Research Article

Separation and Enrichment of Omega 3, 6, and 9 Fatty Acids from the By-Products of Vietnamese Basa Fish Processing using Deep Eutectic Solvent

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Omega 3, 6, and 9 fatty acids were separated and enriched successfully from the by-products of Vietnamese Basa fish processing by the deep eutectic solvent. The total amounts of omega fatty acids were about 57% in the raw material, and they were amounted to 91% after the first separation by DES. The optimal mass ratio is 20 g methyl ester with 200 g methanol and 15–20 g DES. Moreover, the ionic liquid-DES was successfully synthesized with the molar ratio of choline chloride/urea of 1 : 1 and 2 : 1. The characteristics of DES were determined and demonstrated by FTIR, TGA, DSC, ¹H-NMR, and ¹³C-NMR analysis methods.

1. Introduction

The scientific name of Basa fish is *Pangasius bocourti*, a catfish in the family *Pangasiidae* of high economic value, raised in many countries around the world. This species is native to the Mekong Delta in Vietnam and to the Chao Phraya River basin in Thailand [1]. This fish is an important food in the international market. In the Mekong Delta, catfish provide for essential demands over the country, and they are exported to other countries. It is promoting the development of Vietnamese fisheries industry. Average annual production of Vietnamese Basa-catfish from 2013 to 2017 is from 1.1 to 1.2 million tons. However, the main part of exporting fish is fillet meat and the other parts such as head, bone, fat, and skin (total ~60–65%) are still not fully utilized. The by-products of Basa-fish processing including viscera, skin, scales, bones, and skeletons are becoming a major problem for industries, causing environmental pollution and the loss of large quantities of high-value nutrients such as omega 3, 6, and 9 unsaturated fatty acids.

Our studies [2] show that the by-product of Basa-fish processing is 59%. Thus, there will be about 65–70 thousand tons of waste each year. Of which, 8.4% are fatty acids, equivalent to 546.000–588.000 tons. Omega 3, 6, and 9 fatty acids of by-product are 57%, meaning that 300.000–335.000 tons of omega 3, 6, and 9 annually are not used properly for its role.

Omega 3, 6, and 9 play an important role and is necessary for the human body. The body cannot self synthesis these omegas which can prevent cardiovascular diseases, cancer, lower blood pressure, lower cholesterol, and triglycerides in blood, brain accretion, and cardiac regulation and prevent thrombosis [3, 4].

The production process of omega 3, 6, and 9 has been done by many methods as distillation in high temperature to give the useful compounds [5], crystalline at low temperature in ethanol, acetone or solvent mixtures [6], the supercritical fluid used as carbon dioxide (CO₂) at 31.1°C and 72.9 atm to achieve the all of fatty acids [7, 8]. Silver-coated chromatography and high-performance chromatography methods

may isolate polyunsaturated fatty acids [9–11], using the complex of urea is the method to concentrate polyunsaturated fatty acids from waste of poultry and fish [12–15].

Deep eutectic solvents (DESs) are now widely acknowledged as a new class of ionic liquid analogues due to their characteristics and properties that are similar to ionic liquids. DESs show great promise as green solvents because they have the important properties such as biodegradability, low toxicity, and easy preparation [16]. DES was successfully used to efficiently extract baicalin from *Scutellaria baicalensis* Georgi by Wang et al. [17]. In this work, we introduce some initial results when using DES to separate and enrich omega 3, 6, and 9 in the fat of Vietnam's Basa fish processing waste.

2. Materials and Methods

2.1. Materials. The ionic liquid-DES is synthesized from the choline chloride and urea that were supported by Acros Organics Company.

A volume 1750 g of Basa fish was processed according to the exporting fish processing method. The average percentages of the fat, fillet meat, and by-products are shown in Table 1. In which, the by-product was dominant (58.8%) and was used to separate and enrich the omega 3, 6, and 9.

2.2. Synthesis of the Ionic Liquid-DES

2.2.1. Sample Preparation. The general procedure to synthesize ionic liquid-DES based on choline chloride and urea [18, 19] as follows: choline chloride and urea are mixed together at a molar ratio of 1 : 1 (and 2 : 1) in a flask fitted with a heating element and magnetic stirrer. The mixture was heated to 60°C for 5 to 7 minutes until it becomes homogeneous. Cooling at room temperature to prevent the crystallization occurs. When changing the molar ratio between choline chloride and urea to 1 : 2 and performing the same as described above, then cooling the reaction mixture down to room temperature, the mixture rapidly crystallizes into solids.

