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Extraction and Enrichment of n-3 Polyunsaturated Fatty Acids and Ethyl Esters through Reversible $\pi-\pi$ Complexation with Aromatic Rings Containing Ionic Liquids

Ling-Zhi Cheong, Zheng Guo, Zhiyong Yang, Seong-Chea Chua, and Xuebing Xu*

Department of Molecular Biology, Aarhus University, DK-8000 Aarhus, Denmark

ABSTRACT: The present study investigated the potentials of ionic liquids (ILs) containing aromatic rings in extracting and enriching n-3 polyunsaturated compounds. The relationship between extraction efficiency, selectivity, and structure of ILs was studied and elucidated. Ionic liquids containing aromatic rings such as the *N*,*N*′-dialkylimidazolium and *N*-alkylpyridinium are found to selectively extract and enrich n-3 polyunsaturated compounds. The IL extraction process reached equilibrium relatively quickly (<30 min), and the extraction efficiency can be further enhanced by increasing the volume ratio of IL to solvent, addition of silver tetrafluoroborate, and usage of double bond containing solvents such as 1-hexene as stripping solvent. At optimal conditions, 1-butyl-3-methylimidazolium hexafluorophosphate [bmim]PF_6 and 1-butyl-3-methylpyridinium dicyanamide [BuMePyr]DCN had increased extraction capabilities of n-3 polyunsaturated fatty acids (PUFA) (16.19%) and n-3 polyunsaturated fatty acid ethyl esters (PUFAEE) (144.54%), respectively. Multiple-step reverse extraction whereby n-3 PUFAs were first enriched through IL/hexane extraction followed by addition of fresh hexane repeatedly up to three times to the IL phase to desorb the saturated FAs resulted in a higher purity of n-3 PUFAs. Following this reverse extraction operation, the purity of n-3 PUFA and n-3 PUFAEE in the IL phase increased from the initial 15.88 to 38% (step 1) and further to 45% (step 3) and from the initial 72.56 to 82% (step 1) and further to 89% (step 3), respectively. The results from this study strongly suggested the correlation of n-3 PUFA extraction with aromatic/delocalized cation structure of ILs, which provides the basis for future research in designing novel ILs task-specified for efficient long-chain PUFA concentration.

KEYWORDS: ionic liquids, n-3 polyunsaturated fatty acids (n-3 PUFAs), reverse extraction, aromatic,

INTRODUCTION

Marine omega-3 polyunsaturated fatty acids (n-3 PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have long been recognized for their role in reducing the risk of heart disease and inflammatory disease and ameliorating brain function and mental health.[1] Although n-3 PUFA can be synthesized endogenously from α-linolenic acid (ALA), this biosynthetic pathway has been shown to be inefficient.[2] Thus, it is recommended that these healthful n-3 PUFA be obtained through dietary means. The Food Standards Agency (FSA) has recently recommended a daily consumption of 0.9 g of n-3 PUFA for the general population.[3] This has led to increased interest in producing n-3 PUFA concentrates.

At present, there are a number of different methods to produce n-3 PUFA concentrates, namely, enzymatic concentration,[4] urea inclusion complexation,[5] low-temperature fractional crystallization,[6] liquid−liquid extraction,[7] and supercritical fluid extraction. Some of the aforementioned methods, particularly liquid−liquid extraction, involve the usage of water-immiscible organic solvents, which are toxic, flammable, or volatile. In addition, usage of these solvents will eventually raise the overall production cost due to the ever-rising waste disposal cost. Coupled with the growing awareness of "green" manufacturing and processing methods, research on replacement of toxic solvents with less noxious but equally efficient alternatives is desirable.

One such alternative is ionic liquids (ILs). ILs are organic salts comprising a bulky asymmetric cation in combination with any of a wide assortment of anions. Some of the frequently used cations include *N*,*N*′-dialkylimidazolium, *N*-alkylpyridinium, quaternary ammonium, and alkylphosphonium. Meanwhile, tetrafluoroborate and hexafluorophosphate are some of the preferred anions.[8] The high degree of asymmetry in ILs frustrates packing and, hence, inhibits crystallization. In addition, they have a near absence of vapor pressure. Thus, ILs are able to remain in the liquid state over an incredibly large temperature range, from below ambient to well over 300−400 °C. Hence, they are known as "green" solvents. Another interesting feature of ILs is the extraordinary tunability of their physical and chemical properties. For example, the choice of cation and anion can affect the solute's solubility in ILs. Cations with increasing alkyl chain length have increased hydrophobicity and, thus, are more efficient in dissolving hydrophobic materials.[9] These features provide a good basis for ILs to be used as a replacement for toxic solvents in extraction processes. In fact, ILs have been employed as "green" and environmentally benign solvents in the extraction of various simple organic compounds,[10,11] biomolecules,[12,13] and metal ions.[14]

