Production of a Health-Beneficial Food Emulsifier by Enzymatic Partial Hydrolysis of Phospholipids Obtained from the Head of Autumn Chum Salmon

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Abstract: Phospholipids and their partial hydrolysates, namely lysophospholipids (LPLs), have been widely used in food, pharmaceutical, and cosmetic products as highly efficient emulsifiers. This study was conducted to produce docosahexaenoic acid (DHA)-esterified LPLs by enzymatic modification of phospholipids obtained from the head of autumn chum salmon (Oncorhynchus keta). The emulsifying properties of the obtained LPLs were also evaluated. Two different types of substrates of salmon head phospholipids were prepared via silica gel and cold acetone precipitation. Enzymatic partial hydrolysis was carried out using immobilized phospholipase A₁ (PLA₁) and Lipozyme RM IM. Results showed that the increase in DHA in the LPLs was much higher in the silica-separated phospholipids than in the acetone-precipitated phospholipids. When silica-separated phospholipids were used as the substrate, the DHA content of the LPLs increased from 23.1% to 40.6% and 42.6% after 8 h of partial hydrolysis with Lipozyme RM IM and immobilized PLA₁, respectively. The yield of the LPLs was comparatively higher in the Lipozyme RM IM than in the immobilized PLA₁, hydrolysis reaction. The critical micelle concentration values of the LPLs and purified lysophosphatidylcholine (LPC) were 100 mg/L and 5 mg/L, respectively. The surface tension values of the LPLs and LPC were reduced to 30.0 mN/m and 30.5 mN/m, respectively. The hydrophilic-lipophilic balance of the LPLs and LPC were 6.0 and 9.4, respectively. Based on the emulsifying properties observed, we conclude that LPLs derived from the phospholipids of salmon head lipids could be used as a health-beneficial emulsifier in the food industry.

Key words: Oncorhynchus keta, docosahexaenoic acid, lysophospholipids, enzymatic partial hydrolysis, emulsifying properties

1 INTRODUCTION

Phospholipids from marine sources have received significant attention from the nutrition community owing to the health benefits they provide, which include improved immune function, and prevention of heart disease and certain cancers¹⁴. Moreover, phospholipids and their partial hydrolysates, lysophospholipids (LPLs), have good emulsifying properties, and therefore they can be used as potential natural emulsifiers to prepare emulsions⁵⁶. This is due to their unique molecular structure that contains both lipophilic fatty acid groups and a hydrophilic head group.

The docosahexaenoic acid (DHA)-esterified LPLs not only have high-performance emulsifying food components but also have high-performance health-beneficial functions owing to their high bioavailability⁷. It has been reported that the blood-brain barrier cells release components favoring DHA transfer, and DHA is quickly taken up by the brain when incorporated into lysophosphatidylcholine (LPC)⁸; in particular, DHA-esterified lysophosphatidylserine seemed to be the most effective for embryo brain DHA accretion⁹. It has recently been elucidated that major facilitator superfamily domain containing protein 2a (Mfsd2a) is a transporter of DHA-esterified LPC¹⁰. Moreover, sn-2 DHA-esterified phospholipids are the only functionally available molecules in the form of lipids to prevent apoplexy¹¹. Because of increasing consumer demand for natural products, natural emulsifiers have become increasingly im-

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important. Emulsifying agents naturally present in foods have been used in many food products. For example, casein and egg yolk proteins are excellent natural emulsifiers. Phospholipids from egg and soybean have found many applications in food products. However, purification of egg lecithin is too costly for the food industry. Therefore, soy lecithin and its enzymatic derivatives have been widely used as natural emulsifiers in the food industry. The dominant fatty acid component of soy lecithin is mostly linoleic acid. Although linoleic acid is an essential fatty acid for humans, excess intake of it can aggravate various kinds of diseases\(^{12, 13}\). Therefore, DHA-esterified LPLs derived from marine resources could be used as a substitute to prevent excess intake of soy lecithin.

