J. Fish. Sci. Technol. 12(1), 16-23, 2009

JOURNAL OF FISHERIES SCIENCE AND TECHNOLOGY
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Characterization of Fatty Acids Extracted from *Brachionus rotundiformis* Using Lipase-catalyzed Hydrolysis

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Lipids were extracted from marine rotifer, *Brachionus rotundiformis* in order to examine the functionality of lipid enzymatic modification. The fatty acids, palmitic, linoleic, oleic and stearic acids were the dominant forms accounting for approximately 35.8%, 21.5%, 15.9% and 7.7% of the total lipid content, respectively. Lipid fractions were categorized as neutral lipids (38.5%), glycolipids (45.9%) and phospholipids (17.6%), and after extraction from the rotifer were isolated by thin-layer chromatography (TLC) as free fatty acids (FFA), monoacylglycerol (MAG), diacylglycerol (DAG) and triacylglycerol (TAG). The production of polyunsaturated fatty acid (PUFA) concentrate from rotifer lipids was studied using lipase-catalyzed hydrolysis. In addition, rotifer lipids were modified by hydrolysis using lipases such as porcine pancreas, *Candida rugosa* and *Rhizomucor miehei*. The lipase from *Rhizomucor miehei* was effective in extracting linoleic acid (C18:2), while the lipase from *Candida rugosa* was effective in palmitic acid (C16:0) extraction.

Key words: Marine zooplankton, Rotifer, Lipids, Lipase hydrolysis, Fatty acids.

Introduction

In recent years, thraustochytrids have been established as vital in the production of polyunsaturated fatty acids (PUFAs). Particularly, much focus has been on the production of arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), due to their beneficial effects on human health (Periasamy et al., 2007).

PUFAs possess many physiological functions. For instance, ethyl ester of EPA has been used for treating arteriosclerosis and hyperlipidemia since 1990 in Japan (Shimada et al., 2001). While DHA is involved in disease prevention in humans, inhibiting cardiovascular disease, inflammation and cancer, it also been reported to serve important functions in preterm infant development (Shimada et al., 2001). Moreover, monoacylglycerols (MAG) and diacylglycerol (DAG) are the most widely used emulsifiers in the food, pharmaceutic and comestic industries DAG pharmaceutical industries, and often a mixture of MAG and DAG is used for these applications because it produces effective performance with low cost (Fuerby et al., 1997).

Recent investigations have focused on the development of functional lipids from marine organism. Lipase-catalyzed hydrolysis has been used to isolate functional lipids from various fish waste, such as sardine oil (Tomoko and Michael, 2007), cod (Per-Avrid et al., 2007), Atlantic salmon (Marijana et al., 2008), sea urchin (Enroque et al., 2008) and micro algae (Gaelle et al., 2007).

In general, DAG and MAG were induced reaction of between excessive soybean-oil and glycerol at high temperature conditions of more than 200°C and metal- or alkali-catalytic reactions by stimulate chemical esterification. However, when attempting to obtain MAG and DAG mixture at high temperature distillation reaction, the product made has the disadvantage of fading colors, high energy consumption and foul odor. Therefore, the MAG and DAG synthetic complements the inherent disadvantages of synthetic chemical reactions with enzymes (Park and Lee, 2004).

Compared to chemical methods, MAG and DAG synthesis using enzymes can reduce costs and energy consumption while obtaining high product yields at

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lower temperatures. Moreover, production is possibly efficient using enzymatic glycerolysis (Rosu et al., 1999; Noureddini and Harmeier, 1998). MAG can be synthesized through the glycerolysis of fatty oils. In addition, lipase-catalyzed enzymatic production of EPA and DHA concentrate from fish oil has shown potential in producing high quality product due to the mild condition of the process (Tomoko and Michael, 2007).

Marine zooplankton are commonly used as live feed for fish larvae cultures, which generally have high demands for dietary protein due to their rapid growth rates and extensive catabolism of amino acids required in metabolic energy production (Rønnestad et al., 2003). Rotifers in particular are regarded as a good food source appropriate for large quantity cultivation because of their small size and high content of rich nutrients (Helland et al., 2003). Furthermore, rotifers are rich in essential fatty acids (EFA), which are vital for growth and survival of fish larvae (Park et al., 1999). To date, production of PUFAs has not been reported from rotifers.

The objectives of this study therefore were to investigate and characterize lipids extracted from a rotifer, and produce PUFAs concentrate using lipase-catalyzed hydrolysis.

