Journal of Chromatography B, 878 (2010) 2243–2248

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Task-specific ionic liquid-assisted extraction and separation of astaxanthin from shrimp waste

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ARTICLE INFO

Article history:
Received 26 March 2010
Accepted 25 June 2010
Available online 3 July 2010

Keywords:
Astaxanthin
Ionic liquid
Extraction
Separation

ABSTRACT

Astaxanthin, as an outstanding antioxidant reagent, was successfully extracted from shrimp waste by the ionic liquids based ultrasonic-assisted extraction. Seven kinds of imidazolium ionic liquids with different cations and anions were investigated in this work and one task-specific ionic liquid in ethanol with 0.50 mol L^{-1} was selected as the solvent. At the optimized ultrasonic extraction conditions, the extraction amount of astaxanthin increased 98% (92.7 $\mu g\,g^{-1}$) compared to the conventional method (46.7 $\mu g\,g^{-1}$). Furthermore, the extracted solution was isolated through the solid-phase extraction with a molecularly imprinted polymer sorbent. After loading the samples on molecularly imprinted polymer cartridge, the different washing and elution solvents, such as water, methanol, n-hexane, acetone and dichloromethane, were evaluated, and finally, astaxanthin was separated from the shrimp waste extract.

1. Introduction

Shrimp processing is an important marine industry that generates large quantities of shrimp waste consisting of the heads, shells and tails of the shrimp [1]. This waste can be comprised of up to 65% of the initial shrimp weight, and it generates environmental problems [2]. At the same time, several bioactive compounds in the dry shrimp waste, such as proteins, chitins, minerals and carotenoids were wasted [3]. In this case, the recovery of these bioactive components from the shrimp waste can improve the economy of shrimp processing industry.

Astaxanthin (Fig. 1) is the most valuable carotenoid with a selling price of ~$160 per 500 μg of pure astaxanthin from Sigma (St. Louis, MO, USA). It has been applied in the functional food, animal feed additive, cosmetics and food industries [4]. Astaxanthin may be beneficial to cardiovascular, immune, inflammatory and neurodegenerative diseases because of its antioxidant activity. Some research has also demonstrated its potential as an anti-cancer agent [5]. Several studies have reported to extract astaxanthin from shrimp waste employ oils [3,6], organic solvent [1,7] and fermentation process [8].

The exploration of ionic liquids (ILs) for extraction has shown great promise [9,10]. ILs can improve the selectivity and the extraction yields of bioactive compounds in samples as well as alleviating the environmental pollution compared to the conventional organic solvents. Additionally, ultrasonic-assisted extraction (UAE) using ILs as the solvents or co-solvents is an attractive and rapid technique [11,12].

Solid-phase extraction (SPE) has been developed as an acceptable alternative to liquid–liquid extraction for the separation, purification, and solvent exchange of solutes from a solution [13]. One of the most important SPE techniques is molecularly imprinted solid-phase extraction (MISPE) which takes advantage of the shape selectivity of the cavity for the separation of certain compounds from a solution.

Task-specific ILs based UAE (ILs-UAE) was combined with MISPE in order to extract and separate astaxanthin from shrimp waste. The influential parameters of the ILs-UAE and MISPE procedures were systematically optimized. The ILs-UAE approach that was proposed in this study was compared to conventional solvent extraction approaches. MISPE and non-imprinted SPE (NISPE) were also investigated and compared. At the same time, the ILs-UAE and MISPE mechanisms were discussed.

2. Experimental

2.1. Chemicals and standards

Astaxanthin (98%) and ethylene glycol dimethacrylate (EDMA) were purchased from Sigma (St. Louis, MO, USA). 1-Methylimidazole (≥99.0%, GC) was obtained from Fluka (Steinheim, German). 1-Butyl-3-methylimidazolium tetrafluoroborate ($[C_4MIM][BF_4]$), 1-ethyl-3-methylimidazolium tetrafluoroborate ($[C_2MIM][BF_4]$), 1-hexyl-3-methylimidazolium tetrafluoroborate ($[C_6MIM][BF_4]$), 1-butyl-3-methylimidazolium methylsulfate ($[C_4MIM][MS]$), 1-butyl-3-methylimidazolium

