Preparation of Pinolenic Acid Concentrates from Pine Nut Oil Fatty Acids by Solvent Fractionation

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Abstract: Pinolenic acid (PLA), which is a fatty acid (FA) exclusively found in the oils of edible pine nuts, has an appetite-suppression effect, thereby being effective to reduce body weight in humans. PLA concentrates would be suitable for use in functional foods and nutraceuticals due to the health benefits of PLA. PLA concentrates were prepared from free FA (FFA) obtained from pine nut oil using solvent fractionation. Siberian pine nut oil containing 18.3 wt% PLA was used as the starting material for the fractionation. The fractionation was performed in n-hexane at ultra-low temperatures down to −85℃. The PLA concentrates produced under the optimal conditions established in this study (temperature, −85℃; n-hexane-to-FFA ratio (v/w), 30:1; fractionation time, 36 h) contained 69.8 wt% PLA. The yield of PLA was 77.4 wt% of the initial PLA weight in the FFA. These results suggest that solvent fractionation is a more effective approach to prepare PLA concentrates with higher PLA contents at a particular yield of PLA than published methods using urea crystallization (e.g., PLA content = ~47 wt%, yield of PLA = ~77 wt%, Woo et al. (2016)) or lipase-catalyzed reactions (e.g., PLA content = ~30 wt%, yield of PLA = ~61 wt%, Lee et al. (2011)). The resulting PLA concentrates contained 11 of the 12 different species of FA present in the FFA, thereby indicating that the PLA concentrates prepared by solvent fractionation have more diverse FA profiles than those prepared by urea crystallization (e.g., 7 species of FA, Woo et al. (2016)).

Key words: appetite suppressants, concentrates, pine nut oil, pinolenic acid, solvent fractionation

1 INTRODUCTION

Pinolenic acid (PLA; c5,c9,c12-18:3) is a polyunsaturated fatty acid (PUFA) with a polymethylene-interrupted double bond at the Δ5 carbon. PLA is exclusively found in the oils of the edible nuts of pines, such as Siberian pine nut (Pinus sibirica; PLA content, ~19 wt%) and Korean pine nut (P. koraiensis; PLA content, ~14 wt%) [1]. PLA intake is effective for reducing body weight because PLA provides an appetite-suppression effect derived from the increased secretion of gut satiety hormones, such as cholecystokinin-8 and glucagon-like peptide-1 in humans [2-3]

Recently, interest in methods for preparing PLA concentrates has increased owing to the health benefits of PLA. PLA concentrates can be prepared using physical methods [4,5], enzymatic reactions [6,7], or both [8]. Urea crystallization is the most common physical method for preparing PLA concentrates [4,5,8]. Using urea crystallization, PLA concentrates can be prepared from free fatty acid (FFA) mixtures obtained from pine nut oil because fatty acids (FA) that are more saturated than PLA form crystals with urea, allowing PLA to be separated from the crystals by filtration. However, owing to the use of urea, further procedures, including acidification and extraction, are required to recover PLA from the filtrate. Alternatively, PLA concentrates can be prepared enzymatically from pine nut oil or FFA mixtures from the oil [6,8]. These enzymatic reactions involve the use of lipases that have positional specificity or selectivity toward specific FA. The Candida antarctica lipase B-catalyzed ethanolysis of pine nut oil, in which most PLA found in the oil is esterified at the sn-3 position, is a possible approach to prepare PLA concentrates because the lipase has specificity toward FA in the sn-3 position of triacylglycerols (TAG) in the presence of ethanol [9]. No et al. [8] used a Candida rugosa lipase, which has specificity toward unsaturated FA (USFA) with a double bond at the Δ9 carbon, including oleic (18:1n-9) and linoleic acids (18:2n-6), to prepare PLA concentrates by esterification of FFA mixtures obtained from pine nut oil. The lipase-catalyzed reaction leads to the elimination of 18:2n-6 and 18:1n-9, which are the two principal FA in the FFA mix-
tures from the oil, thereby enriching PLA. They further increased the PLA content in the enzymatically treated PLA concentrates using urea crystallization. However, compared with urea crystallization, published enzymatic methods are not effective for increasing the PLA content and yield in PLA concentrates.