Materials and Methods

Materials

Marine zooplankton and rotifer (*Brachionus rotundiformis*) used in this study were purchased from Aquanet Co. Ltd (Tong-young, Gyeong-nam). Rotifers were freeze-dried and stored at -80°C until use. Enzymatic hydrolysis of rotifer lipids was conducted with commercial lipases [porcine pancreas (187 U/mg-solid), *Candida rugosa* (1170 U/mg-solid) and *Rhizomucor miehei* (20,000 U/g)] purchased from Sigma Chemical Co. (St. Louis, MO, USA). Standard and BF_3/methanol were purchased from Sigma (St. Louis, MO, USA). Silica gel (70-230 mesh) was purchased from Merck (Darmstadt, Germany). All other reagents were of the highest commercial grade available.

Analysis of proximate composition

The moisture, lipid, protein and ash contents of the rotifer were determined using AOAC methods (Helrich, 1999). Moisture was determined by oven-drying at 105±1°C. Crude lipid was measured in a Soxhlet system by extraction with diethyl ether solvent. Total nitrogen content was analyzed according to the Kjeldahl procedure (Kjeltec1035, Foss, Sweden). Crude protein content was calculated using a conversion factor of 6.25. Ash content was determined by incineration of samples at 550°C in a muffle furnace (F6000, Barnstead Thermolyne Co., USA).

Extract of total lipid

Total lipids were extracted from freeze-dried rotifer using the Bligh and Dyer method (1959). Briefly, 100 g of sample was mixed with 100 mL of chloroform and 200 mL of methanol for 2 min to obtain a monophasic system. Chloroform (100 mL) was added to this monophasic ternary system and the mixture was blended for 30 seconds. Next, 100 mL of water was added followed by blending for 30 seconds. The homogenate was filtered through Whatman No. 1 and filtrate was collected in a separatory funnel. After separation of the filtrate into two layers, the volume of the chloroform layer was measured.

Separation of neutral lipids, glycolipids and phospholipids

Total lipid was separated into neutral lipids, glycolipids, and phospholipids by passing solution through a glass column (ID 20×300 mm) packed with slurry of activated silica gel (70-230 mesh; Merck, Germany) and chloroform, according to the method of Rouser et al. (1967). The eluents for neutral lipids, glycolipids and phospholipids were chloroform, acetone and methyl alcohol, respectively. The solvents were evaporated using a rotary evaporator and the percentage of each fraction was determined gravimetrically.

Hydrolysis reaction of rotifer lipid

The hydrolysis of rotifer lipid by microbial lipases was conducted in a batch system. Commercial lipases from porcine pancreas (187 U/mg-solid), *Candida rugosa* (1,170 U/mg-solid) and *Rhizomucor miehei* (20,000 U/g) were used to catalyze the hydrolysis reaction. The reaction mixture consisted of lipase 10,000 U, rotifer oil 500 mg and 20 mL of 1:1 (v/v) distilled water:hexane solution. The temperature was controlled at 45°C and shaken at 200 rpm.

Thin-layer chromatography

The various lipid groups were separated and fractionated by thin-layer chromatography (TLC, 0.25 mm silica Gel 60 F_{254}, Merck, Co., Ltd.). The lipids were fractionated using hexane/diethyl ether/ acetic acid (80:20:2, v/v/v). The developed TLC plates were sprayed with a 50% sulfuric acid solution and then heated at 110°C for 20 min. The neutral lipid

standards consisted of MAG, 1,2-DAG, 1,3-DAG, FFA, triacylglycerol (TAG), cholesterol ester and wax. The classes of neutral lipids were detected by TLC-Scanner 3 (Cammag, Switzerland).

Analysis of fatty acid composition

After TLC separation of the lipase-hydrolyzed rotifer oil, acylglycerols and FFA fractions were saponified with 0.5 M-NaOH in methanol for 15 min at 100°C. Fatty acids were methylated with 14% BF_3 in methanol for 20 seconds at 100°C, followed by measurement with a gas chromatograph (GC, Shimadzu QP-5050A, Kyoto, Japan) equipped with a HP-INNOWax capillary column (ID 0.25 mm×30 m). Nitrogen was used as the carrier gas at a 0.5 mL/min flow rate and split ratio of 30:1. The initial temperature of 210°C was increased to 240°C at a rate of 1°C/min, which was maintained for 17 min. The injector and detector temperatures were 250 and 300°C, respectively. Finally, a 1 μL sample of methyl esters was injected into the GC column. The fatty acids were identified by comparing their retention times with those of known fatty acid composition standards.