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doi:10.1016/j.jchromb.2010.06.034

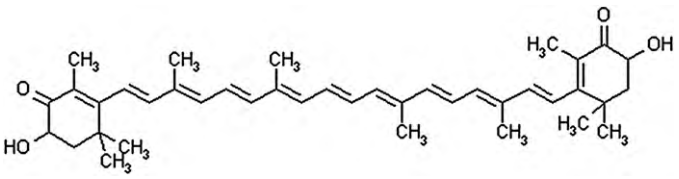


Fig. 1. Chemical structure of astaxanthin.

chloride ([C_4MIM][Cl]) and 1-butyl-3-methylimidazolium bromide ([C_4MIM][Br]) were from Kowoon Institute of Technology (Whasung-gu, Korea). 3-Bromopropylamine Hydrobromide was purchased from Tokyo Chemical Industry CO. LTD. (Tokyo, Japan). α,α′-Azobis(isobutyronitrile) (AIBN) was the product of Junsei Chemical Co., Ltd. (Tokyo, Japan) and was recrystallized prior to use. Methacrylic acid (MAA) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Methanol, ethanol, n-hexane, ethyl acetate, acetone, acetonitrile, dichloromethane and tetrahydrofuran were from DUKSAN Pure Chemical CO., LTD. (Ansan, Korea). Distilled water was filtered using a vacuum pump (Division of Millipore, Waters, USA) and a filter (HA-0.45, Division of Millipore, Waters, USA). All solvents used in the experiment were HPLC or analytical grade. All the samples were filtered by a filter (MFS-25, 0.2 μm TF, WHATMAN, USA) before injected into the HPLC system.

2.2. Preparation of shrimp waste

The shrimp waste was collected from the local market. This waste was frozen when it was transported to the laboratory. The frozen shrimp waste was washed repeatedly in water, freeze-dried, ground and sieved. The freeze-dried shrimp waste particles were stored in a refrigerator.

2.3. Preparation of 1-propylamine-3-methylimidazolium bromide ([C_3NH_2MIM][Br])

The 1-propylamine-3-methylimidazolium bromide IL was synthesized as the reference [14]. First, 100.0 mmol of 1-methylimidazole and 100.0 mmol of 3-bromopropylamine were mixed in 50.0 mL of dry ethanol with stirring under nitrogen atmosphere, and the mixture was refluxed for 24 h. After ethanol was removed under vacuum, the solid residue was dissolved in minimized water. Then the pH value of the solution was adjusted to 8.0 through the addition of solid sodium hydroxide. The obtained solution was concentrated and extracted with ethanol/tetrahydrofuran. A yellow viscous liquid of [C_3NH_2MIM][Br] was obtained after the solvent was removed under vacuum at 80 °C.

2.4. Extraction of shrimp waste

The conventional solvent extraction was carried out by mixing 0.2 g shrimp waste particles with 2.0 mL of the degassed solvent under ultrasonic conditions. The ILs-UAE was performed in ultrasonic water baths. First, 0.2 g of the dried sample powder was mixed with 2.0 mL of different concentrations of the degassed IL solutions in sealed vials, and then the suspensions were ultrasonically extracted. The IL solutions were prepared by dissolving different types of ionic liquids in ethanol at concentrations ranging from 0.25 to 1.5 mol L^{-1}. The optimum extraction conditions were systematically studied in this work. The suspensions that were obtained after extraction were cooled to room temperature and filtered through a 0.45 μm filter prior to the HPLC analysis.

2.5. Preparation of molecularly imprinted polymer (MIP)

According to Ref. [15], the astaxanthin imprinted polymer was prepared through the thermal-initiated polymerization in a 20.0 mL glass vial. The polymerization mixture, which was composed of 23.88 mg of astaxanthin, 55.07 mg of MAA, 5.0 mL of EDMA, and 60.0 mg of AIBN, was dissolved in the appropriate porogenic solvent (dichloromethane). The solution was purged with helium for 10 min in order to remove oxygen, and then polymerized at 60 °C in a water bath for 24 h. After the polymerization, the polymers were ground and sieved through a 32.0 μm sieve. Then the particles were washed with acetone and dichloromethane to remove the templates. Finally, the particles were dried at 50 °C for 12 h, and the obtained particles were stored at ambient temperature until they were use. A non-imprinted blank polymer (NIP, in the absence of the template) was prepared and treated in the same way.