2.2.2. DES's Characteristics. The used analysis methods include FTIR on EQUINOX 55 (Bruker), frequency from 400 to 4000 cm^{-1} , KBr template, the NMR technology by Bruker AC 500, and TGA-DSC by LABSYS evo SETARAM equipments. Viscosity is determined by DV III Ultra (Brookfield). Water concentration is determined by SCHOTT Instruments TitroLine KF trace.

2.3. Omega 3, 6, and 9 Separation and Enrichment

2.3.1. Sample Preparation. The by-product was added with 2 liters of water and boiled for 30 minutes. The mixture was allowed to cool down to 4°C. The mixture was separated into two parts after cooling. The above part is a white grease layer that was separated and weighed 86.10 g. The rest was filtered, dried at 45–50°C in vacuum, and weighed 942.80 g. The fatty acid was extracted from both above parts by methanol and n-hexane. In 86.6 g of the grease, the obtained result was 75.175 g of fatty acid, corresponding to a yield of 87.3%. In

TABLE 1: Parts of catfish details.

No.	Products	Weight (g)	Percentage (%)
1	Fat	106.01	6.057
2	Fillet meat	615.00	35.142
3	By-products	1028.99	58.800
4	Error		0.001

942.80 g of the extracted solution, the fatty acid was not found; i.e., there was not fatty acid in this section.

The obtained fatty acids were esterified by methanol with an FA : methanol ratio of 10 : 1. The mixture was heated to 60°C and stirred at 120 rpm for 3 hours. Next, the mixture was cooled down to room temperature. Then, the ester products were separated and washed several times. Finally, Na_2SO_4 was used to remove water from the methyl ester of FA. The final products were labeled “methyl esters.” The chemical compositions of methyl esters were analyzed by the GC-FID method.

2.3.2. Omega 3, 6, and 9 Separation and Enrichments from Methyl Esters by DES. Methyl ester, methanol, and DES with a ratio of 20 (g) : 200 (g) : m (g) were added to a 3-stage vial equipped with a backwash, heating element, and stirrer at 120 rpm and heated in a temperature of 45°C for 1 hour. The obtained homogeneous mixture was allowed to cool down to 4°C and then cooled at 4°C for 8 hours. The resulting mixture was divided into two layers: the upper layer was liquid and the bottom layer was the crystal solid. The crystal part was washed with cold methanol. The washed methanol was added into the liquid part above, then was evaporated methanol in a vacuum evaporator, dried by Na_2SO_4 , and analyzed the chemical compositions.

The crystal solid was added with 200 ml H_2O , boiled, and stirred until they dissolved and formed two layers. The upper layer contained the fatty acids. These FAs were separated and then removed water by Na_2SO_4 . Finally, the obtained fatty acids were analyzed for the chemical compositions by GC/FID method. The lower layer contained methanol, water, and DES. The methanol and water were removed by the evaporation in vacuum. DES rechecked the physical properties for reusable purpose. In this work, the mass of DES has been changed from 5 g to 10, 15, 20, 25, and 30 g.

2.4. Determination of Fatty Acids. The fatty acids were obtained from the by-products, and the unsaturated fatty acids by DES were analyzed by GC-FID method (GC-Agilent 6890N, flame ionization detector (FID), HP-INOWAX GC column: 30 m $\times$ 0.25 mm $\times$ 0.25 μm , the temperature range of 159–245°C; nitrogen).

3. Results and Discussion

3.1. Synthesis Ionic Liquid-DES

3.2.1. Synthesis DES Base on Choline Chloride and Urea. Both choline chloride and urea are soluble in water. The melting points of choline chloride and urea were at 302°C and 133°C, respectively. However, their mixture becomes a homogeneous solution as increasing the temperature to

60–75°C. The products of reaction depend on the mass ratio of choline chloride and urea. Moreover, some by-products such as ammonium cyanide, NH_3 , HOCN , and biurea can be formed at reaction temperature, as below:

Ammonium cyanide is destroyed, and NH_3 will be formed as follows:

In addition, two urea molecules can combine to form biurea [20]:

Therefore, the reaction temperature and mass ratios of choline chloride and urea must be selected to prevent the formation of by-product.

(1) *Choline Chloride : Urea (1 : 1) Solution.*

(2) *Choline Chloride : Urea (2 : 1) Solution.*

By-products in both cases are water, ammonium chloride, and ammonium hydroxide. These can be removed by heating in vacuum at low temperature (<60°C). Anionic NCO^- and cation choline combine by weak static bond, so

this mix will melt at low temperature and does not crystallize at room temperature (32–33°C).