Results and Discussion

Rotifer compositions

The approximate composition of the rotifer is shown in Table 1. Crude protein content was 63.5%, while lipid, carbohydrates and ash were 17.7%, 7.7% and 4.7%, respectively. In general, the total lipid content obtained from rotifer is between 10.79-14.55% dry matter (Lubzens et al., 1989). The magnitude of variation in zooplankton lipid content is relatively high and inversely related to environmental temperature (Georgi and Guileromo, 2007). Table 2, which summarizes the fatty acid composition of rotifer lipids, demonstrates palmitic acid, linoleic acid, oleic acid and stearic acid were the dominant fatty acids, accounting for 35.8%, 21.5%, 15.9% and 7.7% of the total lipid content, respectively. This was in contrast to the exceedingly low levels exhibited by EPA and DHA. Fatty acid composition of rotifers is highly affected by chlorella, a major food source for rotifer culture (Georgi and Guileromo, 2007). Fatty acid composition of chlorella has been reported similar in qualitative composition, containing palmitic acid, linoleic acid, oleic acid and stearic acid (Georgi and Guileromo, 2007). Palmitic acid or hexadecanoic acid is one of the most common saturated fatty acids (SFA) found in animals and plants (Beare-Rogers et al., 2001). As its name indicates, it is a major component of palm tree oil (palm oil and palm kernel oil) (Beare-Rogers et al., 2001). Linoleic acid is a member of the EFA group omega-6 fatty acids, hence named because they are an essential dietary requirement for all mammals. Symptoms for omega-6 deficiency include dry hair, hair loss and poor wound healing (Cunnane and Anderson, 1997). However, meeting the daily requirement for these fatty acids is relatively easy as a normal diet provides plenty of omega-6 fatty acids by consuming polyunsaturated plant oils (Ruthing and Meckling, 1999).

Table 1. Composition of the rotifer

Compositions	Contents (%)
Moisture	6.5 ± 0.1
Ash	4.7 ± 0.1
Crude lipid	17.7 ± 0.6
Crude protein	63.5 ± 0.2
Carbohydrate	7.7 ± 0.7
Total	100.0

Table 2. Fatty acid composition of the rotifer

Fatty acids	Contents (%)
Caproic (C6:0)	3.4
Myristic (C14:0)	1.9
Pentadecanoic (C15:0)	1.1
Palmitic (C16:0)	35.8
Palmitoleic (C16:1)	0.7
Margaric (C17:0)	0.8
Margaroleic (C17:1)	0.2
Stearic (C18:0)	7.7
Oleic (C18:1)	15.9
Linoleic (C18:2)	21.5
α-linolenic (C-18:3 n-3)	1.6
Arachidic (C20:0)	0.5
Gadoleic (C20:1 n-9)	2.4
Eicosadienoic (C20:2)	2.7
γ-linolenic (C20:3 n-6)	0.6
Arachidonic (C20:4 n-3)	0.8
EPA (C20:5 n-3)	0.0
Behenic (C22:0)	0.6
Erucic (C22:1 n-9)	0.6
DHA (C22:6 n-3)	0.0
Lignoceric (C24:0)	0.6
Nervonic (C24:1)	0.5
Total	100.0

Extraction of rotifer lipids and lipid class composition

The yield of lipids extracted from rotifer was 12.5% (data did not shown), and it is approximately 70% of overall crude lipid contents of the rotifer (17.7%).

The percentage of neutral lipids, glycolipids and phospholipids extracted from the rotifer are shown in

Table 3. Lipid class composition of oil extracted from rotifer byproducts

Compositions	Contents (%)
Glycolipid	38.5 ± 1.3
Neutral lipid	43.9 ± 2.6
Phospholipid	17.6 ± 1.7
Total	100.0

Table 3. Based on total lipid content, neutral lipid composition was 43.9% and glycolipid and phospholipid compositions were 38.5% and 17.6%, respectively. Composition of the neutral lipid was separated by TLC. TAG and FFA were the major constituents of the rotifer lipids. MAG, 1,2- and 1,3-DAG, cholesterol esters and wax were the minor components. Copeman et al. (2004) reported that phospholipids constituted 54.9% of cod flesh oil. Passi et al. (2002) reported sardine oil contained 40.8% TAG and 18.4% phospholipids.