ILs, particularly those with cationic aromatic rings, contain delocalized π electrons and hence are strong π electrons acceptor. They form $\pi-\pi$ complexation with electron donors

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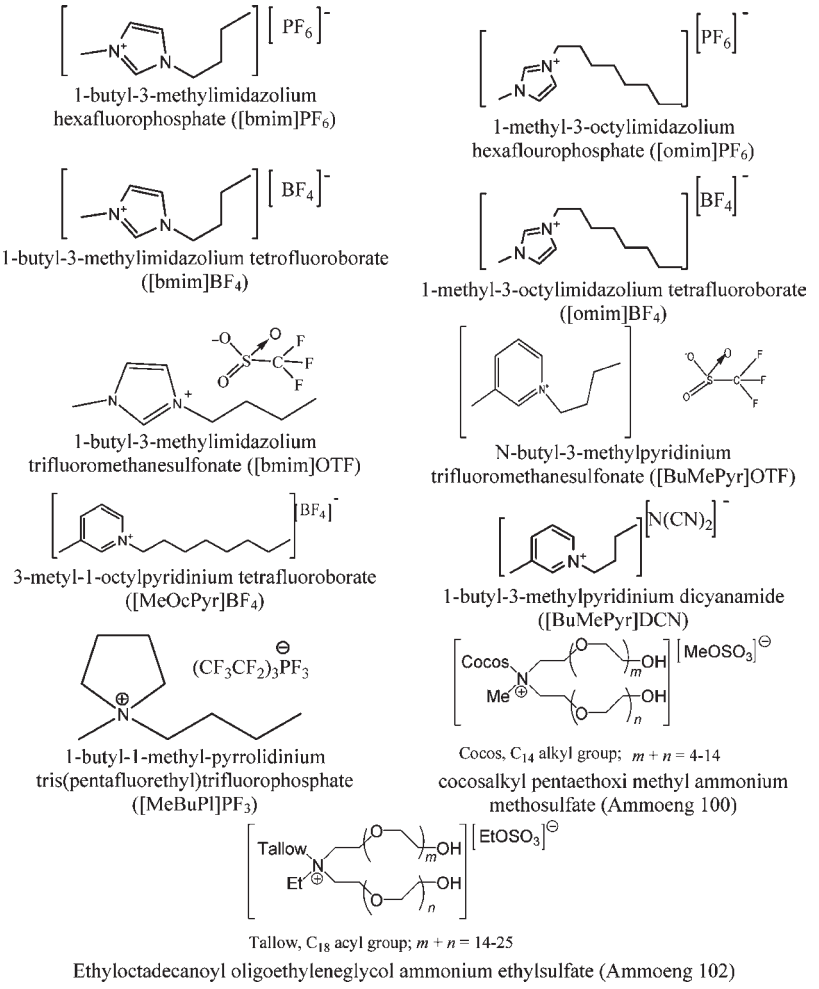

Figure 1. Structures of the ILs used in extraction of n-3 PUFA and n-3 PUFAEE.

such as double bond containing compounds, resulting in a charge transfer complex. The $\pi-\pi$ complexation is weak and thus can be easily reversed to release the initial compounds. This concept can be employed in selective extraction and enrichment of n-3 PUFAs from a bulk lipidic phase. When added to a bulk lipidic phase, ILs with aromatic rings may selectively attract n-3 PUFAs into the IL phase by forming π bonds with the n-3 PUFA. Following that, a stripping solvent can be used to break such bondings to release the n-3 PUFAs. In fact, this concept has been employed for selective extraction and enrichment of n-3 polyunsaturated fatty acid methyl esters (n-3 PUFAME)[15−17] and also polyunsaturated triacylglycerols (TAG).[18] In these studies, silver salt is added to strengthen π-complexing capacity to improve the extraction and enrichment capacities of n-3 PUFAs. However, in addition to being pricey, silver salts are toxic and corrosive, which is not suitable for food application.

Thus, the present study aims to investigate the possibility of solely employing ILs without silver salt in extracting and enriching n-3 PUFA and n-3 polyunsaturated fatty acids ethyl esters (n-3 PUFAEE). A variety of ILs with (imidazolium and pyridinium) and without (pyrrolidinium and tetrammonium) aromatic structure were investigated to examine the dependency of extraction efficiency and selectivity on IL structures (cation, anion, and substituents). The extraction processing conditions, namely, dissolving and stripping solvents, amount of extractant that is IL in the present case, and extraction duration, are also evaluated. This study also introduced a novel multiple-step reverse extraction concept where fresh solvents are added to the IL phase from the extraction to desorb the saturated FAs from the IL phase, which resulted in further enrichment of n-3 PUFAs.