Fractionation, also referred to as fractional crystallization, is a physical process that separates edible lipids, such as TAG or FA, into solid and liquid fractions based on the solubility of lipid components with different degrees of unsaturation at a controlled temperature. The two main types of industrial-scale fractionation processes are dry and solvent fractionation. The main advantage of solvent fractionation is a high separation efficiency and an enhanced yield of the targeted fraction compared with that obtained by dry fractionation. Solvent fractionation is usually utilized for the production of high-value-added lipid products because it requires high equipment investment and has high operating costs. Solvent fractionation using organic solvents, such as n-hexane or acetone, permits more saturated lipid components with high melting points to crystallize, whereas more unsaturated components with low melting points remain dissolved in the solvent. Solvent fractionation has been successfully applied to enrich health-beneficial FA, such as eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3), from different oil sources. However, to date, no published studies have used a solvent fractionation technique to prepare PLA concentrates.

The aim of this study was to prepare PLA concentrates from FFA mixtures obtained from pine nut oil using solvent fractionation. The solvent fractionation process was performed at ultra-low temperatures down to −85°C to achieve crystallization of the two principal FA (namely, 18:2n-6 and 18:1n-9) in the FFA mixtures, which could then be separated from PLA remaining in the liquid phase. Our present study revealed that solvent fractionation is an effective approach to prepare PLA concentrates containing a greater amount of PLA with a higher yield of PLA compared with published methods involving urea crystallization or lipase-catalyzed reactions. We investigated the effects of solvent amount and temperature on the PLA content and the yield of PLA in PLA concentrates. Then, we established the optimal conditions to maximize the PLA content in PLA concentrates while increasing the yield of PLA to enhance the economic feasibility of the fractionation system.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

Siberian pine nut oil (PLA content, 18.3 wt%) was obtained from Komega Co. (Eumseong, Korea). Anhydrous n-hexane (purity, >95%) was purchased from Avantor Performance Materials (Center Valley, PA, USA). The FA methyl ester (FAME) standards were obtained from Supelco (Bellefonte, PA, USA). All other reagents were of analytical grade.

2.2 Preparation of FFA from pine nut oil

Pine nut oil (100 g) was saponified with 400 mL of 10 wt% sodium hydroxide in 75 vol% ethanol. Distilled water (200 mL) was added to the saponified mixture, and the resulting aqueous layer was acidified by adding 250 mL of 6 N hydrochloric acid. n-Hexane (500 mL) and distilled water (300 mL) were added, and the aqueous layer was removed using a separatory funnel; the n-hexane layer containing the FFA was washed five times with distilled water (5 × 100 mL). The n-hexane layer was dried over anhydrous sodium sulfate, and n-hexane was removed using a rotary vacuum evaporator at 40°C to obtain the FFA fraction, which was then stored at −80°C.

2.3 Solvent fractionation of FFA from pine nut oil

PLA concentrates were prepared from the FFA fraction obtained from pine nut oil by solvent fractionation. The FFA fraction (2 g) and the desired amount of n-hexane/n-hexane-to-FFA ratios (v/v) of 10:1, 20:1, 30:1, and 40:1 were loaded in a 100 mL glass bottle. The bottle was heated to 40°C using a water bath to completely melt the FFA in n-hexane prior to starting the fractionation. After the melting process, the solution was immediately placed in an ultra-low temperature freezer (model 705, Thermo Fisher Scientific, Marietta, OH, USA). The solution was cooled to the desired temperature (from −85 to −65°C, every 5°C) at a rate of −2.0 to −1.5°C/min and held at that temperature. The fractionation time was considered to start the moment the solution was placed in the freezer. Fractionation of the FFA was performed without agitation of the solution. After the fractionation process, the liquid fraction was separated from the solid fraction by vacuum filtration through a Whatman no. 1 filter paper in a Buchner funnel, which was precooled to the fractionation temperature to avoid partial melting of the crystals during filtration. The liquid fraction was dried using a rotary vacuum evaporator at 40°C and subsequently by flushing under nitrogen.