Marine (fisheries) by-products are rich in DHA or eicosapentaenoic acid (EPA). Squid skin, squid muscle, squid connective tissues, and testis as well as the ovary of fish and shellfish are rich in DHA-containing phospholipids\(^{14, 15}\), while the body wall and internal organs of sea cucumber (\textit{Apostichopus japonicus}), and internal organs and gonads of starfish (\textit{Asterias amurensis}) are rich in EPA-bound phospholipids\(^{16, 17}\). However, typical sources for both DHA- and EPA-abundant phospholipids are giant scallop internal organs, salmon heads, and krills\(^{14, 16}\). To decrease by-products and wastes from bioresources, various ways of efficient utilization of fisheries by-products are gaining importance around the world, including Japan. Each year, more than 2000 to 3000 metric tons of the head of autumn chum salmon (\textit{Oncorhynchus keta}) are transferred to a processing industry located in Ishinomaki city, Japan. There, the cartilage is separated from the head of salmon, but other parts that are rich in lipids are not utilized. It is thought that salmon head is rich in sn-2 position DHA-containing phospholipids. In this study, we produced DHA-esterified LPLs from phospholipids obtained from the head of autumn chum salmon by carrying out hydrolysis reactions using phospholipase A\(_1\) (PLA\(_1\)) and sn-1,3 positional-specific lipase (Lipzyme RM IM). The enzymatic modification reaction such as the lipase-mediated partial hydrolysis was carried out to cleave off the fatty acid moiety bound at the sn-1 position of marine phospholipids, because most of the naturally occurring marine source phospholipids are known to contain DHA at the sn-2 position. The emulsifying properties of the obtained LPLs were also evaluated.

Lipids were extracted from the cartilage-separated residues using ethanol; however, 2-propanol can also be used to extract total lipids reducing the solvent cost to 1/3-1/4, which is more practically valuable for large-scale operations. Lipzyme RM IM (79.2 BIU/g), a commercial 1,3-specific lipase from \textit{Rhizomucor miehei} immobilized on an anion exchange resin, was a generous gift from Novozymes A/S ( Bagsvaerd, Denmark). PLA\(_1\) from \textit{Aspergillus oryzae}, was a gift from Mitsubishi Chemical Foods Co. Ltd. (Tokyo, Japan). Duolite A568 was purchased from Sumika Chemtex Co. Ltd. (Osaka, Japan). All other chemicals used were of analytical or chromatographic grade.

2.2 Preparation of Substrates

Phospholipids were separated from the total lipids of salmon head using Sep-Pak Vac 35cc silica cartridge (Waters Corporation, Milford, MA, USA) as described by Juuneda and Rocquelin\(^{19}\). The lipids (200 mg of total lipid) were loaded onto the top of the cartridges. Then, simple lipids and phospholipids were eluted using chloroform and methanol, respectively, in sequential order. In this study, phospholipids were also separated using the acetone precipitation method. More than 20-fold volume of acetone was added to the total lipids that were kept overnight in a freezer (−20°C). The phospholipids fraction (precipitate) was collected by decanting the upper portion that contained simple lipids. The obtained phospholipids were dissolved in n-hexane and stored under argon gas in the dark at −50°C until use for the enzymatic reaction.

2.3 Immobilization of PLA\(_1\)

The immobilized enzyme was prepared using the optimal conditions determined by Garcia et al.\(^{20}\). In brief, commercially available PLA\(_1\) was suspended into 0.1 M Tris-HCl buffer (pH 7.0) at a protein concentration of 1.5% as enzyme suspension. In a typical immobilization trial, 1 g of support (Duolite A568) was mixed with 10 mL of enzyme suspension and placed in a reciprocal shaker operating at 125 strokes per min at 50°C. After 24 h, the suspension was filtered through a Buchner funnel to recover the resulting immobilized enzyme preparation from the Whatman #1 filter paper. The solids were rinsed with 50 mL of 0.1 M Tris-HCl buffer (pH 7) and dried overnight at 30°C in a vacuum oven. The dried immobilized PLA\(_1\) was stored at 4°C in a desiccator for further use.