MATERIALS AND METHODS

Materials. ILs 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim]PF_6), 1-methyl-3-octylimidazolium hexafluorophosphate ([omim]-PF_6), 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim]BF_4), 1-methyl-3-octylimidazolium tetrafluoroborate ([omim]BF_4), 1-butyl-3-methylpyridinium dicyanamide ([BuMePyr]DCN), *N*-butyl-3-methylpyridinium trifluoromethanesulfonate ([BuMePyr]OTF), 3-methyl-1-octylpyridinium tetrafluoroborate ([MeOcPyr]BF_4), cocosalkyl pentaethoxymethyl ammonium methylsufate (Ammoeng 100), and tetraalkylammonium sulfate (Ammoeng 102) were obtained from Solvent Innovation GmbH, Germany. ILs 1-butyl-3-methylimidazolium trifluoromethanesulfonate ([bmim]OTF) and 1-butyl-1-methylpyrrolidinium tris(pentafluoroethyl)trifluorophosphate ([MeBuPl]PF_3) were obtained from Sigma Aldrich and Merck, respectively. Figure 1 shows the structures of the aforementioned ILs. Salmon oil from byproduct of farmed salmon containing approximately 15% of n-3 PUFA was provided from Marine Bioproducts A/S, Norway. Fish oil ethyl esters (EPQX 6000EE) with 72.56% n-3 PUFAEE was procured from EPAX, Norway, and used as received. Lipase Novozym 435 (*Candida antarctica* lipase B immobilized

on a macroporous acrylic resin) was kindly donated by Novozymes A/S (Bagsvaerd, Denmark). Boron−trifluoride−methanol complex solution (Sigma-Aldrich), diethyl ether (Sigma-Aldrich, 98%), heptane (Sigma-Aldrich, 99%), hydrochloric acid, methanol (Sigma-Aldrich, 99%), 1-hexene (Fluka, 96%), potassium hydroxide (Merck, 96%), silver tetrafluoroborate (Sigma-Aldrich, 99%), and sodium hydroxide (Merck, 96%) were used as received.

Hydrolysis of Salmon Oil and Purification of the Marine-Based Free Fatty Acids (FFA). Salmon oil containing 15.88% of n-3 PUFAs was hydrolyzed at a temperature of 65 °C using lipase in phosphate buffer (pH 7.0) to obtain the FFA for use in extractions. First, 100 g of salmon oil was mixed with 100 mL of 10 mM phosphate buffer in a 500 mL brown flask. The reaction mixture was then heated to a temperature of 65 °C in a water bath. The hydrolysis was initiated by adding in 25 g of lipase Novozym 435. After 24 h of reaction time, the reaction was stopped by filtering out the lipase. The aforementioned reaction conditions are based on previous knowledge to obtain a complete or nearly complete hydrolysis for high yield of marine-based FFA. The degree of salmon oil hydrolysis in present study is 90%.

As the final reaction product contained a mixture of FFA, monoacylglycerol (MAG), diacylglycerol (DAG), and triacylglycerol (TAG), purification of the marine-based FFA fraction is required. First, 50 mL of 0.5 M ethanolic potassium hydroxide was added to the final reaction product. Following that, the glycerides fraction was extracted twice by using 100 mL of hexane. The remaining marine-based FFA fraction contained in the water phase was then acidified by adding 25 mL of 2 M hydrochloric acid. Finally, the marine-based FFA fraction was extracted twice by using 100 mL of hexane. The hexane phase containing marine-based FFA was dried by activated molecular sieves, and the FFAs were obtained after evaporation of solvent. Despite the high degree of salmon oil hydrolysis, up to 90%, significant FFA losses are encountered during the purification process. A total of 60 g of marine-based FFA per 100 g of salmon oil was obtained at the end of the purification process.

Ionic Liquid Extractions of n-3 PUFA and PUFAEE. Ionic liquid extractions of n-3 PUFA and PUFAEE were performed in 2 mL tubes, which were vibrated at room temperature using a microtiter shaker (IKA, GmbH). First, 100 μL of either marine-based FFA or fish oil ethyl esters was dissolved in 200 μL of hexane in the 2 mL tubes. Two hundred microliters of ILs was then added into the tubes. Two separate phases can be observed. The tubes were then vibrated at room temperature using a microtiter shaker at a speed of 1100 rpm for 30 min. At the end of the extraction duration, the tubes were centrifuged at 12000 rpm for 10 min and left standing for 15 min for phase separation. The upper hexane phase was removed and methylated directly for analysis of fatty acid composition using gas chromatography. To strip the fatty acid or ethyl esters from the IL phase, 200 μL of diethyl ether was added to the lower IL phase. This mixture was then vibrated at room temperature using a microtiter shaker at a speed of 1100 rpm for 15 min. Following that, the tubes were centrifuged at 12000 rpm for 10 min and left standing for 15 min for phase separation. The upper diethyl ether phase was sampled and methylated for analysis of fatty acid composition. The stripping fatty acids or ethyl esters procedure was carried out twice. All of the ionic liquid−solvent extractions of n-3 compounds were carried out in similar manner unless otherwise specified.