2.4 Analysis of FA composition

The FA compositions of the lipid samples were analyzed according to the method described by Kang et al. Lipid samples (20 mg) were saponified with 3 mL of 0.5 N methanolic sodium hydroxide solution at 85°C for 10 min and then cooled to room temperature (23°C). After methylating the saponifiable lipids with 3 mL of 14% methanolic boron trifluoride solution at 85°C for 10 min, the mixture was cooled to room temperature, 3 mL of isooctane and 5 mL
of a saturated sodium chloride solution were added, and the mixture was vortexed. The upper isooctane layer containing the FAME was collected and passed through an anhydrous sodium sulfate column. The FAME were analyzed by gas chromatography using an Agilent Technologies 7890A gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionization detector and a fused silica column (SP-2560, 100 m × 0.25 mm i.d. × 0.2 μm film thickness, Supelco). FAME samples (1 μL) were injected in split mode with a split ratio of 200:1. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The injector and detector temperatures were maintained at 225 and 285°C, respectively. The oven temperature was held at 100°C for 4 min, increased to 240°C at a rate of 3°C/min, and finally held at 240°C for 17 min. The FAME were identified by comparing their retention times with those of the standards, and their contents (wt%) were calculated. The yield of PLA was calculated using the following equation:

\[ \text{Yield of PLA (wt%)} = \left( \frac{W_2 \times C_2}{W_1 \times C_1} \right) \times 100 \]  

where \( W_1 \) is the weight of FFA from pine nut oil, \( C_1 \) is the PLA content in the FFA, \( W_2 \) is the weight of the liquid fraction obtained by solvent fractionation of the FFA, and \( C_2 \) is the PLA content in the liquid fraction.

2.5 Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics (version 20) software (IBM Corp., Chicago, IL, USA). All data are presented as mean ± standard deviation. The difference between lipid samples was assessed using a two-tailed Student's \( t \)-test or one-way analysis of variance followed by Duncan's multiple range test. The difference was considered significant if the \( p \)-value was less than 0.05.

3 RESULTS AND DISCUSSION

3.1 FA composition of pine nut oil FFA

Table 1 gives the FA compositions of pine nut oil and the FFA fraction obtained from the oil, which was used as the starting material to prepare PLA concentrates. The content of each FA, including PLA, in the FFA fraction was not significantly different from that in pine nut oil. The FFA fraction was mainly composed of 18:2n-6 (45.1 wt%) and 18:1n-9 (23.6 wt%), which constituted ~69 wt% of the total FA. Saturated FA (SFA) containing 16–20 carbons, namely palmitic (16:0, 4.2 wt%), stearic (18:0, 2.6 wt%), and arachidic acids (20:0, 0.4 wt%) accounted for ~7 wt% of the total FA. The PLA fraction in the FFA was 18.3 wt%, Taxoleic acid (c5,c9-18:2, 1.7 wt%) and sciadonic acid (c5,c11,c14-20:3, 1.0 wt%), minor PUFA with a poly-methylene-interrupted double bond at the 5Δ carbon, were also found in the FFA fraction. All FA (total content, ~80 wt%) present in the FFA fraction were less saturated than PLA, except \( \alpha \)-linolenic acid (18:3n-3, 0.5 wt%) and c5,c11,c14-20:3 (1.0 wt%). Thus, the FA profile results clearly suggest that PLA can be separated from other FA in the FFA fraction using fractionation techniques. Solvent fractionation is a more appropriate approach than dry fractionation for the preparation of PLA concentrates from the FFA fraction owing to the relatively small differences in the number of carbons (16–20) and number of double bonds (0–3) between FA in the FFA fraction.

