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Research Article

Application of ionic liquid-based microwave-assisted extraction of anthocynin from lycium ruthenicum

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Abstract: The anthocyanin of lycium ruthenicum was extracted using ionic liquid combined with microwave. The effects of ionic liquid species, ionic liquid concentration, microwave power and extraction time on the anthocyanin yield of lycium ruthenicum were investigated. The results showed that the application of ionic liquid-based microwave-assisted extraction might effectively increase the extraction rate of anthocyanin of lycium ruthenicum. The most suitable conditions were 1.0 mol/L of [Bmim]Br and 210 W of microwave power for 12 seconds with the sample to solvent ratio of 1:30. Under the best condition, the anthocyanin yield of lycium ruthenicum was 2.92mg/g.
Keywords: Lycium ruthenicum, Anthocyanin, Ionic liquid, Microwave, Extraction.

INTRODUCTION

Anthocyanin is a water soluble natural pigment, widely distributed in the organs of flowering plants (angiosperms), such as flower, fruit, stem, leave, root, and so on. It usually exists at glycosidic form. Anthocyanin glycoside nucleus is 2- phenyl benzopyrane cation that belongs to flavonoids compound [1]. As a natural and edible pigment, anthocyanin is safe, non-toxic and rich in resources. Moreover, it has certain nutritional and pharmacological effects [2]. Extraction is main step for separation, purification and utilization of anthocyanin. At present, there are many methods to assisted extract anthocyanin, such as ultra high pressure, microwave, ultrasonic, high pressure pulse electric field, and so on [3,4,5]. lycium ruthenicum, a Lycium plant of Solanaceae, is a perennial bush wild plant, living in the northwest drought area China [6]. Its ripening fruit is rich in anthocyanin contents, which were 1.75 and 1.91 times of blueberry and purple cabbage, respectively. lycium ruthenicum is a new wild resource developed in recent years. [7]. Modern pharmacological research showed that lycium ruthenicum pigment has the effects of antioxidant, activation of macrophages, anti-aging effect and so on [8].

Ionic liquid is entirely composed of anion and cation, being a new media and soft functional material. Under the framework of green chemistry, it acquires fast development in recent years, possessing particular characteristics such as no volatilization, wide liquid range, strong solubility and conveniently design regulation. Extraction with ionic liquid is environmentally friendly and improves selectivity, integrating the advantages of efficient extraction and preconcentration [9]. Currently, many food functional ingredients such as flavonoids, resveratrol and chlorogenic acid, had been extracted using Ionic liquids as extraction solvents [10, 11, 12]. Microwave, an electromagnetic wave with the wavelength of 1 mm ~1 meter, acquires widely application in phytochemistry field as a new emerging technology in recent years [13]. It has the charistics of strong penetrability and selectivity, high heating efficiency. Because microwave is an instantaneous penetration heating, it might accelerate extraction rate and effectively increase ingredient yield [14]. At present, microwave extraction technology has been applied in pectin, polysaccharide, polyphenols and other ingredients [15, 16, 17].

In this experiment, the anthocyanin of lycium ruthenicum was extracted using ionic liquid combined with microwave. The effects of ionic liquid species, ionic liquid concentration, microwave power and extraction time on the anthocyanin yield of lycium ruthenicum were investigated. We hope this research might provide relevant reference for effective development of lycium ruthenicum production.

MATERIALS AND METHODS

Materials and Reagents

Lycium ruthenicum was originated of Qaidam, Qinghai Province, China; 1-Butyl-3-methylimidazolium tetrafluoroborate (purity≥97%), abbreviated with [Bmim]BF4, was purchased from Xiya Chemical Technology Co. Ltd., Chengdu, China; 1-Butyl-3-

methylimidazolium Chloride (purity≥98%), abbreviated with [Bmim]Cl, was purchased from TCI development Co. Ltd., Shanghai, China; 1-Butyl-3-methylimidazolium Bromide (purity≥97%), abbreviated with [Bmim]Br, was purchased from Aladdin Industrial Corporation, Shanghai, China; Potassium chloride, hydrochloric acid, sodium acetate and glacial acetic acid (analytical grade) were purchased from Beichen Fangzheng Chemical Reagent Factory, Tianjin, China.

Equipments and instruments

GZX-9246 MBE Digital blast drying box, Shanghai Boxun Industrial Co., Ltd. medical equipment factory, Shanghai, China; UV-1100 spectrophotometer, Shanghai Meipuda Instrument Co., Ltd., Shanghai, China; 80-2 Centrifuge, Guohua Electric Appliance Co., Ltd, Changzhou China; G70D20CN1P-DI(SO) Microwave Oven, Galanz microwave oven electric appliance manufacturing Co., Ltd., Guangdong, China; MJ-25BM04B Mill, Guangdong Midea premium appliances manufacturing Co., Ltd., Guangzhou, China.