2.4 Partial Hydrolysis of Phospholipids using Immobilized PLA\(_1\)

Partial hydrolysis of phospholipids with immobilized PLA\(_1\) was carried out following the method of Ono et al.\(^{21}\) and Kim et al.\(^{22}\). For the optimization of enzyme loading, three different amounts (53 mg, 80 mg, and 106 mg) of immobilized PLA\(_1\) were used. Immobilized PLA\(_1\) was equilibrated with saturated NaNO\(_3\) solution (Water activity, \(a_w=\)
Production of health beneficial food emulsifier through bioconversion

2.5 Partial Hydrolysis of Phospholipids using Lipozyme RM IM

Partial hydrolysis of phospholipids using Lipozyme RM IM was carried out following the method of Ono et al.\textsuperscript{20}. In brief, Lipozyme RM IM (53 mg) was equilibrated with saturated K\textsubscript{2}CO\textsubscript{3} solution (a\textsubscript{o} = 0.44) in a desiccator at 25°C for 24 h. To initiate the reaction, a\textsubscript{o} adjusted immobilized PLA\textsubscript{1} was added to 2 mL of distilled n-hexane solution, which contained 20 mg of silica-separated phospholipids. The reaction mixtures in screw-capped vials were incubated at 50°C with vigorous stirring under argon gas atmosphere. The reaction was terminated by removing the immobilized PLA\textsubscript{1} with a 0.45 μm polytetrafluoroethylene (PTFE) membrane filter (PALL Corporation, NY, USA) using chloroform:methanol (1:1, v/v) as an eluent. The recovered reaction mixtures were applied on a Sep-Pak Vac 12cc silica cartridge. Free fatty acids were first removed with chloroform:methanol (10:1, v/v); then, the remaining LPLs were recovered with methanol and used for further analysis. After optimization of the enzyme loading, a partial hydrolysis reaction was carried out using the acetone-precipitated phospholipids. All trials were conducted in triplicates.

2.6 Analysis of Fatty Acid Composition

Fatty acid methyl esters were derived from the substrates and LPLs following the method described by Prevot and Mordret\textsuperscript{23}. In brief, dried substrates or LPLs were dissolved in 1 mL of n-hexane, and 0.2 mL of methanolic 2N-NaOH solution was added. The mixture was shaken using a vortex mixer and kept at 50°C for 20 s and then 0.2 mL of methanolic 2N-HCl solution was added to neutralize the solution. The n-hexane layer was collected, concentrated, and subjected to gas chromatography (GC 353, GL Science Inc., Tokyo, Japan) connected with a 0.5 μm PEG-20M liquid phase-coated 40 m × 1.2 mm i.d. G-300 column (Chemicals Evaluation and Research Institute, Saitama, Japan) with flame ionization detection. The temperature of the column, detector, and injector were 190°C, 250°C, and 250°C, respectively. The fatty acids were identified by comparing the peak retention times to authentic standards (GL Sciences Inc., Tokyo, Japan) and by following the theory of linear relationship between the carbon number unit or the number of double bonds of fatty acid and the logarithm of the corresponding retention times.

2.7 Lipid Composition Analysis

The lipid compositions of the substrates and LPLs were determined using a commercial silica gel 60 F\textsubscript{254} (Merck, Darmstadt, Germany) thin-layer chromatograph (TLC) plate with the solvent system consisting of chloroform:methanol:water (60:25:4, v/v/v). The plate was sprayed with 8% phosphoric acid containing 3% cupric acetate and heated at 150–160°C for 15 min. Each spot was identified by authentic lipid standards and then lipid compositions were analyzed using a scanner and TLC analysis software (JustTLC version 4.0.3, Sweden, Lund, Sweden).