3.2 Effects of solvent amount

The use of a greater amount of solvent during solvent fractionation of lipids enhances the yield of a targeted fraction because the addition of solvent lowers the viscosity of the lipids, thereby improving the separation efficiency during filtration\(^{10,12,14} \). However, an excessive amount of solvent increases the production cost of the targeted fraction\(^{12} \). We evaluated the effects of the amount of \( n \)-hexane used for solvent fractionation of FFA from pine nut oil on the PLA content in the obtained liquid fraction and the yield of PLA. The investigated \( n \)-hexane-to-FFA ratios (v/w) were 10:1, 20:1, 30:1, and 40:1. For these trials, the temperature was maintained at ~80°C. Figure 1a shows the effects of the amount of \( n \)-hexane on the PLA content in the liquid fraction as a function of fractionation time (1–48 h). The PLA content in the trial with an \( n \)-hexane-to-FFA ratio of 10:1 was significantly greater or tended to be greater than those of the trials with \( n \)-hexane-to-FFA ratios of 20:1, 30:1, and 40:1. The maximum PLA content (70.7 ± 0.7 wt%, at 24 h) in the trial with an \( n \)-hexane-to-FFA ratio of 10:1 was not significantly different from that of the trial with an \( n \)-hexane-to-FFA ratio of 20:1 (69.7 ± 1.1 wt%, at 36 h), but was significantly greater than those of the trials with \( n \)-hexane-to-FFA ratios of 30:1 (68.2 ± 1.2 wt%, at 36 h) and 40:1 (66.3 ± 0.9 wt%, at 48 h). These results indicate that the maximum PLA content increased and the fractionation time required to achieve the maximum PLA content decreased as the amount of \( n \)-hexane decreased. The effects of the amount of \( n \)-hexane on the yield of PLA as a function of fractionation time are shown in Fig. 1b. At an \( n \)-hexane-to-FFA ratio of 10:1, the yield of PLA ranged from 37.1 to 44.1 wt% and was significantly lower than those obtained in the other trials throughout the fractionation process. This behavior resulted from a low separation efficiency during filtration owing to an insufficient amount of \( n \)-hexane. When the \( n \)-hexane-to-FFA ratio was increased from 10:1 to 20:1, a significant increase in the yield of PLA (range of 69.6–80.0 wt%) was observed throughout the fractionation process. An increase in the \( n \)-hexane-to-FFA ratio from 20:1 to 30:1 caused a significant increase in the yield of PLA after 12 h, with the exception of 36 h, with a yield of PLA in the range of 77.9–86.5 wt%. However, further increasing the \( n \)-hexane-to-FFA ratio to 40:1 did not significantly increase the yield of
Fig. 1 Effects of $n$-hexane-to-free fatty acid (FFA) ratio (w/w) on the pinolenic acid (PLA) content in the liquid fraction obtained by the solvent fractionation of the FFA fraction from pine nut oil (A) and the yield of PLA (B) as a function of fractionation time (1, 2, 4, 6, 8, 10, 12, 18, 24, 36, and 48 h), and the correlation between the PLA content and the yield of PLA (C). The fractionation process was performed in $n$-hexane at $-80\,^\circ C$. The weight of the FFA fraction was 2 g. All trials were conducted in triplicate. Means indicated by the same letter (a–d) are not significantly different ($p > 0.05$).

PLA (range of 80.3–87.7 wt%) throughout the fractionation process over that obtained at an $n$-hexane-to-FFA ratio of 30:1. To enhance the economic feasibility of the fractionation system for industrial applications, it is preferable to reduce the amount of $n$-hexane used while maximizing the PLA content. The correlation between the PLA content and the yield of PLA for different amounts of $n$-hexane is illustrated in Fig. 1c. The points closer to the upper-right corner of the graph correspond to greater PLA contents at a given yield of PLA or greater yields of PLA at a desired PLA content. The points corresponding to the trial with an $n$-hexane-to-FFA ratio of 10:1 are farthest away from the upper-right corner of the graph, indicating that this ratio is not suitable for the economic preparation of PLA concentrates, even though the maximum PLA content was achieved. Instead, the point closest to the upper-right corner of the graph (PLA content = 68.2 wt%, yield of PLA = 78.0 wt%) was obtained in the trial with an $n$-hexane-to-FFA ratio of 30:1. This result suggests that the most suitable $n$-hexane-to-FFA ratio for the fractionation process is 30:1 when considering both the PLA content in the PLA concentrates and the yield of PLA.