(3) *Choline Chloride : Urea (1 : 2) Solution.*

TABLE 2: Physical index of DES solution at 30°C.

No.	Index	Choline chloride : urea ratio	
		1:1 ratio	2:1 ratio
1	pH	6.65	5.92
2	Density (g/ml)	1.197	1.167
3	Viscosity (cP)	61.5	57.3
4	Electronic conductivity (mS)	1.64	2.69
5	Water concentration (%)	0.65	0.81

FIGURE 1: FTIR of DES solutions were synthesized with the choline chloride/urea molar ratios: a-(1:1), b-(2:1), c-(1:2).

With 1 : 2 ratio of choline chloride : urea, the by-products include by-products in case of 1 : 1 ratio and isocyanic acid. This is the factor which changes products' comeback initial state and crystallize at room temperature.

Based on this initial survey, we choose DES solution with 1:1 and 2:1 ratio of choline chloride:urea to continue optimization to determine the best ratio.

3.2.2. Physical Properties of DES Solution. Physical properties of DES solution with choline chloride : urea ratio in 1 : 1 and 2:1 are shown in Table 2.

Electronic conductivity of urea in water is 0.01 mS, while electronic conductivity of choline chloride in water is 5.6 mS. After the reaction, electronic conductivity of solution decreases clearly. In case of 2:1 ratio, the higher value of electronic conductivity can be explained by the remaining quality of choline chloride after reaction. This result contributes in confirming the bond of choline chloride and urea

as diagram (1) and to form new product with different electronic conductivities. The conductivity of urea in water is 0.01 mS, while the conductivity of choline chloride in water is 5.6 mS. When creating new products, the conductivity decreases considerably, where a 2:1 solution has a greater electrical conductivity, it is explained by the excess choline chloride content, which results in higher conductivity of the solution. This result confirms that choline chloride is chemically related to urea according to the reaction scheme (1) and produces a new product with conductivity different from that of the original.

3.2.3. Analysis and Identification

(1) FTIR Analysis. Figure 1 shows the FTIR spectra of DES solutions that were synthesized with the molar ratio of choline chloride/urea: a-(1:1), b-(2:1), c-(1:2). In FTIR of solutions (a) and (b), vibrational frequencies at 3230–3550 cm^{-1} and 3445.63 cm^{-1} can be attributed to the

FIGURE 2: TGA of (a) choline chloride, (b) urea, and (c) choline chloride:urea 1:1.

FIGURE 3: DSC of (a) choline chloride, (b) urea, and (c) choline chloride:urea (1:1).

vibration of the hydrogen bond between OH groups with average intensity. The vibrational frequencies at $3180\text{--}3220\text{ cm}^{-1}$ and 3194.28 cm^{-1} are the frequencies of N-H in the amide group of urea. The vibrational frequencies at $1660\text{--}1705\text{ cm}^{-1}$ and 1661.19 cm^{-1} are the frequencies of C=O in urea. And the frequencies of $1590\text{--}1620\text{ cm}^{-1}$ and 1607.64 cm^{-1} indicate the average vibration of N-H in the amide group that combines with vibration of C-N in urea.

In $2800\text{--}3300\text{ cm}^{-1}$, the longer vibration of N-H bond in amide of urea and O-H shows that the amide group of urea bond to O-H of choline. The range from $2700\text{ to }3000\text{ cm}^{-1}$ and 2966.11 indicates the strong vibration of --NH_2 . The range from $2280\text{ to }3380\text{ cm}^{-1}$ is the average vibration of N-H bond in --NH_3^+ .

Especially, in the range of $2100\text{--}2225\text{ cm}^{-1}$, 2158.95 cm^{-1} contributes to vibration of $\text{N}\equiv\text{C-O}^-$ (cyanide ionic). Vibration at 629.28 cm^{-1} is also the strong vibration of NCO^- . In $1150\text{--}1210\text{ cm}^{-1}$, 1161.79 cm^{-1} , C-N bond vibrates with average intensity. The 1135.60 cm^{-1} , 1082.56 cm^{-1} , and

FIGURE 4: NMR spectrum of DES (1:1): ^1H NMR (a) and ^{13}C NMR (b).

1054.91 cm^{-1} positions are the little strong vibration of C-N in choline chloride.

The FTIR spectra of solutions show unclearly because of the crystallization. Results of chemical-physical properties and FTIR show that both solutions are the good ionic liquids.