For screening of volume ratio of ILs to hexane, four different ratios (1:1, 1.5:1, 2:1, and 2.5:1) were used. Four different extraction durations (30, 45, 60, and 75 min) were also studied. Besides diethyl ether, 1-hexene was also employed as a stripping solvent. In the study to investigate the effect of silver salt addition, approximately 20−25 mg of silver tetrafluoroborate was first dissolved in the IL phase prior to the start of the extraction process. In reverse extraction procedure, instead of stripping the FAs or ethyl esters after the first extraction step, fresh hexane (200 μL) was added to the IL phase and the extraction process continued for another 30 min. This procedure was repeated three times. Following the reverse extraction process to desorb the saturated FAs from the IL phase, the n-3 PUFA or n-3 PUFAEE were stripped from the IL phase using stripping solvent and methylated for fatty acid composition analysis.

Determination of Fatty Acid Composition. Fatty acid composition was determined according to AOCS official method Ce 2-66.[19] The marine-based FA and ethyl esters were first added to 1 mL of 0.5 M methanolic sodium hydroxide. The resultant mixture was then refluxed for 10 min at 80 °C. Once the mixture was sufficiently cooled to room temperature, 1 mL of 20% boron−trifluoride−methanol complex solution and 0.2 mL of 0.1% hydroquinone in methanol were added to the mixture. This solution was then refluxed for 5 min at 80 °C. Following cooling to room temperature, 1 mL of heptane and 0.2 mL of salt solution (sodium chloride and potassium carbonate in deionized water) were added and mixed. The solution was then dried with sodium sulfate anhydrous and centrifuged. Finally, the supernatant was removed and analyzed for fatty acid composition.

Fatty acid composition was determined using a Thermo Scientific Trace GC Ultra gas chromatograph equipped with a flame ionization detector and a Supelco OmegaWax 320 fused silica capillary column (30 m × 0.32 mm × 0.2 μm film thickness). Helium was used as a carrier gas at a flow rate of 1 mL/min. The injector and detector temperatures were set at 250 and 270 °C, respectively. The oven temperature was initiated at 170 °C. Then, the oven temperature was increased to 215 °C at 1 °C/min. The final temperature of 215 °C was held for 20 min. The total run time was 65 min. The peaks were identified by comparison of retention time with fatty acid methyl ester standard. All measurements were conducted in duplicate.

The distribution coefficient is defined as the ratio of the weight percent (W) of the solute in the IL phase and the solvent phase. Thus, the distribution coefficient for the n-3 compounds and non n-3 compounds can be calculated according to the following equations:

$$D_{\text{n-3 compounds}} = \frac{W_{\text{IL}}^{\text{n-3 compounds}}}{W_{\text{solvent}}^{\text{n-3 compounds}}} \qquad D_{\text{non n-3 compounds}} = \frac{W_{\text{IL}}^{\text{non n-3 compounds}}}{W_{\text{solvent}}^{\text{non n-3 compounds}}}$$

The IL selectivity of n-3 compounds over non n-3 compounds is defined as the ratio of the distribution coefficients (D) of n-3 compounds and non n-3 compounds. It can be calculated according to the following equation:

$$\text{selectivity} = \frac{D_{\text{n-3 compounds}}}{D_{\text{non n-3 compounds}}}$$

The extraction capability of the IL is defined as the percentage increment of the n-3 compounds in IL phase as compared to the n-3 compounds in the unextracted FA or ethyl esters.

$$\begin{aligned}\text{extractioncapability} = [&(\text{wt \% of n-3 compounds in IL}\\ &- \text{wt \% of n-3 compounds in unextracted fraction})\\ &/\text{wt \% of n-3 compounds in unextracted fraction}] \times 100\end{aligned}$$

RESULTS AND DISCUSSION

Fatty Acid Compositions of the Salmon Oil Fatty Acids and Fish Oil Ethyl Esters. Table 1 shows the fatty acid compositions of the salmon oil fatty acids and fish oil ethyl esters. The salmon oil fatty acids contained 15.88% of n-3 PUFA (C18:3, C20:3, C20:4, C20:5, C22:5, C22:6); meanwhile, the fish oil ethyl esters contained a high amount, 72.56%, of n-3 PUFAEE (C18:3, C20:3, C20:4, C20:5, C22:5, C22:6). Unlike n-3 PUFA, n-3 PUFAEE contain an ethyl group attached to the carboxylic group of the fatty acids. Both n-3 PUFA and n-3 PUFAEE

Table 1. Fatty Acid Composition of Purified Salmon Oil Fatty Acids and Fish Oil Ethyl Esters

fatty acid	salmon oil fatty acids	fish oil ethyl esters
C14:0	5.68	0.25
C14:1	0.22	0.02
C16:0	17.78	1.53
C16:1	5.87	0.70
C18:0	3.28	4.38
C18:1	33.14	8.96
C18:2	10.41	0.82
C18:3 (n-3)	3.69	0.48
C20:0	0.00	0.95
C20:1	3.58	2.82
C20:2	0.37	0.41
C20:3 (n-3)	0.26	2.29
C20:4 (n-3)	0.00	0.14
C20:5 (n-3)	6.30	41.14
C22:1	2.24	0.52
C24:0	1.55	6.00
C24:1	0.00	0.08
C22:6 (n-3)	2.84	27.90
C22:5 (n-3)	2.79	0.61
total	100	100
total n-3 fatty acids	15.88	72.56