2.8 Determination of Emulsifying Properties

The emulsifying properties, such as surface tension, critical micelle concentration (CMC), and hydrophilic-lipophilic balance (HLB) of the obtained LPLs were determined. LPC was also prepared to compare its emulsifying properties to those of LPLs. To isolate LPC, LPLs were subjected to preparative thin-layer chromatography (TLC). Isolation of LPC was carried out on a 0.5-mm thick silica gel 60 F\textsubscript{254} TLC plate. TLC plates were developed using chloroform:methanol:water (60:40:5, v/v/v) to 70% Rf height, followed by 100% development using n-hexane/diethyl ether/acetic acid (70:30:1, v/v/v). The lipid composition was also analyzed through TLC using the same solvent combination. TLC plates were charred at 150°C for 10 min after being sprayed with 8% phosphoric acid containing 3% cupric acetate.

The surface tensions of the aqueous DHA-esterified LPL and LPC suspensions were measured with a CBVP-Z tensiometer (Kyowa Interface Science Co. Ltd., Saitama, Japan) employing the Wilhelmy plate technique\textsuperscript{24}. Measurements were made at 25°C in an equilibrium state; i.e., the surface tension was measured after the change in the observed value was less than 0.1 mN/m for 1 h. The CMC was determined from the plot of the surface tension against the logarithm of the concentration (log C). The values reported are the mean of three measurements. HLB was calculated following the method described by Griffin\textsuperscript{25}.

3 RESULTS AND DISCUSSION

3.1 Yield and Fatty Acid Composition of Phospholipids

The fatty acid compositions of silica-separated phospholipids and acetone-precipitated phospholipids are shown in Table 1. For industrial applications, phospholipids are mainly separated using the acetone precipitation method.

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\textsuperscript{19} JustTLC version 4.0.3, Sweden, Lund, Sweden.

\textsuperscript{20} Ono et al. (2017).

\textsuperscript{21} Prevot and Mordret (1989).

\textsuperscript{22} Griffin (1958).

\textsuperscript{23} Wilhelmy plate technique.

\textsuperscript{24} CBVP-Z tensiometer.

\textsuperscript{25} Griffin (1958).
Simple lipids dissolve in acetone; in contrast, more polar compounds such as phospholipids are precipitated at lower temperatures ($\approx 20^\circ$C). Polar lipids (phospholipids and glycolipids) are mostly insoluble in cold acetone at $\approx 26^\circ$C. For this reason, we prepared two types of phospholipids, which were used as substrates, and compared for their increase in DHA content and yield of LPLs after partial hydrolysis reactions with Lipozyme RM IM and immobilized PLA1. In this study, the yield of phospholipids was much higher in the acetone precipitation method ($\approx 31.5^\circ$C) than in the silica separation method ($\approx 24.7^\circ$C); however, silica-separated phospholipids contained comparatively higher amount of DHA ($\approx 23.1^\circ$C), followed by palmitic acid ($\approx 21.9^\circ$C), oleic acid ($\approx 16.3^\circ$C), and EPA ($\approx 9.5^\circ$C). On the other hand, the major fatty acids in the acetone-precipitated phospholipids were palmitic acid ($\approx 19.5^\circ$C), oleic acid ($\approx 18.4^\circ$C), DHA ($\approx 13.5^\circ$C), and EPA ($\approx 5.9^\circ$C).