2.6. Procedure of SPE cartridges

The MIP and NIP (150.0 mg) were packed into empty polypropylene cartridges, and preconditioned with dichloromethane. Then 0.2 mL of the standard solution of astaxanthin (0.5 mg mL^{-1} in dichloromethane) was loaded onto the SPE cartridges, and the samples were washed and eluted with different solvents. The solutions (0.2 mL) that were from the shrimp waste through ILs-UAE were loaded onto the SPE cartridges. The washing and elution steps were performed under the optimal conditions. The filtrate solvent was removed under vacuum and reconstituted in 0.2 mL of the mobile phase for further HPLC analysis.

2.7. HPLC analysis

The HPLC system was comprised of a M930 solvent delivery pump (Young Lin Co. Korea), a UV detector (M 720 Absorbance Detector, Young-In Scientific Co., Korea) and an integrated data system (Autochrowin. Ver. 1.42, Young Lin Co., Korea). Injection valves with 20.0 μL sample loops were used. The HPLC analysis was performed with a commercial C_{18} column (4.6 mm × 150 mm, 5 μm) purchased from RStech Co. (Daejeon, Korea). The mobile phase was dichloromethane/methanol/acetonitrile/water (5/85/5.5/4.5, v/v) [16], the flow rate was set at 0.5 mL min^{-1}, the UV wavelength was set at 476 nm, and the injection volume was 5.0 μL.

3. Result and discussion

3.1. Extraction of shrimp waste

3.1.1. Effect of solvents

The solvent selection was carried out using water, methanol, ethanol, n-hexane, ethyl acetate, acetone and dichloromethane. During this analysis, 2.0 mL of the solvent was used to extract 0.2 g shrimp waste for 60 min at room temperature. The ultrasonic power was set at 75 W. Comparing the extracted amounts of the astaxanthin in the different solvents (Fig. 2), ethanol was proved to be the best solvent, and, therefore, ethanol was selected for the following experiments.

3.1.2. Effect of ILs

The ILs had a strong dissolving power and the charged environment protected astaxanthin against oxidation. The IL structure significantly influenced its physicochemical properties, which affected the extraction efficiency of the target compounds [17]. ILs with different cations and anions were investigated in order to determine the optimal ILs and it evaluates their performance in the ILs-UAE of astaxanthin (Table 1).

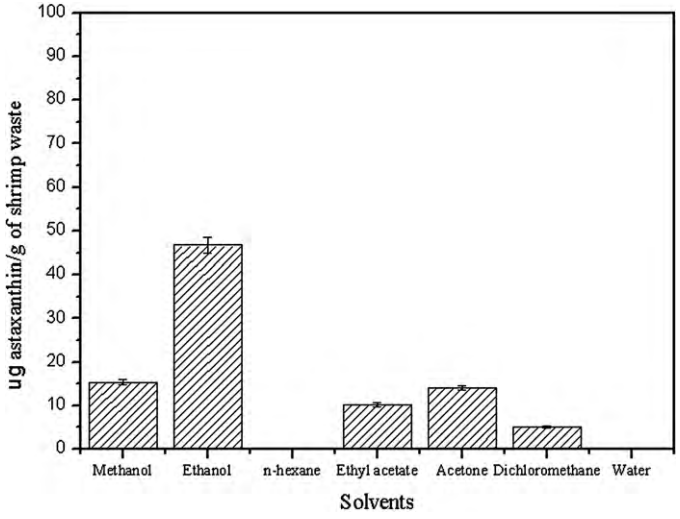


Fig. 2. Effect of solvents on the extracted amounts of astaxanthin. (Solid/liquid ratio ($g\,mL^{-1}$) = 1/10, ultrasonic power = 75 W, time = 60 min, room temp.).