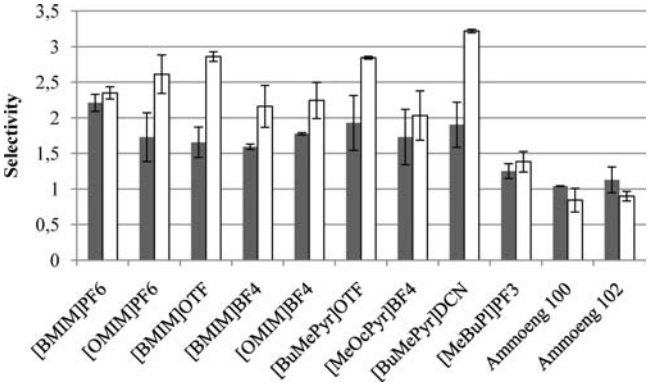

Figure 2. Selectivity of different ILs for n-3 PUFA (white bars) and n-3 PUFAEE (gray bars).

contain double bonds in their structures. The presence of the double bonds in their structures enables the n-3 PUFA and n-3 PUFAEE to form reversible π-bonds with the delocalized electrons of the aromatic rings containing ILs.

Dependency of Extraction Performance on IL Structures. Figures 2 and 3 show the selectivity and extraction capability of different ILs for n-3 PUFA and n-3 PUFAEE, respectively. Figure 2 shows imidazolium- and pyridinium-based ILs had a selectivity of more than 1.5 and 2.0 for extraction of n-3 PUFAs and n-3 PUFAEEs, respectively. Meanwhile, pyrrolidinium- and tertraammonium-based ILs ([MeBuPl]PF_3, Ammoeng 100, Ammoeng 102) had a selectivity of less than 1.5 for both n-3 PUFAs and n-3 PUFAEE. Similar findings can be observed from Figure 3 in which both pyrrolidinium- and tertraammonium-based ILs exhibited significantly ($P < 0.05$) lower extraction

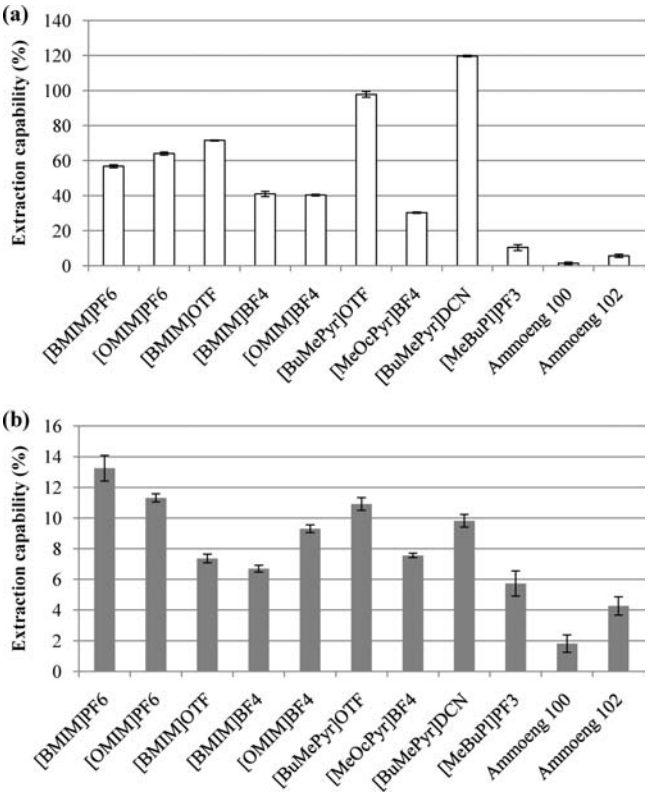

Figure 3. Extraction capability of different ILs for (a) n-3 PUFA (white bars) and (b) n-3 PUFAEE (gray bars).