### 3.2 Optimization of Immobilized PLA1 Loading

The effects of immobilized PLA1 loading on the increase in DHA content and yield of LPLs of the silica-separated phospholipids were investigated by monitoring the time course reaction; the results are shown in Fig. 1. Three different amounts of water activity ($a_w = 0.65$) adjusted immobilized PLA1 ($53$ mg, $80$ mg, and $106$ mg) were used for these trials. The amount of DHA and total EPA plus DHA in the LPLs increased gradually while EPA decreased when the amount of enzyme increased up to $80$ mg. However, the amount of EPA, DHA, and total EPA plus DHA in the LPLs increased up to $8$ h of reaction, and then decreased when the amount of enzyme increased up to $106$ mg. The yield of LPLs decreased gradually during the first $4$ h of reaction, and then decreased sharply up to $8$ h for all the amounts of enzyme tested. A comparatively lower yield of LPLs was

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**Table 1** Fatty acid composition of phospholipids (PLs) obtained from the head of autumn chum salmon.$^a$

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Silica-separated PLs</th>
<th>Acetone-precipitated PLs</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>$3.5 \pm 0.12$</td>
<td>$6.3 \pm 0.24$</td>
</tr>
<tr>
<td>16:0</td>
<td>$21.9 \pm 0.37$</td>
<td>$19.5 \pm 0.47$</td>
</tr>
<tr>
<td>16:1</td>
<td>$5.0 \pm 0.22$</td>
<td>$6.4 \pm 0.15$</td>
</tr>
<tr>
<td>16:2</td>
<td>$1.3 \pm 0.05$</td>
<td>$1.3 \pm 0.04$</td>
</tr>
<tr>
<td>18:0</td>
<td>$3.9 \pm 0.11$</td>
<td>$4.4 \pm 0.17$</td>
</tr>
<tr>
<td>18:1</td>
<td>$16.3 \pm 0.33$</td>
<td>$18.4 \pm 0.35$</td>
</tr>
<tr>
<td>18:2</td>
<td>$0.3 \pm 0.03$</td>
<td>$0.3 \pm 0.03$</td>
</tr>
<tr>
<td>18:3</td>
<td>$0.4 \pm 0.01$</td>
<td>$0.5 \pm 0.05$</td>
</tr>
<tr>
<td>20:1</td>
<td>$4.7 \pm 0.12$</td>
<td>$10.7 \pm 0.25$</td>
</tr>
<tr>
<td>20:3</td>
<td>$1.2 \pm 0.01$</td>
<td>$0.7 \pm 0.02$</td>
</tr>
<tr>
<td>20:4</td>
<td>$0.5 \pm 0.03$</td>
<td>$0.4 \pm 0.02$</td>
</tr>
<tr>
<td>20:5</td>
<td>$9.5 \pm 0.18$</td>
<td>$5.9 \pm 0.13$</td>
</tr>
<tr>
<td>22:1</td>
<td>$3.4 \pm 0.09$</td>
<td>$8.1 \pm 0.22$</td>
</tr>
<tr>
<td>22:5</td>
<td>$2.9 \pm 0.11$</td>
<td>$1.9 \pm 0.08$</td>
</tr>
<tr>
<td>22:6</td>
<td>$23.1 \pm 0.71$</td>
<td>$13.5 \pm 0.42$</td>
</tr>
<tr>
<td>Others</td>
<td>$2.1 \pm 0.10$</td>
<td>$1.7 \pm 0.09$</td>
</tr>
</tbody>
</table>

$^a$ Percentage of total fatty acids. Results represent mean $\pm$ SD ($n = 6$).

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**Fig. 1** Effects of immobilized PLA1 loading ($a = 53$ mg, $80$ mg, and $106$ mg) on the increase in DHA content and yield of lysophospholipids after partial hydrolysis reaction of silica-separated phospholipids. Reaction conditions: amount of substrate, $20$ mg; $a_w = 0.65$; temperature, $50^\circ$C.
obtained when the enzyme loading was higher. It has been reported that the incorporation of n-3 polyunsaturated fatty acid (PUFA) into phosphatidylcholine (PC) increased when higher amounts of enzyme loadings were used\(^{27,28}\). Overall, the yield of LPLs decreased significantly, although the amount of DHA and total EPA plus DHA increased in the LPLs during the partial hydrolysis reaction. For enzyme loading, 80 mg were selected as the optimum amount for further study of the increase in DHA content and yield of LPLs with the cost of the enzyme taken into consideration. It is important to reduce the amount of enzyme loading to reduce production cost, although increasing the amount of enzyme loading increases the incorporation of n-3 PUFA proportionately\(^{29,30}\).