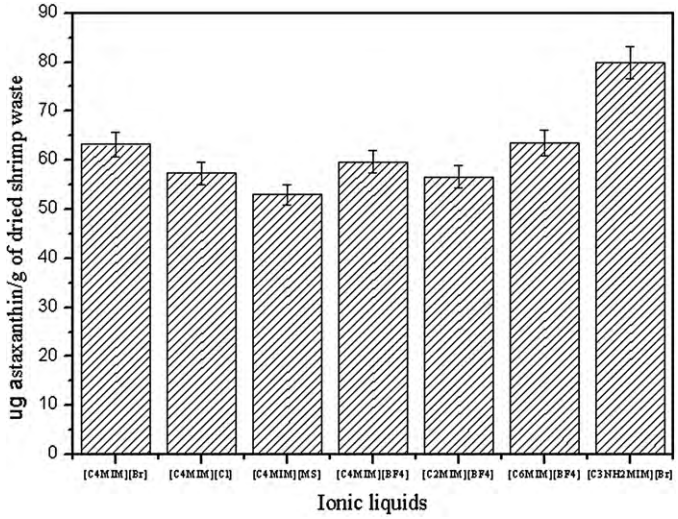
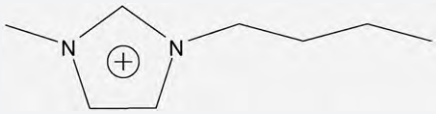
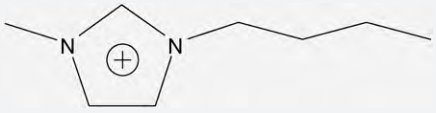
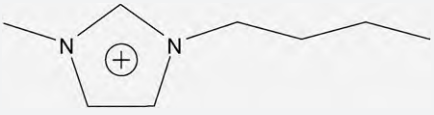
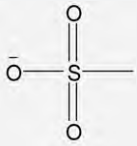
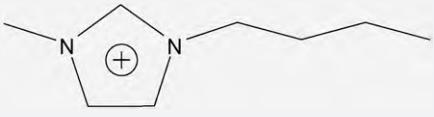
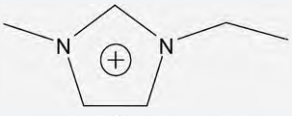
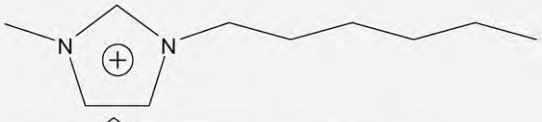
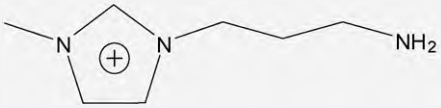


Fig. 3. Effect of ILs on the extracted amounts of astaxanthin (0.50 mol L^{-1} $[C_3NH_2MIM][Br]$ in ethanol, solid/liquid ratio ($g\,mL^{-1}$) = 1/10, ultrasonic power = 75 W, time = 60 min, room temp.).

Fig. 3 shows that the addition of the ILs to the ethanol obviously increased the extracted amount of astaxanthin compared to the extraction using ethanol as the solvent in the UAE. Meanwhile, these results suggested that the cations and anions of the ILs influenced the extraction of astaxanthin from the shrimp waste. 1-n-butyl-3-methylimidazolium ionic liquids with Br^-, Cl^-, BF_4^- and MS^- were tested, and the results indicated that Br^- was more efficient than the others for the ILs-UAE of astaxanthin because of the different miscibility of the four 1-butyl-3-methylimidazolium ILs with ethanol.

$[C_2MIM][BF_4]$, $[C_4MIM][BF_4]$ and $[C_6MIM][BF_4]$ with the same anion of BF_4^- were used to investigate the effects of cations (Fig. 3). The results suggested that increasing the alkyl chain length influenced the extraction, and $[C_6MIM][BF_4]$ was more efficient than the other two ILs. The hydrogen bond ability [18] and the hydrophobicity of the three cations increased from ethyl to hexyl for 1-position

Table 1
Chemical structures of studied ILs.