capability for n-3 PUFAs (<20%) and n-3 PUFAEEs (<6%) as compared to imidazolium and pyridinium-based ILs. On the basis of these findings, the conclusion can be drawn that a strong correlation exists between the enrichment efficiency of n-3 PUFAs and the aromatic structure of ILs. ILs containing aromatic structure are able to form temporary weak $\pi-\pi$ interactions with polyunsaturated bonds in n-3 PUFA and n-3 PUFAEE, which resulted in enrichment of such compounds in the IL phase. In the present case, it is found that ILs with cations containing aromatic rings, namely, the *N*,*N*′-dialkylimidazolium ([bmim]PF_6, [omim]PF_6, [bmim]BF_4, [omim]BF_4, and [bmim]OTF) and *N*-alkylpyridinium ([BuMePyr]DCN, [BuMePyr]OTF, and [MeOcPyr]BF_4) types have higher selectivity and extraction capability for n-3 PUFA and n-3 PUFAEE as compared to those without aromatic structure. Besides that, the alkyl chain length of the cation of ILs is found to have a positive effect on the selectivity and extraction capability of n-3 PUFA and n-3 PUFAEE. Increasing the alkyl chain length from C_4 to C_8 resulted in higher selectivity and extraction capability of the aromatic rings containing ILs with the exception of *N*,*N*′-dialkylimidazolium PF_6 ILs. [bmim]PF_6 had higher selectivity and extraction capability for n-3 PUFA as compared to [omim]PF_6. This finding is similar to those by Li and Li.[14] In short, it is difficult to conclude which cation had better selectivity. This is because other aspects of the IL structures such as anions also have some effects on the interaction between IL and extract molecules.

Comparison between ILs with the same cation but different anions shows anions play important roles in extraction capability and selectivity for n-3 PUFA and n-3 PUFAEE (Figures 2 and 3). However, it is difficult to draw unambiguous conclusions

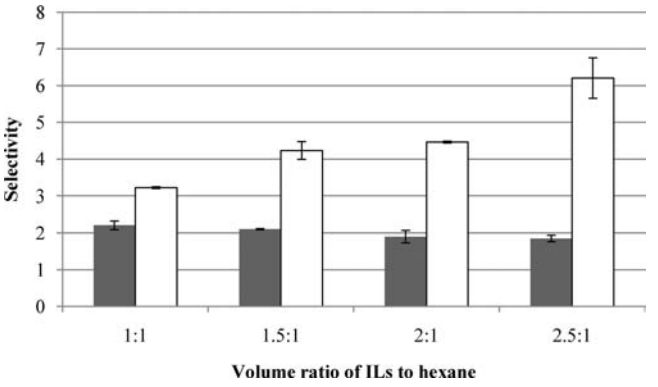

Figure 4. Effect of volume ratio of IL to hexane on selectivity for n-3 PUFA (white bars) and n-3 PUFAEE (gray bars).

regarding their effects either by polarity or ion size. For example, for [bmim]-type ILs, the polarity ($OTF^- > BF_4^- > PF_6^-$) or molecular size ($OTF^- > PF_6^- > BF_4^-$) cannot be correlated with selectivity ($PF_6^- > OTF^- > BF_4^-$) or extraction capability ($PF_6^- > OTF^- > BF_4^-$) for n-3 compounds. Ionic liquids have been identified as associated nonmolecules between ionic salts and molecular solvents, which show multiple interactions simultaneously. Anions contribute to the property of ILs including total polarity of IL, packing pattern, and viscosity. Good selectivity and extraction capability of [BuMePyr]DCN might be ascribed to smaller ionic size (CN)2N− (small hindrance for bigger n-3 PUFA access to aromatic ring) and lower viscosity (faster mass transfer).

On the basis of the above findings, the 119.7% increment in n-3 PUFA by [BuMePyr]DCN and 13.5% enhancement in n-3PUFAEE by [bmim]PF_6 obtained, respectively, are selected for the following studies with different extraction conditions. The lower increment of n-3 PUFAEE may probably be due to the fact that the starting material of ethyl esters used in the present study has a high concentration of 72.6% of n-3 PUFAEE as compared to the starting material of fatty acids, which contained 15.8% of n-3 PUFA. The IL may have already reached their maximum π-bonding and loading capacity; hence, the lower extraction efficiency was obtained (Figure 3b).

Effects of Operation Variables. Extraction conditions, namely, volume ratio of extractants (ILs) to dissolving solvent (hexane), presence or absence of silver salt, stripping solvents, and extraction duration, are studied with the selected ILs for the possibility of further omega-3 enrichment. The volume ratio of ILs to hexane is found to have a positive effect on selectivity (Figure 4) and extraction capability of n-3 PUFAs (Figure 5). Increasing the ratio of ILs to hexane from 1:1 to 2.5:1 resulted in increased extraction capability of n-3 PUFA from 119 to 152%. Nevertheless, this effect is not seen with n-3 PUFAEE. The increase in volume ratio of ILs to hexane did not result in increased extraction capability of n-3 PUFAEE. This indicates ILs have a maximal π-bonding and loading capacity. Once this capacity has been reached, further increment in the volume ratio of ILs will not enhance the selectivity and extraction capability of the IL. As the increment in extraction capability is not significant, the volume ratio of ILs to hexane is fixed at 1:1 in the following study.