3.3. Fatty Acid Composition and Yield of the LPLs

To produce DHA-esterified LPLs, partial hydrolysis reactions of silica-separated and acetone-precipitated phospholipids were carried out using Lipozyme RM IM and immobilized PLA\(_1\). The DHA content of the recovered LPLs increased up to 49.4% and 46.2% after 24 h of partial hydrolysis reactions with Lipozyme RM IM and immobilized PLA\(_1\), respectively, when silica-separated phospholipids were used as substrate (Fig. 2). The increase in DHA content in the LPLs coincides with the results of Ono et al.\(^{21}\). It has been reported that Lipozyme RM IM preferentially acts on fatty acid ester bonds at the sn-1 position of PC\(^{31}\). Thus, DHA is mostly esterified at the sn-2 position in the phospholipids obtained from the head of salmon. In contrast to DHA, palmitic acid and oleic acid content decreased, and stearic acid content remained constant during the partial hydrolysis reaction using Lipozyme RM IM. However, palmitic acid, stearic acid, oleic acid, and EPA contents decreased after immobilized PLA\(_1\)-mediated hydrolysis reaction of silica-separated phospholipids (Fig. 2).
The yield of LPLs was comparatively higher in the Lipozyme RM IM-mediated reaction (41.5%) than in the immobilized PLA1-mediated reaction (40.5%) after 24 h of reaction. However, 8 h were sufficient for enzymatic reaction if we consider the increase in DHA content and the yield of LPLs. In terms of bioconversion, Lipozyme RM IM was superior to immobilized PLA1 when silica-separated phospholipids were used as substrate. Similarly, the DHA content of LPLs increased up to 37.5% and 41.8% after partial hydrolysis reaction of acetone-precipitated phospholipids for 24 h with Lipozyme RM IM and immobilized PLA1, respectively (Fig. 3). The increase in DHA content and yield was comparatively higher in the LPLs obtained after partial hydrolysis reaction of the acetone-precipitated phospholipids using immobilized PLA1 than Lipozyme RM IM. However, the yield of LPLs decreased with increasing reaction time. Overall, the increase in DHA content and yield of LPLs was much higher in the silica-separated phospholipids than in the acetone-precipitated phospholipids.

### 3.4 Lipid Composition of LPLs

The lipid composition of the silica-separated and acetone-precipitated phospholipid and lysophospholipid fractions after partial hydrolysis reactions using Lipozyme RM IM and immobilized PLA1, Reaction conditions were the same as those for the trials in Fig. 2. Abbreviations: PE, Phosphatidylethanolamine; PC, Phosphatidylcholine; LPE, Lysophosphatidylethanolamine; LPC, Lysophosphatidylcholine.

![Fig. 4](image_url)

**Fig. 4** Lipid composition of phospholipids and lysophospholipids of (a) silica-separated phospholipids and (b) acetone-precipitated phospholipids after partial hydrolysis reactions using Lipozyme RM IM and immobilized PLA1. Reaction conditions were the same as those for the trials in Fig. 2. Abbreviations: PE, Phosphatidylethanolamine; PC, Phosphatidylcholine; LPE, Lysophosphatidylethanolamine; LPC, Lysophosphatidylcholine.
amounts produced in Lipozyme RM IM-mediated reactions. In the case of acetone-precipitated phospholipids, the Lipozyme RM IM reaction produced a comparatively higher amount of LPC (35.7%) and LPE (21.6%) than the immobilized PLA1 reaction after partial hydrolysis for 24 h. In terms of the yield of LPLs, silica-separated phospholipids were superior to acetone-precipitated phospholipids. Considering the LPC content and yield of LPLs, silica-separated phospholipids and immobilized PLA1 were selected for producing LPLs for further experiments.