Hydrolysis reaction of rotifer lipid

Three commercial lipases were obtained for the hydrolysis of MAG, DAG, TAG and FFA at 45°C in a batch reactor. Fig. 1, Fig. 2 and Fig. 3 show MAG, DAG, TAG and FFA fractions produced by lipases (porcine pancreas, *Candida rugosa* and *Rhizomucor miehei*). The three lipases reached a steady state reaction during 12 hr. However, incremental responses were observed in our results as well. Fig. 1 shows changes in the rotifer lipid fraction after hydrolysis by porcine pancreas. The FFA fraction increased from 36.0% to 53.4% after 12 hr of hydrolysis, yet other lipid fractions such as the TAG fraction decreased. In general, DAG is absorbed without re-synthesis to TAG after the body transports it to liver. Thereafter it is used as an energy source throughout beta-oxidation and thus does not accumulate in the body. However, TAG is absorbed into the body through a different process (Yamamoto et al., 2005).

A similar tendency was observed in the *Candida rugosa* lipase. Changes in levels of MAG, DAG, TAG and FFA are shown in Fig. 2. The TAG fraction was significantly reduced from 27.7% to 15.4% after 2 hr of hydrolysis followed by a gradual decrease during the 12 hr to 7.7%. The FFA fraction increased from 36.0% to 49.2% after 2 hr and steadily increased until 12 hr of hydrolysis. MAG and DAG fraction also increased during the 12 hr. When sardine oil was subjected to enzymatic hydrolysis using *Candida rugosa* lipase in a previous report, a similar trend was observed. In that case, TAG was decreased from 86.20% to 70.31% after 1.5 hr of hydrolysis, while DAG increased from 13.40% to 26.89% after 1.5 hr and again to 32.33% after 9 hr (Tomoko and Michael, 2007). Tamotsu and Tsuneo (1990) reported the *Candidia rugosa* lipase is most desirable because it rapidly yields fish oil containing the desired composition of TAG, although the recovery of said TAG is only 30-40%. In Fig. 3 showing the lipase from *Rhizomucor miehei*, TAG significantly was declined from 27.1% to 13.8% after 2 hr and finally to 4.9% after 12 hr, while DAG increased after 12 hr from 14.5% to 20.5%. The levels of FFA also increased from 36.0% to 60.2% during the 12 hr. Therefore, our results state lipid fractions are changed

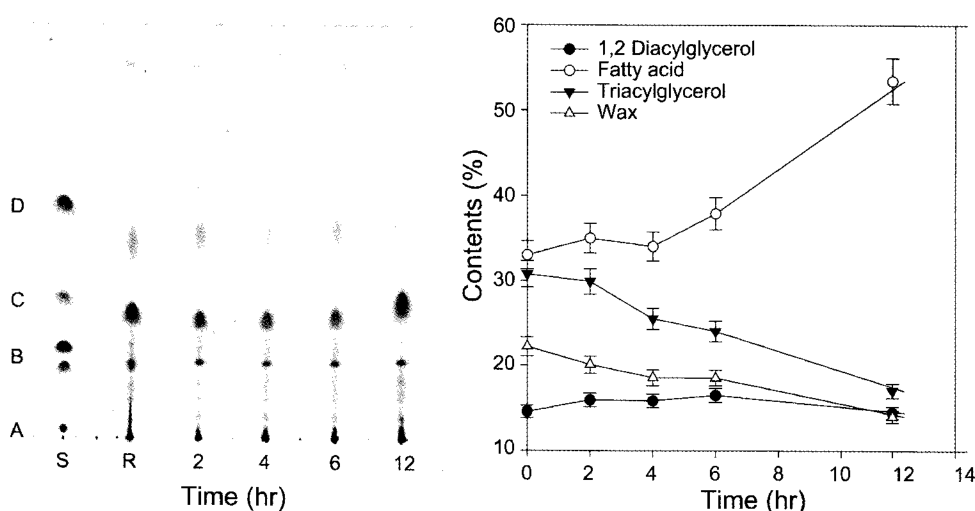

Fig. 1. Lipid classification of rotifer lipid hydrolysates obtained after treatment with the lipase from porcine pancreas and detected by TLC-Scanner. A, Mono (cis-9-monolein); B, Di (cis-9-1,2-diolein, cis-9-1,3-diolein); C, Fatty acid; D, Triglyceride (cis-9-triolein), S ; Standard mixture.