Several studies[14−17] have shown that the presence of salt in the ionic liquid−solvent extraction system had positive effects on selectivity and extraction capability of omega-3 compounds. The

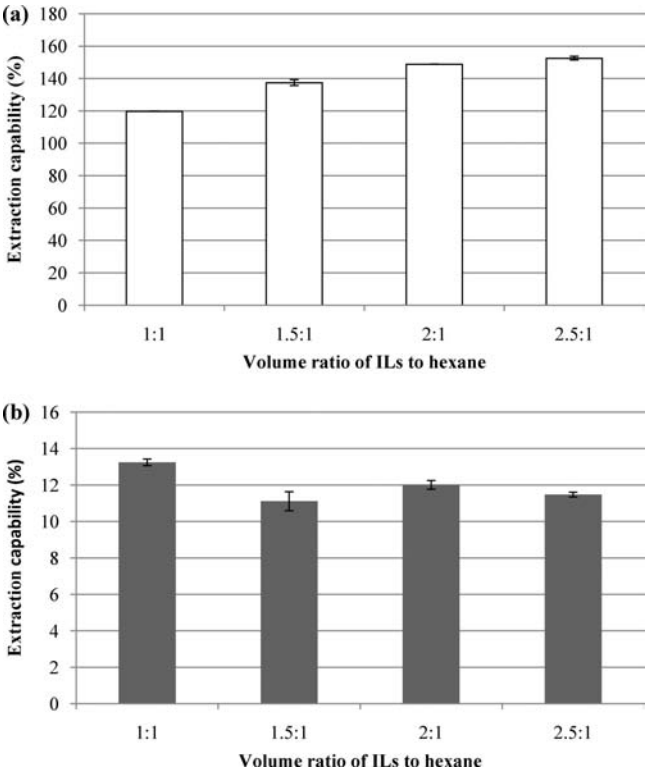

Figure 5. Effect of volume ratio of IL to hexane on extraction capability for (a) n-3 PUFA (white bars) and (b) n-3 PUFAEE (gray bars).

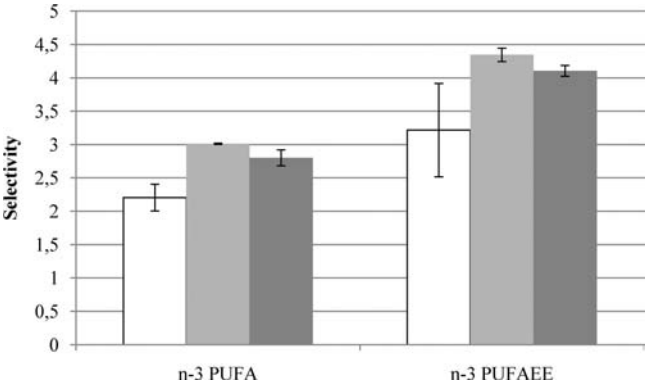

Figure 6. Effect of salt addition and stripping solvent on selectivity of IL for n-3 PUFA and PUFAEE [(white bars) without addition of salt and diethyl ether as stripping solvent; (light gray bars) with addition of salt and 1-hexene as stripping solvent; (dark gray bars) with addition of salt and diethyl ether as stripping solvent].

present study had a similar finding; however, the effect was not as significant as reported.[14−17] In the presence of silver salt, IL demonstrated increased selectivity (Figure 6) and extraction capability. Stripping solvent is equally important to obtain the extracted fatty acids fraction from the IL phase. A double bond containing stripping solvent, namely, 1-hexene, is more effective in stripping the unsaturated compounds from the ILs as compared to diethyl ether. It is possible that 1-hexene with its double bond is able to form π-bonding with the ILs, resulting in dispelling of the unsaturated compounds from the IL phase into the stripping solvent phase. In fact, Li and Li[14] found diethyl

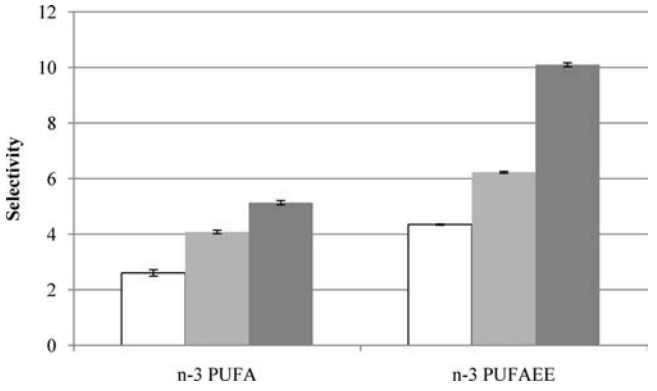

Figure 7. Effect of multiple extraction on IL selectivity for n-3 PUFA and PUFAEE [(white bars) step 1 in multiple extraction; (light gray bars) step 2 in multiple extraction; (dark gray bars) step 3 in multiple extraction.

ether to be more effective in stripping and concentrating the saturated FAs. In the presence of silver salt and 1-hexene as stripping solvent, [bmim]PF_6 and [BuMePyr]DCN had increased extraction capability to 16.19% (n-3 PUFAEE) and 144.54% (n-3 PUFA), respectively. As previously discussed, [bmim]PF_6 showed a smaller increment in extraction capacity as it may have reached its maximal π-bonding and loading capacity.

