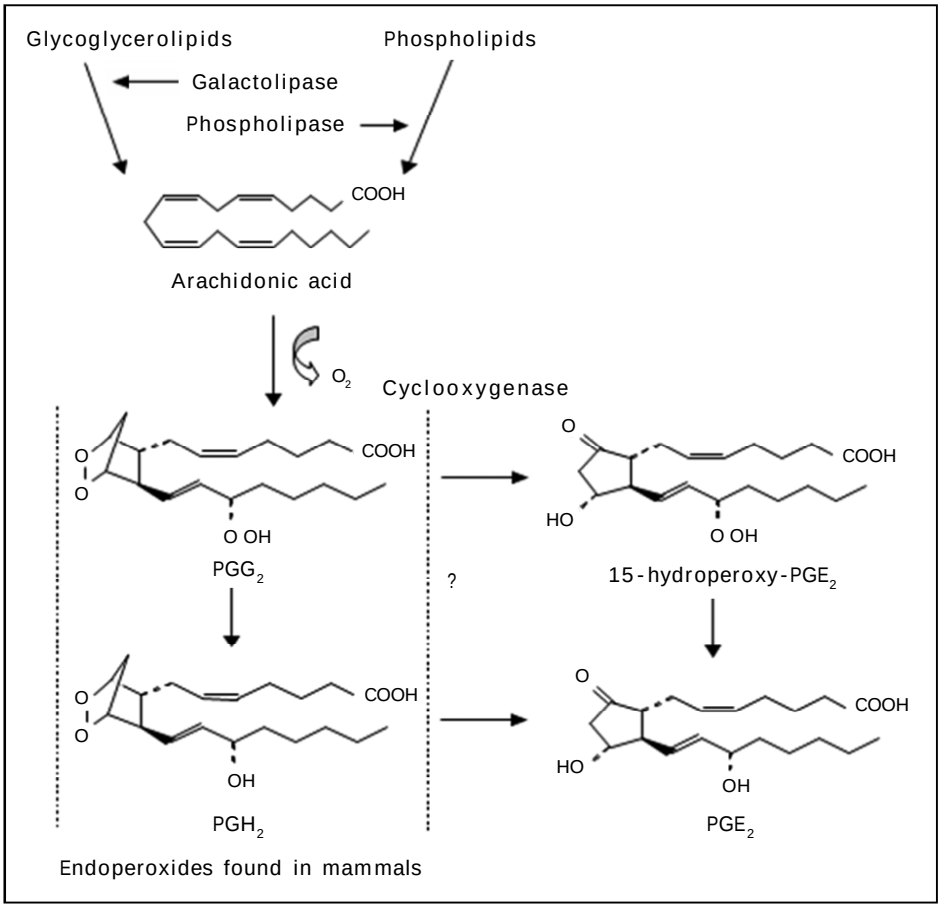

Figure 4. Proposed biosynthesis pathway of prostaglandin in the red alga *G. vermiculophylla* (Illijas, 2008)

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