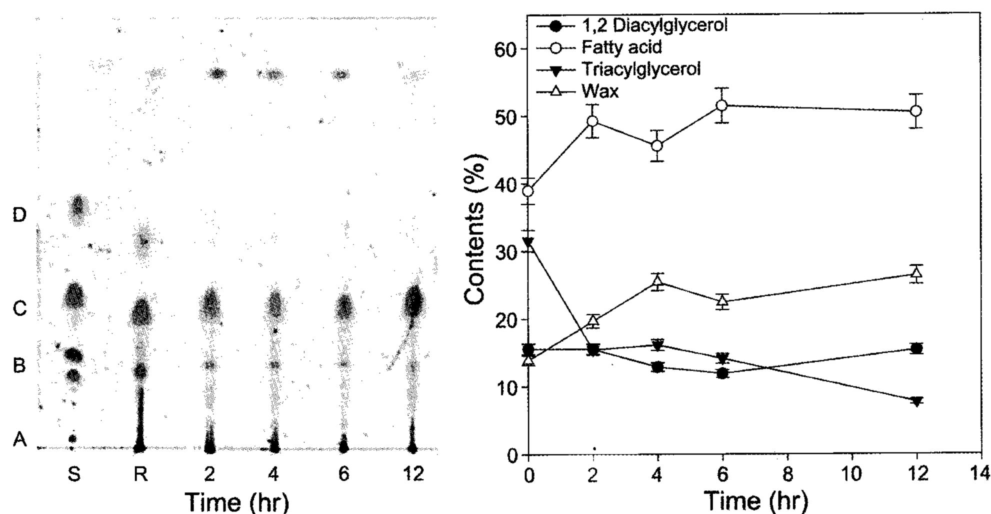

Fig. 2. Lipid classification of rotifer lipid hydrolysates obtained after treatment with the lipase from Candida rugosa and detected by TLC-Scanner. A, Mono (cis-9-monolein); B, Di (cis-9-1,2-diolein, cis-9-1,3-diolein); C, Fatty acid; D, Triglyceride (cis-9-triolein), S; Standard mixture.

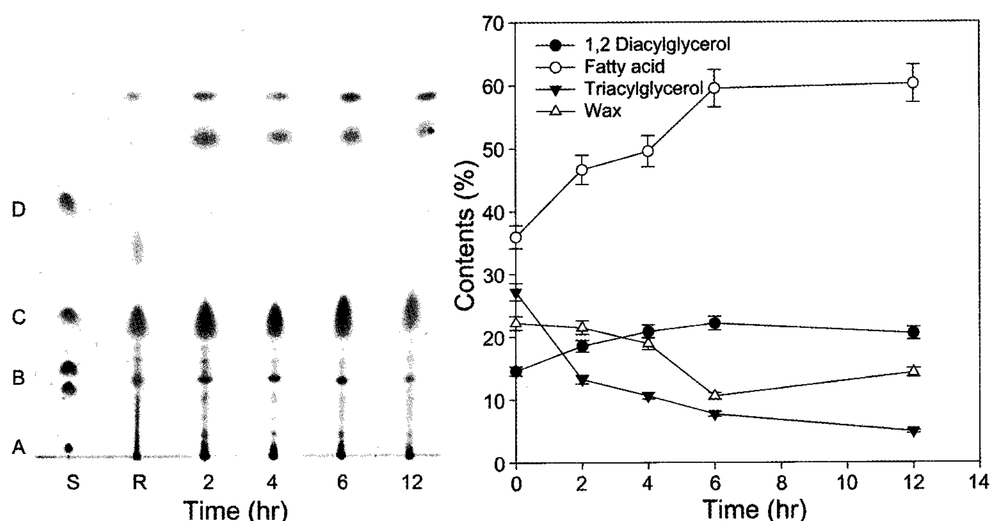

Fig. 3. Lipid classification of rotifer lipid hydrolysates obtained after treatment with the lipase from Rhizomucor miehei and detected by TLC-Scanner. A, Mono (cis-9-monolein); B, Di (cis-9-1,2-diolein, cis-9-1,3-diolein); C, Fatty acid; D, Triglyceride (cis-9-triolein), S; Standard mixture.

by lipase hydrolysis. Specifically, the FFA fraction was increased by lipases depending on reaction time. In general, enzymatic activity followed by FFA removal increases the concentrations of EPA and DHA while reducing SFA and monounsaturated fatty acid (MUFA) (Tomoko and Michael, 2007).