(2) *TGA and DSC Analysis of DES (1:1)*. The clear difference between thermal gravity of choline chloride, urea, and product mixes is displayed in Figure 2. In $350\text{--}400^\circ\text{C}$ range, choline chloride (a) and urea (b) are both completely decomposed. But in their mix, at 250°C , there is fast changing mass including increasing and decreasing mass. After that, their mix's mass decreased slowly to 80°C . This demonstrates that between choline chloride and urea, there is bonding, not to mechanical mixing.

Similar to TGA analysis, DSC result of choline chloride, urea, and their products is also clearly different (Figure 3).

FIGURE 5: Chromatogram of GC/FID analysis of the fatty acids in methyl ester (a) and in methyl ester-DES (b).

Mix's crystallization temperature is about 300°C, while choline chloride crystallizes at 200°C and urea crystallizes at 100, 250, and 400°C.

(3) *NMR Analysis of DES (1:1)*. Figure 4 shows the NMR spectrum of DES solution that was synthesized with the molar ratio of choline chloride/urea: (1:1). Results are

shown as follows: (a) ^1H NMR (CDCl_3 , 500 MHz): 3.2 (1H), 3.3 (5H), 3.5 (5H), 5.4 (7H), 5.8 (1H), 5.7 (1H) and (b) ^{13}C NMR (CDCl_3 , 125 MHz): 58.1, 59.5, 63.0, 63.0, 118, and 163.5.

3.2. Determination of Fatty Acid in Samples. The composition of the crude materials was determined by GC/FID method (Figure 5(a)) and is shown in Table 3.

TABLE 3: The chemical compositions of fatty acids before separation and enrichment by DES.

No.	Fatty acids	Raw methanol extract (%)	Methyl ester (%)
1	Saturated fatty acids	31.37	35.58
2	Unsaturated fatty acid	2.85	3.35
3	Omega 3	2.00	1.84
4	Omega 6	16.44	14.48
5	Omega 9	40.71	40.63
6	Undefined	6.63	4.12
Total		100.00	100.00

TABLE 4: Classification of saturated fatty acids, unsaturated fatty acids, and omega in methyl ester.

Classification	Name of compounds	Percentage (%)
SFA	Myristic acid (C14:0)	1.96
	Palmitic acid (C16:0)	26.55
	Stearic acid (C18:0)	6.78
	Arachidic acid (C20:0)	0.29
USFA	Palmitoleic acid (C16:1)	3.35
OM-3	<i>cis</i> -5,8,11,14,17-Eicosapentaenoic acid (C20:5n3) (EPA)	0.42
	α -Linolenic acid (C18:3n3) (ALA)	0.46
	<i>cis</i> -11,14,17-Eicosatrienoic acid (C20:3n3)	0.15
	Nervonic acid (C24:1n9) + <i>cis</i> -4,7,10,13,16,19-docosahexaenoic acid (C22:6n3) (DHA)	0.63
OM-6	Linoleic acid (C18:2n6c) (LA)	12.41
	γ -Linolenic acid (C18:3n6) (GLA)	1.05
	<i>cis</i> -8,11,14-Eicosatrienoic acid (C20:3n6)	0.18
	Arachidonic acid (C20:4n6) (AA)	0.48
OM-9	Oleic acid (C18:1n9c)	40.21
	<i>cis</i> -11-Eicosenoic acid (C20:1n9)	0.42
Undefined		4.11
Total		100.00

The chemical compositions of methyl esters were not significantly different to one's before esterification reaction. The total content of omega 3, 6, and 9 in the raw materials is 57%; 39% are non-omega 3, 6, and 9 fatty acids; 4% are unidentified substances. Omega 3, 6, and 9 and non-omega are identified in Table 4 and Figure 5(a).

3.3. Separation and Enrichment of Omega 3, 6, and 9 by DES. In this work, we use DES with a choline chloride/urea ratio of 1 : 1 to separate and enrich omega 3, 6, and 9 because it has a pH of nearly 7, small electronic conductivity (1.64), and water low content (0.65%). These properties will not cause side effects with multiple coupling compounds in the sample. Figure 6 illustrates the process of enriching omega from the sample.

Effectiveness of process delamination during the participation of DES is shown in Table 5.

The performance of omega FA separation from the liquid layer varies from 12% to 19.5%, depending on the mass of DES (5–30 g per 20 g of methyl ester). It changes insignificantly when increasing the mass of DES. For omega 3, 6, and 9, the separation efficiency is between 17% and 29%, depending on the mass of DES. This performance can reach over 50% for omega 3 and 6 as shown in Table 6.