3.3 Effects of temperature

Temperature is a key factor in the solvent fractionation of lipids because the solubility of a lipid component in a given solvent typically depends on temperature. The two principal FA in the FFA fraction from pine nut oil, 18:2$n$-6 and 18:1$n$-9, are sparingly soluble in $n$-hexane at temperatures below $-50\,^\circ C$—the solubilities (per 100 g of $n$-hexane) of 18:2$n$-6 and 18:1$n$-9 are $\sim 3.0$ g at $-50\,^\circ C$ and $\sim 0.1$ g at $-40\,^\circ C$, respectively. By contrast, FA with three double bonds, including PLA, 18:3$n$-3, and $c$5,$c$11,14:20-3, are expected to be more soluble than 18:2$n$-6 or 18:1$n$-9 in $n$-hexane at temperatures below $-50\,^\circ C$, although there are no literature reports on the solubility of these FA at such temperatures. We evaluated the effects of temperature on the PLA content in the liquid fraction obtained by solvent fractionation of the FFA from pine nut oil and the yield of PLA. In these trials, temperatures from $-85$ to $-65\,^\circ C$ were tested using a $5\,^\circ C$ interval at an $n$-hexane-to-FFA ratio of 30:1. Figure 2a shows the effects of temperature on the PLA content in the liquid fraction as a function of fractionation time (1–48 h). A significant increase or tendency to increase was found for the PLA content with decreasing temperature throughout the fractionation process, except at 48 h. For the trial at $-85\,^\circ C$, the maximum PLA content (69.8 ± 0.6 wt%) was achieved at 36 h. This PLA content was not significantly different from that achieved at $-80\,^\circ C$ (68.2 ± 1.2 wt%, at 36 h), but was significantly greater than those achieved at $-75\,^\circ C$ (67.1 ± 1.9 wt%, at 48 h), $-70\,^\circ C$ (64.1 ± 0.1 wt%, at 48 h), and $-65\,^\circ C$ (57.8 ± 0.6 wt%, at 36 h). However, no distinct differences were observed among the yields of PLA (77.4–82.8
Solvent Fractionation to Prepare PLA Concentrates


wt\% obtained at fractionation times of 36–48 h for all the trials, as shown in Fig. 2b. The correlation between the PLA content and the yield of PLA at different temperatures is illustrated in Fig. 2c. The point closest to the upper-right corner of the graph (PLA content = 69.8 wt\%, yield of PLA = 77.4 wt\%) was obtained in the trial at −85°C. This result indicates that by considering both the PLA content in the PLA concentrates and the yield of PLA, the most suitable temperature for the fractionation process is −85°C.

3.4 Optimal fractionation conditions

From the experimental results in sections 3.2 and 3.3, we concluded that the optimal conditions for the preparation of PLA concentrates from the FFA fraction obtained from pine nut oil are a temperature of −85°C, an n-hexane-to-FFA ratio of 30:1, and a fractionation time of 36 h. Under these conditions, PLA concentrates containing 69.8 wt\% PLA were obtained with a yield of 77.4 wt\% based on the initial weight of PLA in the FFA fraction. Woo et al.\(^5\) used urea crystallization to prepare PLA concentrates containing ~47 wt\% PLA with a yield of PLA of ~77 wt\% from the FFA fraction obtained from Korean pine nut oil with a PLA content of ~13 wt\%. Lee et al.\(^6\) and Zhao et al.\(^7\) found that ethyl ester forms of PLA concentrates can be prepared by C. antarctica lipase B-catalyzed ethanolation of pine nut oil. From Korean pine nut oil (PLA content, ~14 wt\%), they obtained PLA concentrates containing ~30 wt\% PLA with a yield of PLA of ~61 wt\%\(^6\) and PLA concentrates containing ~36 wt\% PLA with a yield of PLA of ~40 wt\%\(^7\). These results indicate that solvent fractionation is a more effective approach for preparing PLA concentrates containing more PLA at a particular yield of PLA than published methods involving urea crystallization or lipase-catalyzed reactions.