Fatty acid composition of the rotifer lipid TLC fraction

The rotifer lipid hydrolysis using the two lipases, *Candida rugosa* and *Rhizomucor miehei*, was examined because of increases in 1,2 DAG and TAG. Table 4 shows the changed fatty acid content after hydrolysis by the *Candida rugosa* lipase. The SFA of DAG and TAG fractions increased 4.2% and 2.0%, respectively. The predominant fatty acid in rotifer lipid and hydrolysate accounting for SFA was C16:0, comprising approximately 22.1% of each. After hydrolysis, MUFA of the DAG, TAG and FFA fractions was increased by 3.6%, 5.1% and 19.3%, respectively, with the predominant fatty acid being C16:1 followed by C18:1. In addition, in the MAG fraction C18:2 increased 13.2% and C16:1 declined 13.8%, while PUFA was increased by 10.2%. The predominant fatty acid in rotifer lipid and hydrolysate accounting for PUFA was C18:2, comprising appro-

Table 4. Fatty acid composition of MAG, DAG, TAG and FFA fractions from rotifer lipids hydrolyzed with lipase from Candida rugosa (%)

FAMEs	Rotifer Oil				Lipase from *Candida rugosa*			
	A	B	C	D	A	B	C	D
C16:0	23.0	24.6	22.6	25.9	17.1	25.1	20.8	25.7
C18:0	4.9	10.5	5.3	5.2	11.4	15.8	3.7	8.3
SFA	33.0	39.4	31.1	37.2	28.5	43.6	29.8	39.2
C16:1	13.8	0.0	0.6	0.7	0.0	0.0	0.7	0.0
C18:1	3.7	4.8	3.6	4.9	16.9	9.3	6.7	9.5
C20:1	2.2	1.8	0.9	1.6	0.0	0.0	0.8	2.1
C22:1	2.8	1.5	0.5	1.1	0.0	2.4	0.5	1.8
MUFA	22.5	8.1	5.9	8.3	16.9	11.7	9.2	13.4
C18:2	30.8	36.7	55.5	44.4	48.0	37.5	52.4	34.1
C18:3	0.0	1.2	2.1	1.3	6.6	2.8	2.0	1.2
C20:4	0.0	0.0	0.3	0.0	0.0	0.0	0.5	0.0
C22:2	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0
C20:5	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0
PUFA	44.4	52.6	63.0	54.4	54.6	44.6	61.1	47.4

A, monoacylglycerol; B, 1,2- and 1,3-diacylglycerol; C, fatty acid, D: triacylglycerol; FAMEs, Fatty acid methylester; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Table 5. Fatty acid composition of MAG, DAG, TAG and FFA fractions from rotifer lipids hydrolyzed with lipase from Rhizomucor miehei (%)

FAMEs	Rotifer Oil				Lipase from *Rhizomucor miehei*			
	A	B	C	D	A	B	C	D
C16:0	23.0	24.6	22.6	25.9	24.4	22.4	22.7	25.9
C18:0	4.9	10.5	5.3	5.2	14.7	11.3	4.4	6.1
SFA	33.0	39.4	31.1	37.2	43.8	36.4	30.6	35.8
C16:1	13.8	0.0	0.6	0.7	0.0	0.0	0.6	0.0
C18:1	3.7	4.8	3.6	4.9	11.6	5.3	3.4	5.6
C20:1	2.2	1.8	0.9	1.6	2.6	1.5	1.0	1.6
C22:1	2.8	1.5	0.5	1.1	2.3	1.7	0.1	1.3
MUFA	22.5	8.1	5.9	8.3	16.5	8.5	5.6	8.5
C18:2	30.8	36.7	55.5	44.4	30.1	43.7	55.8	41.6
C18:3	0.0	1.2	2.1	1.3	0.0	2.4	2.3	2.8
C20:2	3.0	3.7	3.5	6.2	3.1	3.5	4.2	5.8
C20:4	0.0	0.0	0.3	0.0	0.0	0.0	0.1	0.0
C20:5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C22:2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PUFA	44.4	52.6	63.0	54.4	39.7	55.1	63.8	55.7

A, monoacylglycerol; B, 1,2- and 1,3-diacylglycerol; C, fatty acid; D, triacylglycerol; FAMEs, Fatty acid methylester; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