ILs	Cations	Anions
$[C_4MIM][Br]$		Br^-
$[C_4MIM][Cl]$		Cl^-
$[C_4MIM][MS]$		
$[C_4MIM][BF_4]$		BF_4^-
$[C_2MIM][BF_4]$		BF_4^-
$[C_6MIM][BF_4]$		BF_4^-
$[C_3NH_2MIM][Br]$		Br^-

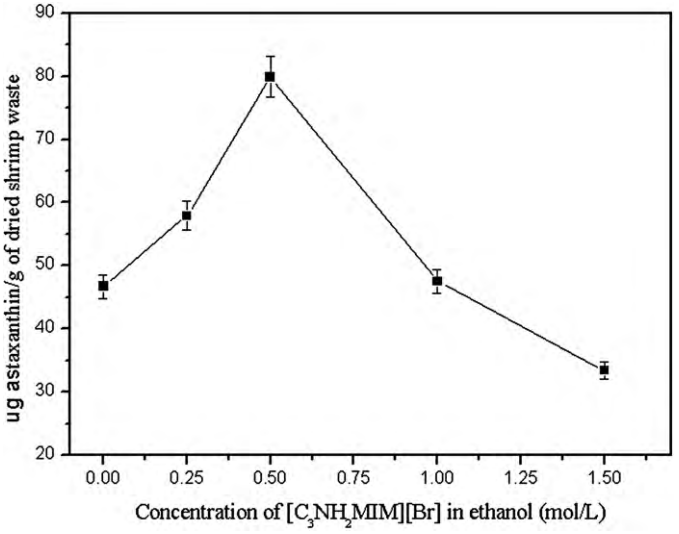


Fig. 4. Effect of ILs concentrations in ethanol on the extracted amounts of astaxanthin. (Solid/liquid ratio ($g\,mL^{-1}$) = 1/10, ultrasonic power = 75 W, time = 60 min, room temp.).

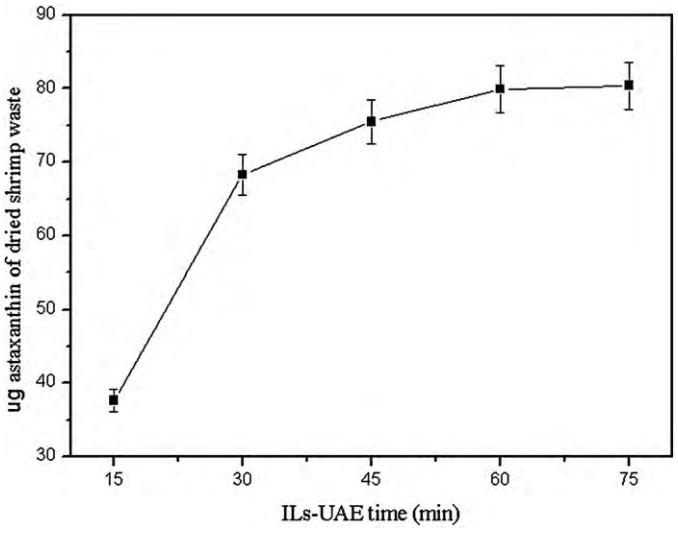


Fig. 5. Effect of ILs-UAE time on the extracted amounts of astaxanthin. (Solid/liquid ratio ($g\,mL^{-1}$) = 1/10, ultrasonic power = 75 W, time = 60 min, room temp.).

of the 1-alkyl-3-methylimidalizium ring resulting in the stronger interactions of $[C_6MIM][BF_4]$ with astaxanthin.

The ILs-UAE exhibited a higher efficiency than the conventional UAE because of the π–π, *n*–π, hydrophobic and hydrogen bond interactions. Moreover, the investigation of the ILs suggests that incorporation of functional group of the ions in the ILs improved their efficiency [19]. A "task-specific" IL was employed to extract astaxanthin from the shrimp waste by analyzing the chemical structure of astaxanthin. $[C_3NH_2MIM][Br]$ maintained the properties of the above ILs and increased the interactions with the functional groups of astaxanthin. Additionally, the existence of the reductive amine group protected the astaxanthin against oxidation. The task-specific IL was expected to exhibit a good extraction of astaxanthin from the shrimp waste because of these advantages. This theory was proven in Fig. 3. After comparing the efficiencies of the different ILs, $[C_3NH_2MIM][Br]$ was selected for the subsequent experiments.