In terms of extraction duration, the present study found ionic liquid−solvent extraction of n-3 compounds to be a relatively fast technique. In fact, it is found that extraction equilibrium had been reached in <30 min. Increasing the extraction time to 75 min had no significant effects on selectivity and extraction capability of ILs (data not shown). Previous studies[14−17] had also reported similar findings in which extraction equilibrium can be reached reasonably quickly in <30 min. Thus, the optimized ionic liquid−liquid extraction systems composed of either [bmim]PF_6 or [BuMePyr]DCN at 1:1 volume ratio of ILs to hexane were used in the following study. Both systems were added with salt and extracted for 30 min. 1-Hexene was finally used to strip the n-3 compounds from the IL phase.

Multiple-Step Reverse Extraction. To obtain a high recovery of the omega-3 compounds, we tested a new concept of multiple-step reverse extraction. The idea is n-3 PUFAs together with some saturated FAs are first extracted in IL phase through specific $\pi-\pi$ interaction with the cationic aromatic ring in the IL. A saturated alkane solvent such as hexane is then added to the omega-3 compound rich IL phase to desorb the saturated FAs, hence increasing the overall omeaga-3 purity of the end product. This reverse extraction operation will also improve the cost efficiency of using ILs for extraction, thus enhancing its feasibility for practical application. Different from previous work by the increased use of absorbents,[16,17] the present study added hexane repeatedly up to three times to desorb the saturated FAs from the IL phase. This resulted in a higher purity of n-3 PUFAs in the IL phase and final stripping solvent phase. Figure 7 shows the effect of reverse extraction on IL selectivity for the n-3 PUFA and n-3 PUFAEE. Reverse extractions are found to increase significantly ($P < 0.05$) the selectivity of IL toward n-3 compounds. By replacing the solvent phase with fresh hexane, the purity of n-3 PUFA and n-3 PUFAEE in the IL phase increased from 38% (step 1) to 45% (step 3) and from 82% (step 1) to 89% (step 3), respectively. The possible reason for this further enrichment may be the saturated FAs; unlike unsaturated FAs, which formed π-bonding with the IL phase, saturated FAs were weakly bonded to the IL. Addition of fresh hexane enabled these saturated FAs to partition into the hexane phase, leading to higher n-3 PUFA purity in the IL phase.

In conclusion, the present study showed ILs containing aromatic rings, namely, the imidazole and pyridinium types, selectively extracted and enriched the healthful n-3 PUFA and n-3 PUFAEE by forming reversible π-bonding with the double bonds of these compounds. Hydrophobic anion and long alkyl chain length of ionic liquids profoundly influence the extraction performance of ILs. This process reached equilibrium relatively quickly and can be further enhanced by increasing the volume ratio of IL to solvent, addition of silver tetrafluoroborate, and usage of double bond containing solvents such as 1-hexene as stripping solvent. At optimal condition, [bmim]PF_6 and [BuMePyr]DCN had an increased extraction capability of n-3 PUFA (16.19%) and n-3 PUFAME (144.54%), respectively. In addition, a multiple-step reverse extraction process can be carried out repeatedly for further enhancement of the n-3 PUFA and n-3PUFAEE. Repeated addition of fresh hexane to the IL phase is found to strip the weakly bonded saturated FAs from the IL phase. Following three steps of repeated extraction, the purity of n-3 PUFA in the IL phase is enhanced from the initial 15.88 to 38% (step 1) and further to 45% (step 3). Meanwhile, the purity of n-3 PUFAEE in the IL phase is enhanced from the initial 72.56 to 82% (step 1) and further to 89% (step 3). Despite the usage of solvents, namely, hexane and diethyl ether, in the present study, the amount of solvent used in ionic liquid extraction of omega-3 compounds is significantly less than those used, for example, in liquid−liquid extraction. According to the U.S. Food and Drug Administration, the presence of trace amounts of hexane (class 2 solvent) and diethyl ether (class 3) solvents poses minimum health hazard.[20] Although ionic liquids are still quite pricey at present, the short processing time (30 min), possibility of recyclability, and simplicity of the procedure resulted in cost efficiency as compared to methods such as low-temperature fractionation and urea inclusion complexation, which require slow cooling for an extended period of time. Thus, ionic liquid extractions of n-3 PUFA and PUFAEE may be a potential alternative to the currently available method.

AUTHOR INFORMATION

Corresponding Author

*Postal address: Department of Molecular Biology, Aarhus University, Gustav Wieds Vej 10, DK-8000 Aarhus C, Denmark. Phone: +45 8942 5089. Fax: +45 86123178. E-mail: xu@mb.au.dk.

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