The omega fatty acid compositions were determined by the GC/FID method (Figure 5(b)) and are shown in Table 7 and Figure 7. It is that although the separation efficiency is low, the purity of omega 3, 6, and 9 was high. The content of omega FA was reached from 83% to 91% for the first separation, and the optimal mass of DES was in a range from 15 to 20 g/20 g of methyl esters (Table 7 and Figure 7(a)).

Eighty percent FA of methyl esters is combined with DES to form the crystal solid, as shown in Section 2.3. In this mixture, there are mainly SFA and UFA. Omega FAs were accounted for only 20–30% as shown in Table 8 and Figure 7(b).

The FA separation process from the crystal solid was also carried out with DES; however, the omega fatty acids were not obtained. This result indicated that DES was not capable of separating omega FA from the non-omega FA when the omega FA/non-omega FA ratio was less than 1.

4. Conclusions

The ionic liquid-deep eutectic solvent was successfully synthesized with the molar ratio of choline chloride/urea of 1 : 1 and 2 : 1. The characteristics of DES were determined and demonstrated by FTIR, TGA, DSC, ^1H -NMR, and ^{13}C -NMR analysis methods. DES's physical properties were

FIGURE 6: (a) DES and methanol extract; (b) DES, methanol extract, and methyl ester after stirring and heating; (c) mixture after 8 hours of cooling; (d) omega-rich fatty acids in the liquid layer; (e) crystal solid under the microscope; (f) fatty acids separated from crystal solid.

TABLE 5: The dependence of the liquid/crystal solid ratios on mass of DES.

Mass of DES (g)	Liquid (g)	Crystal solid (g)	Loss (g)
5	2.40	17.52	0.08
10	2.87	17.08	0.05
15	3.30	16.60	0.10
20	3.60	16.33	0.07
25	3.82	16.09	0.09
30	3.90	16.00	0.10

TABLE 6: The efficiency of omega FA separation from the liquid layer.

Mass of fatty acids	Mass of DES (g)/20 g methyl ester						
	0	5	10	15	20	25	30
Omega 3, 6, and 9 FA (g)	11.39	1.99	2.42	3.00	3.25	3.30	3.34
Efficiency (%)	—	17.47	21.06	26.33	28.53	28.97	29.32
Omega 3 and 6 FA (g)	3.26	1.12	0.89	1.22	1.74	1.21	1.54
Efficiency (%)	—	34.00	27.30	37.42	53.37	37.12	47.24

TABLE 7: Fatty acid compositions of the liquid layer.

Mass of DES (g)	SFA (%)	UFA (%)	Omega 3, 6, and 9 (%)	Undefined (%)	Total (%)
5	11.76	2.90	82.97	2.37	100
10	7.46	3.14	84.35	5.05	100
15	3.30	2.37	91.03	3.30	100
20	2.99	2.64	90.30	4.34	100
25	8.22	2.17	86.60	3.01	100
30	6.66	3.70	85.52	4.12	100

FIGURE 7: The dependence of omega FA and non-omega FA in the content of DES: (a) in liquid layer and (b) in crystal solid.

TABLE 8: Fatty acid compositions of crystal solid.

Mass of DES (g)	SFA (%)	UFA (%)	Omega 3, 6, and 9 (%)	Undefined (%)	Total (%)
5	60.45	13.80	25.32	0.43	100
10	62.22	15.07	22.40	0.36	100
15	65.73	15.12	18.57	0.58	100
20	63.75	14.68	20.08	0.49	100
25	55.43	12.41	31.64	0.52	100
30	54.62	14.30	30.51	0.57	100

determined: the electronic conductivity of DES is 1.64 mS and 2.69 mS and the viscosity is 61.5 cP and 57.3 cP, corresponding to the molar ratio of choline chloride/urea of 1 : 1 and 2 : 1.

DES with a choline chloride/urea ratio of 1 : 1 was used to enrich omega 3, 6, and 9 because it has a pH of nearly 7, small electronic conductivity (1.64), and water low content (0.65%). The results show that the percentage of omega 3, 6, and 9 was increased from 57% in raw materials to 91% for the first separation. The optimal mass ratio is 20 g methyl acetate with 200 g methanol and 15–20 g DES.

DES was only used to separate and enrich the omega FA when the omega FA/non-omega FA ratio is greater than 1.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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