ximately 36.7% of each. The lipase from *Candida rugosa* was non-specific and displays a higher ability to concentrate EPA and DHA than 1,3-specific lipases, which is in agreement with other studies (Carvalho et al., 2002; Sun et al., 2002). One reason why this lipase produces n-3 PUFA concentrate with high efficiency may be its chain-length selectivity, showing higher activity with relatively short-chain fatty acids such as C18 or below (McNeill et al., 1996). However, comparison with rotifer lipid is not possible as rotifers contain little EPA and DHA. In the case of sardine oil, C16:0 content decreased significantly from 24.9% to 17.1% after 1.5 hr and gradually decreased to 13.2% after 6 hr (Tomoko and Michael, 2007). For this study, the fatty acid composition after hydrolysis by the *Rhizomucor miehei* lipase is shown in Table 5. It shows SFA of the MAG fraction increased 10.8%. The predominant fatty acid in rotifer lipid and hydrolysate accounting for SFA was C16:0, comprising approximately 22.8% of each. The MUFA of MAG fraction deceased 6.0%. The predominant fatty acid in the MAG fraction was C16:1 followed by C18:1, despite C16:1 being broken after hydrolysis. The PUFA of MAG fraction

was decreased 4.7% in contrast to the DAG, TAG and FFA fractions, which increased slightly. The predominant fatty acid in rotifer lipid and hydrolysate accounting for PUFA was C18:2, comprising approximately 35.6% of each. Furthermore, the C18:2 content of the MAG, DAG and TAG fractions was 30.1, 43.7 and 55.8%, respectively. In case of Turkish anchovy lipid, levels of PUFA in the glyceride mixture were raised to 40% while the individual TAG and DAG fractions contained 45 and 30% PUFA, respectively (Ustun et al., 1997). The lipase from *Rhizomucor miehei* is 1,3-specific and was found to be more effective at increasing C16:0 concentrations in MAG, DAG and TAG fractions compared to lipases from *Candida rugosa*. However, the lipase from *Candida rugosa* was found to be more effective at increasing C18:2. Finally, no significant changes in EPA or DHA concentrations were observed in rotifer lipids hydrolyzed by lipases from either *Rhizomucor miehei* or *Candida rugosa*.

This study shows the lipase from *Rhizomucor miehei* influences positional specificity more than fatty acid specificity. However, further investigations are required on the products of functional lipids such as conjugated linoleic acid as well as on the synthesis of MAG, DAG and TAG by esterification of rotifer lipid fatty acids with glycerol.

Acknowledgment

This research was supported by a grant from Marine Bioprocess Research Center of the Marine Bio21 project funded by the Ministry of Land, Transport and Maritime, Republic of Korea.

References

Beare-Rogers, J., A. Dieffenbacher and J.V. Holm. 2001. Lexicon of lipid nutrition. Pure Appl. Chem., 73, 685-744.

Bligh, EG. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37, 911-917.

Carvalho, P.D.O., P.R.B. Campos, M. D'Addio Noffs, D.H.M. Bastos and J.G. De Oliveira. 2002. Enzymic enhancement of n-3 polyunsaturated fatty acids content in Brazilian sardine oil. Acta. Pharma. Boner., 21, 85-88.

Conpeman, L. and C.C. Parrish. 2004. Lipids classes, fatty acid, and sterols in seafood from Gilbert Bay, southern Labrador. J. Agric. Food Chem., 52, 4872-4881.

Cunnane, S. and M. Anderson. 1997. Pure linoleate deficiency in the rat: influence on growth, accumulation of n-6 polyunsaturates, and (1-^{14}C) linoleate oxidation. J. Lipid, 4, 805-812.

Enrique, G.D., D.C. John, M.C.R. Shawn and J.B. Tammy. 2008. Effects of dietary lipids on the fatty acid composition and lipid metabolism of the green sea urchin (Strongylocentrotus droebachiensis). Aquaculture, 276, 120-129.

Fuerby, A.M., L. Tian, P. Adlercreutz and B. Mattiasson. 1997. Preparation of diglycerides by lipase-catalyzed alcoholysis of triglycerides. Enzyme Microb. Technol., 20, 198-206.

Gaelle, P., P. Laurent, S. Samin and E. Francoise. 2007. Use of lipases for the production of a DHA-rich lipid fraction from the microalgae (Isochrysis galbana). J. Biotechnol., 131, 74-97.

Georgi, P. and G. Guileromo. 2007. Which are fatty acids of the green alga Chlorella?. Biochem. Syst. Ecol., 35, 281-285.

Helrich, K. 1999. Official methods of Analysis 16th ed., Association of Official Analytical Chemists, Inc. Washington, DC, USA, 69-88.

Helland, S., B.F. Terjesen and L. Berg. 2003. Free amino acid and protein content in the planktonic copepod Temora longicornis compared to Artemia Franciscan. Aquaculture, 215, 213-218.

Lubzens E., A.Tandler and G. Mintoff. 1989. Rotifers as food in aquaculture. Hydrobiologia., 186, 387-400.