3.1.3. Effect of IL concentration

The extraction was carried out with different concentrations of the IL ethanol solution (from 0.25 to 1.50 $mol\,L^{-1}$) in order to determine the optimal IL concentration for the ILs-UAE of astaxanthin from the shrimp waste. Based on the results in Fig. 4, the extracted amounts increased in the IL concentration range of 0.25–0.50 $mol\,L^{-1}$. However, the extracted amount decreased above the concentration. Therefore, 0.50 $mol\,L^{-1}$ of $[C_3NH_2MIM][Br]$ was selected for the following experiments.

3.1.4. Effect of other ILs-UAE conditions

The other factors that might have influenced the ILs-UAE were also investigated, including the time, the ultrasonic power and the solid/liquid ratio. The ILs-UAE was carried out at 75 W from 15 to 75 min in order to optimize the extraction time. In Fig. 5, the extraction amounts of astaxanthin dramatically increased as the extraction time increased from 15 to 60 min. After 60 min, no obvious increase in the extracted amount of astaxanthin was observed.

The effects of the ultrasonic power were examined as an important impact factor. The extractions were carried out at 15, 45, 75 and 105 W, respectively, at a constant time of 60 min. From Fig. 6, the ultrasonic power did not significantly influence the extracted amount of astaxanthin above 75 W. Considering the energy consumption, 75 W was selected as a suitable ultrasonic power.

The solid/liquid ratio was also an important factor with respect to increasing the extracted amounts of astaxanthin. Larger volumes of the solvent not only decreased the economic feasibility but also created unnecessary waste. Fig. 7 shows that the extracted amounts of astaxanthin increased with increasing solvent volume. The highest extracted amount (92.70 μg of astaxanthin/g dried shrimp waste) of astaxanthin was obtained at a solid/solvent ratio of 1/40 ($g\,mL^{-1}$), which corresponded to a ~98% increased with respect to the conventional solvent UAE. The dissolution of the bioactive components into the solvent is a physical process. When the amount of solvent increased, the chance of bioactive components coming into contact with the solvent also increased, leading to higher leaching-out rates. The solid/solvent ratio of 1/10 ($g\,mL^{-1}$) was sufficient for economic considerations.

Based on the above experiments, the most economic ILs-UAE conditions were 0.50 $mol\,L^{-1}$ $[C_3NH_2MIM][Br]$ in ethanol as the extraction solvent with an ultrasonic power of 75 W for 60 min, at a solid/liquid ratio of 1:10 ($g\,mL^{-1}$).

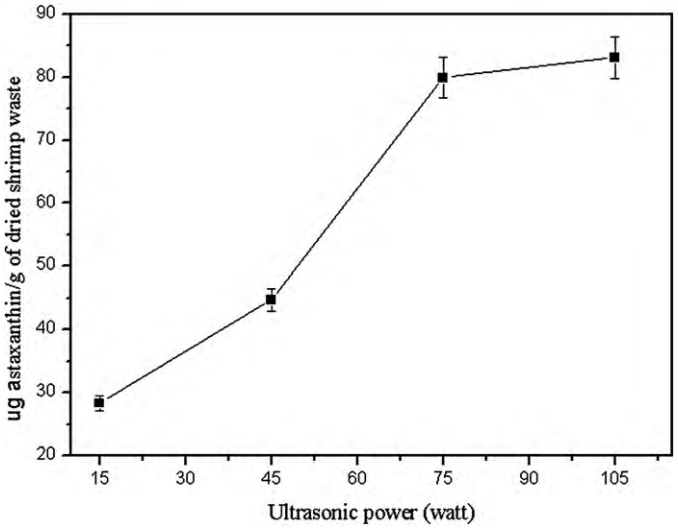


Fig. 6. Effect of ultrasonic power on the extracted amounts of astaxanthin. (0.50 $mol\,L^{-1}$ $[C_3NH_2MIM][Br]$ in ethanol, solid/liquid ratio ($g\,mL^{-1}$) = 1/10, time = 60 min, room temp.).