3.5 FA composition of PLA concentrates

Table 1 shows a comparison of the FA compositions of the PLA concentrates obtained under the optimal conditions established in this study and the FFA fraction used as the starting material to prepare the PLA concentrates. The PLA concentrates contained 69.8 wt\% PLA, 11.2 wt\% 18:2\(^n-6\), 6.5 wt\% c5,c9-18:2, 4.4 wt\% 18:1\(^n-9\), and 3.8 wt\% c5,c11,c14-20:3 as the principal FA. SFA, including 16:0 and 18:0, and USFA, such as vaccenic acid (18:1\(^n-7\)), 18:3\(^n-3\), eicosenoic acid (20:1), and eicosadienoic acid (20:2), were also detected at levels below 1 wt\% in the PLA concentrates. This result shows that the PLA content in the PLA concentrates was increased by 51.5 wt\% relative to the PLA content of 18.3 wt\% in the original FFA fraction. This enrichment of PLA was mainly achieved by significantly decreasing the contents of the two major FA in the FFA fraction—the contents 18:2\(^n-6\) and 18:1\(^n-9\) were reduced by 33.9% and 19.2%, respectively. The contents

\(^a\)–d Means indicated by the same letter are not significantly different (p > 0.05).
Table 1  Fatty acid (FA) compositions (wt %) of pine nut oil and the free FA (FFA) fraction obtained from pine nut oil via saponification, and the pinolenic acid (PLA) concentrates obtained from the FFA fraction under the optimal solvent fractionation conditions.

<table>
<thead>
<tr>
<th>FA</th>
<th>Pine nut oil</th>
<th>FFA fraction obtained from pine nut oil</th>
<th>PLA concentrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>4.2 ± 0.0(^a)</td>
<td>4.3 ± 0.0(^{++})</td>
<td>0.4 ± 0.0(^*)</td>
</tr>
<tr>
<td>18:0</td>
<td>2.6 ± 0.0</td>
<td>2.6 ± 0.0(^{++})</td>
<td>0.1 ± 0.0(^*)</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>23.6 ± 0.1</td>
<td>23.6 ± 0.1(^{++})</td>
<td>4.4 ± 0.1(^*)</td>
</tr>
<tr>
<td>18:1n-7</td>
<td>0.4 ± 0.0</td>
<td>0.4 ± 0.0(^{++})</td>
<td>0.3 ± 0.0(^*)</td>
</tr>
<tr>
<td>c5,c9-18:2</td>
<td>1.7 ± 0.0</td>
<td>1.7 ± 0.0(^{++})</td>
<td>6.5 ± 0.1(^*)</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>45.1 ± 0.0</td>
<td>45.1 ± 0.1(^{++})</td>
<td>11.2 ± 0.4(^*)</td>
</tr>
<tr>
<td>c5,c9,c12-18:3 (PLA)</td>
<td>18.3 ± 0.1</td>
<td>18.3 ± 0.1(^{++})</td>
<td>69.8 ± 0.6(^*)</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.5 ± 0.0</td>
<td>0.5 ± 0.0(^{++})</td>
<td>0.6 ± 0.0(^*)</td>
</tr>
<tr>
<td>20:0</td>
<td>0.4 ± 0.0</td>
<td>0.4 ± 0.0(^{++})</td>
<td>()</td>
</tr>
<tr>
<td>20:1</td>
<td>1.2 ± 0.0</td>
<td>1.1 ± 0.0(^{++})</td>
<td>0.2 ± 0.0(^*)</td>
</tr>
<tr>
<td>20:2</td>
<td>0.6 ± 0.0</td>
<td>0.6 ± 0.0(^{++})</td>
<td>0.9 ± 0.0(^*)</td>
</tr>
<tr>
<td>c5,c11,c14-20:3</td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.0(^{++})</td>
<td>3.8 ± 0.1(^*)</td>
</tr>
<tr>
<td>Unidentified</td>
<td>0.5 ± 0.0</td>
<td>0.5 ± 0.0(^{++})</td>
<td>1.7 ± 0.0(^*)</td>
</tr>
</tbody>
</table>