Marijana, T., V. Anne, G. Tor, S. Hilde, E.T. Bente, A.K. Marte and R. Bente. 2008. Preparation changes in fatty acids metabolism during differentiation of Atlantic salmon preadipocytes; Effects of n-3 and n-9 fatty acids. BBA-Mol. Cell Biol. L., 1781, 326-335.

Noureddini, H. and S.E. Harmeier. 1998. Enzymatic glycerolysis of soybean oil. J. Am. Oil Chem. Soc., 75. 1359-1365.

Park, H.G., K.W. Lee, S.M. Lee, S.K. Kim and H.S. Kim. 1999. Change of fatty acid composition of rotifer according to enrichment diets and methods in the high density culture. J. Korean Fish. Soc., 32, 748-752.

Park, R.K. and K.T. Lee. 2004. Synthesis and characterization of mono- and diacylglycerol enriched functional oil by enzymatic glycerolysis of corn oil. Korea J. Food Sci., 36, 211-216.

Passi, S., S. Cataudella, P.D. Marco, F.D. Simone and L. Rasterlli. 2002. Fatty acid composition and antioxidant levels in muscle tissue of different mediterranean marine species of fish and shellfish. J. Agric. Food Chem., 50, 7314-7322.

Per-Avrid, W., H.R. Katja, L.C. Chantal, Z.I. Jose, R. Jose and K. Elin. 2007. Phospholipids vs. neutral lipids: Effects on digestive enzymes in Atlantic cod (*Gadus morhua*) larvae. Aquaculture, 272, 502-513.

Periasamy, A., D.U. Park, E. J.Jeh, Y.S. Jeong and K.H. Byung. 2007. Investigation of the physiological pro-

perties and synthesis of PUFAs from thraustochytrids and its electrophoretic karyotypes. Biotechnol. Bioprocess Eng., 12, 720-729.

Rosu, R., M. Yasui, Y. Iwasaki and T. Yamane. 1999. Enzymatic synythesis of symmertrical 1,3-diacylglycerols by direct esterifiaction of glycerol in solvent-free system. J.Am. Oil Chem. Soc., 76, 839-843.

Rouser, G., D. Kritchevsky, G. Simon and G.J. Nelson. 1967. Quantitative analysis of brain and spinach leaf lipid employing silicic acid column chromatography and acetone for elution of glycolipids. Lipids, 2, 37-42.

Ruthig, D.J. and K.A. Meckling-Gill. 1999. Both (n-3) and (n-6) fatty acids stimulate wound healing in the rat intestinal epithelial cell line, IEC-6. J. Nutr., 10, 1791-1798.

Rønnestad, I., S.K. Tonheim, H.J. Fyhn, C.R. Rojas-Garcia, Y. Kamisaka, W. Koven, R.N. Finn, B.F Terjesen, Y. Barr and L.E.C Conceição. 2003. The supply of amino acids during stages of marine fish larvae: a review of recent findings. Aquaculture, 227, 147-164.

Shimada, Y., A. Sugihara and Y. Tominaga. 2001. Enzymatic purification of polyunsaturated fatty Acids. J. Biosci. Bioprocess Eng., 6, 529-538.

Sun, T., G.M. Pigott and R.P. Herwing. 2002. Lipase-assisted concentration of n-3 polyunsaturated fatty acids from viscera of farmed Atlantic salmon (*Salmo salar* L.). J. Food Sci., 67, 130-136.

Tamotsu, H. and Y. Tsuneo. 1990. Selective hydrolysis of fish oil by lipase to concentrate n-3 polyunsaturated fatty acids. Agric. Biol. Chem., 54, 1459-1467.

Tomoko, O. and M. Michael .2007. Production of n-3 polyunsaturated fatty acid concentrate from sardine oil by lipase-catalyzed hydrolysis. Food chem., 103, 1411-1419.

Ustun, G., S. Guner, G. Arer, S. Turkay and A.T. Erciyes. 1997. Enzymatic hydrolysis of anchovy oil: Production of glycerides enriched in polyunsaturated fatty acids. Appl. Biochem. Biotech., 68, 171-186.

Yamamoto, K., H. Asakawa, K. Tokunaga, S. Meguro, H. Watanabe, I. Tokimitsu and N. Yagi. 2005. Effect of diacylglycerol administration on serum triacylglycerol in a patient homozygous for complete lipoprotein lipase deletion. Metab. Clin. Exp., 54, 67-71.

(Received 12 January 2009; Reviced 14 February 2009; Accepted 18 March 2009)