3.5 Emulsifying Properties of LPLs

It is well known that the emulsifying properties of LPLs are more beneficial than those of diacylphospholipids. Therefore, the emulsifying properties of LPLs derived from silica-separated phospholipids were studied. The emulsifying properties of LPC, prepared from the obtained LPLs, were also determined. The CMC for the LPLs and LPC was determined by surface tension measurements (Fig. 5). Two break points were observed on the equilibrium surface tension (ŋ) vs. log C plots of the LPLs and LPC. Such double break points have also been shown in some mixed surfactant systems including polymer components. The break point at lower concentration on each surface tension curve is shown as C1. The higher break points agree with the corresponding phase-separation concentration, C2, which was 7 and 12 times higher for C1 for the LPLs and LPC, respectively. The changes of slope at C1 in their ŋ vs. log C plots are attributed to the formation of premicellar aggregates. Moreover, C1 and C2 have been termed as the transition concentrations of aggregation. The onset point of solubilization is widely used for the determination of CMC. Therefore, the CMC values of LPLs and LPC were 100 mg/L and 5 mg/L, respectively. The surface tension values of LPLs and LPC were 30.0 mN/m and 30.5 mN/m, respectively. The higher CMC value of the LPLs and LPC were 100 mg/L and 5 mg/L, respectively. The onset point of solubilization is widely used for the determination of CMC. Therefore, the CMC values of LPLs and LPC were 100 mg/L and 5 mg/L, respectively. In another study, fatty acids from Corynebacterium lepus had a surface tension and CMC values of 30 mN/m and 150 mg/L, respectively. Generally, the surface tensions at the CMCs of various purified biosurfactants have been reported to range from approximately 27–35 mN/m.

HLB is an important parameter to measure the suitability of any emulsifier for use in food products. An HLB system is often used for the selection of emulsifiers and is a measure of the surfactant’s preference for oil or water, with a higher number corresponding to a greater hydrophilicity-to-lipophilicity ratio. A high HLB value is preferred for o/w emulsions. In this study, the HLB values for the LPLs and LPC were estimated at approximately 6.0 and 9.4, respectively. Crude lecithin had an HLB value of 5.0, and the HLB value of soybean lyso-lecithin hydrolyzed using PLA1 increased due to the increase in unsaturated fatty acids and LPLs. Therefore, it is expected that LPLs and LPC result in more stable emulsions than soybean lecithin used for o/w emulsions. Thus, LPLs obtained by partial hydrolysis of phospholipids from the total lipids of salmon head would become more hydrophilic and have an improved function as an emulsifier in o/w emulsions.

4 CONCLUSIONS

The results of this study demonstrated that the amount of DHA and total EPA plus DHA increased while the yield of LPLs decreased during the partial hydrolysis reaction with Lipozyme RM IM and immobilized PLA1. Moreover, the amount of DHA increased up to 40.6% and 42.6% after 8 h of partial hydrolysis reactions with Lipozyme RM IM and immobilized PLA1, respectively, when silica-separated phospholipids were used as substrate. In terms of the yield of LPLs, silica-separated phospholipids were superior to acetone-precipitated phospholipids. Considering the LPC content and yield of LPLs, silica separated-phospholipids and immobilized PLA1 could be used to produce LPLs. The surface tension values of the LPLs and purified LPC were reduced to 30.0 mN/m and 30.5 mN/m at a concentration of 100 mg/L and 5 mg/L, respectively. Considering the advantages of natural emulsifiers, our results suggest that emulsifiers produced from the phospholipids of the head of autumn chum salmon can be used as novel natural emulsi-
fiers in the food industry.

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