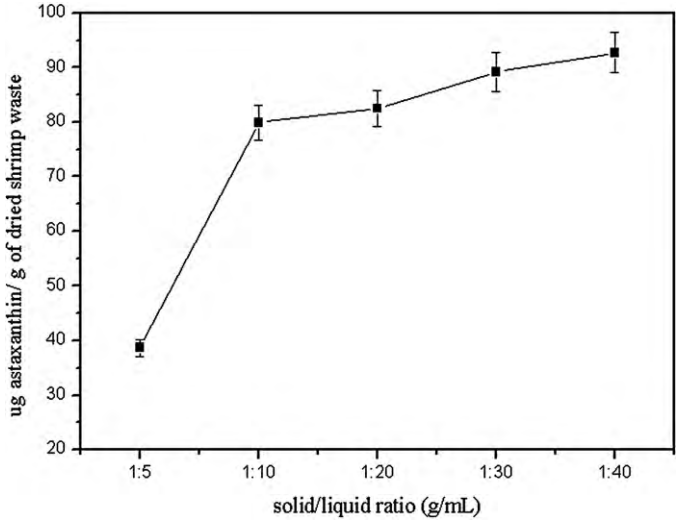


Fig. 7. Effect of solid/liquid ratio on the extracted amounts of astaxanthin. (0.50 mol L^{-1} [C_3NH_2MIM][Br] in ethanol, ultrasonic power = 75 W, time = 60 min, room temp.).

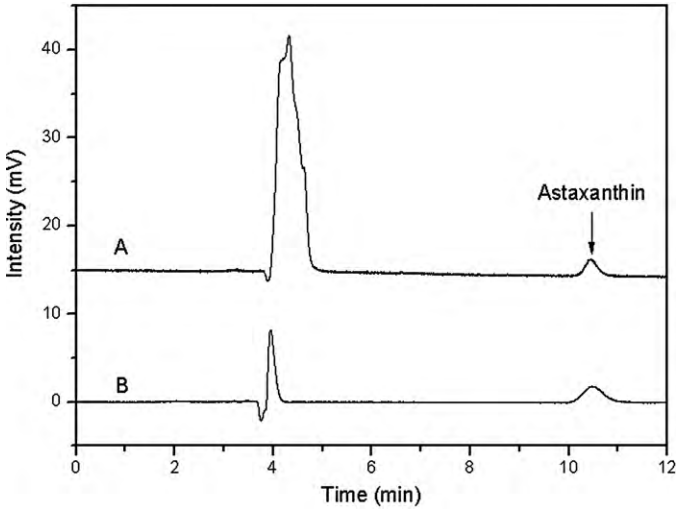


Fig. 8. Chromatograms of MISPE: (A) extract of shrimp waste, (B) astaxanthin elute by dichloromethane. (Mobile phase: dichloromethane/methanol/acetonitrile/water (5/85/5.5/4.5, v/v), flow rate: 0.5 mL min^{-1}, injection volume: 5 μL, UV: 476 nm.).

3.2. SPE of astaxanthin from extract

SPE was utilized in order to isolate astaxanthin from the shrimp waste extract. The imprinting effects were evaluated using MISPE and NISPE with the standard solution (0.50 mg mL^{-1} astaxanthin in dichloromethane). Using the same solvent, more amount of astaxanthin was eluted out from NIP than MIP (Table 2). It revealed that astaxanthin had stronger interaction of MIP than NIP. Therefore, the MIP was undoubtedly selected as the sorbent in the SPE of astaxanthin from shrimp waste extract.