\(^a\) Mean ± standard deviation \((n = 3)\)
\(^{++}\) Not significantly different from pine nut oil, \(p > 0.05\).
* Significantly different from FFA fraction obtained from pine nut oil, \(p < 0.05\).

of all SFA (i.e., 16:0, 18:0, and 20:0) and the other two monounsaturated FA (i.e., 18:1n-7 and 20:1) in the FFA fraction were also significantly reduced in the PLA concentrates. By contrast, except for 18:2n-6, the contents of all PUFA, such as c5,c9-18:2, 18:3n-3, 20:2, and c5,c11,c14-20:3, were significantly greater in the PLA concentrates than in the FFA fraction. Interestingly, the increased content of c5,c9-18:2 in the PLA concentrates suggests that c5,c9-18:2 has a greater solubility in \(n\)-hexane under the fractionation conditions used in this study than 18:2n-6, which is its positional isomer. A similar tendency was also observed for the solubilities of PLA and its positional isomer 18:3n-3—unlike the drastic increase in the PLA content, the 18:3n-3 content was slightly increased from 0.5 wt% (in the FFA fraction) to 0.6 wt% (in the PLA concentrates). Further investigations are required to identify why the solubilities of PUFA with a polymethylene-interrupted double bond at the \(\Delta5\) carbon are greater than those of their positional isomers, which are commonly found in foods. Finally, as shown in Table 1, with the exception of 20:0, 11 different species of FA contained in the original FFA fraction remained in the PLA concentrates. Woo et al.\(^b\) reported that PLA concentrates prepared by urea crystallization from Korean pine nut oil containing 13 different species of FA only contained 7 species of FA, despite the relatively low PLA content of \(\sim\)47 wt%. In particular, all SFA (i.e., 16:0, 18:0, and 20:0) were eliminated from the PLA concentrates. Consequently, PLA concentrates prepared in this study have more diverse FA profile than those prepared by urea crystallization. PLA concentrates prepared by \(C.\) antarctica lipase B-catalyzed ethanolyis of Korean pine nut oil, as reported by Lee et al.\(^b\), contained all 10 species of FA present in the oil. Thus, no distinct difference exists between the diversities of the FA profiles of our PLA concentrates and those prepared by the lipase-catalyzed reaction.

4 CONCLUSIONS

This study is the first to use solvent fractionation for the preparation of PLA concentrates from the FFA fraction obtained from pine nut oil. The solvent fractionation process was performed in \(n\)-hexane using a conventional ultra-low temperature freezer that can be operated down to \(-85^\circ\)C. We demonstrated that PLA concentrates containing a greater amount of PLA with a higher yield of PLA could be obtained under the optimal conditions established in this study when compared with those prepared in previous studies using conventional urea crystallization or lipase-catalyzed reactions. Compared with urea crystallization, solvent fractionation also increased the diversity of the FA profile of the PLA concentrates. This study was limited, as the effects of temperatures below \(-85^\circ\)C on the PLA
content in the PLA concentrates and the yield of PLA could not be investigated, despite the possibility that the PLA content may increase at lower temperatures.

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Conflict of Interest
The authors have declared no conflict of interest.

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