As shown in Table 2, water and n-hexane cannot wash the target compound from MISPE, so both of them were selected as the washing solvents to remove the polar and non-polar interferences, respectively. Most of astaxanthin can be eluted out from the MIP sorbent using dichloromethane, so it was selected as the elution solvent. Under the economic condition, 0.2 mL of the extract from the ILs-UAE was loaded onto the MISPE cartridge, and then different volumes of the solvents (from 1.0 to 4.0 mL) were used to obtain the optimal volume. When the volume of the washing solvents was larger than 2.0 mL, the interference removal did not increase. In addition, the extraction amount of astaxanthin of 79.9 mg g^{-1} did not change at elution solvent volumes larger than 1.0 mL. Therefore, 2.0 mL of water and n-hexane were used as the washing solvents, and 1.0 mL of dichloromethane was used as the elution solvent. The chromatogram is shown in Fig. 8.

Table 2
Extracted amounts of astaxanthin with different elution solvents by MIP and NIP.

Washing solvents (volume: 1 mL)	Amount of astaxanthin (μg mL^{-1})	
	NIP	MIP
Water	0	0
Methanol	35.3	25.2
Acetone	92.4	52.9
n-Hexane	4.45	0
Dichloromethane	96.2	94.4

3.3. Analytical performance of the proposed method

The calibration curves were constructed using the areas from the chromatographic peaks that were measured at seven different concentrations ranging from 5×10^{-4} to 0.50 mg mL^{-1}. Each experiment was repeated three times, and a good linearity was obtained throughout the concentration range. The linear correlation equations was $Y = 0.253X + 0.074$ ($r^2 = 0.994$) for astaxanthin. Where Y is the peak area (mAU × s), and X is the concentration of astaxanthin in the solution (μg mL^{-1}) of the injected samples. The repeatability assays were calculated as the relative standard deviations (RSDs) and were carried out by injecting standard solutions of astaxanthin five times over a 5-day period.

Three astaxanthin concentrations (Table 3) were added to the extracts from shrimp waste, respectively and the recovery rate was determined by comparing the measured concentration with the theoretical concentration using Eq. (1):

$$R = \frac{C_p - C_0}{C_m} \times 100\% \quad (1)$$

In this equation, R is the recovery rate, C_p is the total amount of the compound in the final solvent, C_0 is the amount of the compound from the shrimp waste, and C_m is the amount of the compound that was added.

The standard solutions of astaxanthin was diluted and injected until the limit of detection (LOD) was obtained at a signal/noise ratio of 3. Table 3 lists the RSD of the precision tests, the limit of detections (LOD) on the standard solutions and the recovery rates. These values exhibited an acceptable precision and accuracy compared to the real sample analysis.

Table 3
RSDs and recovery rates of the astaxanthin from shrimp waste.

Compounds	RSD (%)	Recovery rate				LOD (ng mL^{-1})
	Intra-day	Inter-day	Amount added (μg mL^{-1})	Average recovery (%)	RSD (%)	
Astaxanthin	0.33	0.51	6.39	84.1	0.55	398
			7.99	87.2	0.67	
			9.59	86.2	0.51	

4. Conclusion

The task-specific ILs-UAE of astaxanthin from shrimp waste was studied at various conditions. It is concluded that the highest amount of astaxanthin in the extract that was obtained through ILs-UAE increased almost 98% (92.7 $\mu g\,g^{-1}$) compared to the conventional solvent UAE. However, for a moderate astaxanthin content, the economic ILs-UAE condition was found to be: 0.50 mol L^{-1} [C_3NH_2MIM][Br] in ethanol as the extraction solvent with an ultrasonic power of 75 W for 60 min at a solid/liquid ratio of 1:10 ($g\,mL^{-1}$). The astaxanthin in the extract was subsequently separated from the interferences through MISPE. The MISPE exhibited a higher affinity to astaxanthin compared to the NISPE and was successfully applied as a special sorbent. A good linearity was obtained from 5×10^{-3} to 0.50 $mg\,mL^{-1}$ ($r^2 > 0.99$). And 79.90 $\mu g\,g^{-1}$ of astaxanthin was extracted under the obtained conditions. The recovered astaxanthin concentration corresponded well with the previous reported data from 24.22 to 100.0 $\mu g\,g^{-1}$ of carotenoid pigments [1].

Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation (NRF) of Korea, funded by the Ministry of Education, Science and Technology (2010-0015731).

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