Extraction of anthocyanin from lycium ruthenicum

Lycium ruthenicum was dried with blast drying box at 45℃ for 15min in order to remove residual moisture. Afterward, it was milled and sieved with 80 mesh. 200mg of lycium ruthenicum powder was placed into 50 ml-Erlenmeyer flask, and then ionic liquid solution with different concentration of was added into the flask. The conical flask was placed into a microwave oven to extract anthocyanin with different power and time. Subsequently, the extracted mixture was centrifuged with centrifuge at 3000r/min for 15min. the anthocyanin content of supernatant was determined using pH differential method. Each treatment was repeated three times, and the result was showed with mean value.

Anthocyanin determination of lycium ruthenicum

Anthocyanin content was evaluated by pH differential method [18]. 1ml of appropriately diluted supernatant was respectively added into 10 ml of 0.025 M potassium chloride solution (pH 1.0) and 0.4 M sodium acetate buffer solution (pH 4.5), and their absorbance values were measured at 510 and 700nm after 15 min of incubation at 23℃. 1% HCl-methanol solution served as the blank control, four values were A510 (pH1.0), A700 (pH1.0), A510 (pH4.5), A700 (pH4.5) respectively. The content of total anthocyanin was expressed as follows: Anthocyanin content(mg/L) = $[(\Delta A \times Mw)/(\varepsilon \times 1)] \times D_f \times 1000$, where$\Delta A$=（A510-A700）pH1.0－（A510 -A700）pH 4.5, Mw is relative molecular mass of cornflower glucoside (484.82g/mol),ε is the molar extinction coefficient of cornflower glucoside (24825 mol^{-1}) and D_f is dilution factor (the total dilution multiple of sample).

RESULTS AND ANALYSIS

Selection of ionic liquid species

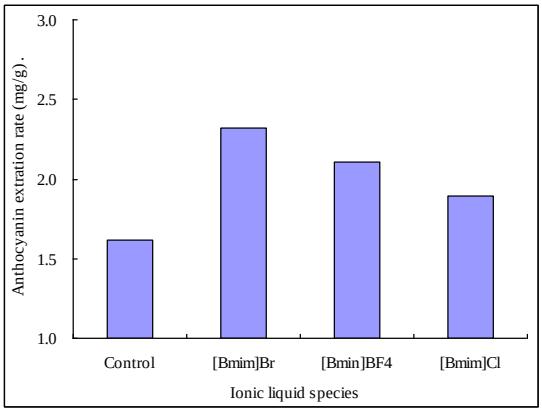

Fig.1 Effect of ionic liquid species on the anthocyanin extraction rate of lycium ruthenicum

200 mg of lycium ruthenicum powder was placed into 50 ml-Erlenmeyer flask, and 4 ml of different ionic liquids with the concentrations of 0.8 mol/L was added into the Erlenmeyer flask. The mixtures respectively were shaken up. Afterward, the mixtures were treated with microwave at 280W power for 10 seconds. Finally, the extracted mixtures were centrifuged at 3000r/min for 15min, and the anthocyanin content of supernatant was determined. As shown in Figure 1, after the addition of ionic liquid, the anthocyanin extraction rate of lycium ruthenicum increased apparently. Among three ionic liquids, the extraction rate of adding [Bmim]Br was the highest, followed by adding [Bmim]BF4. The extraction rate of adding [Bmim]Cl was the lowest. And they were 43.2%, 30.2 %and 16.8% higher than that of control sample. In the following experiment, the extraction conditions of adding [Bmim]Br were further optimized.

Ionic liquid concentration

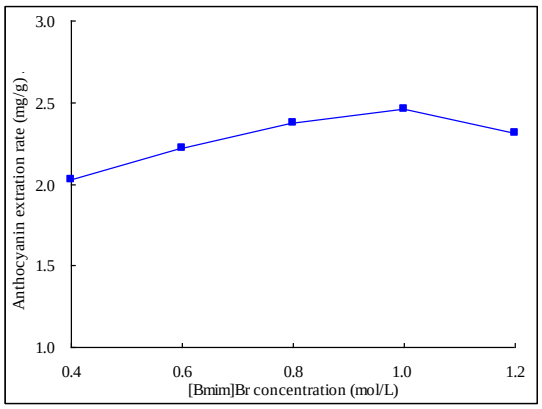

Fig.2 Effect of [Bmim]Br concentration on the anthocyanin extraction rate of lycium ruthenicum

200 mg of lycium ruthenicum powder was placed into 50 ml-Erlenmeyer flask, and 5 ml of [Bmim]Br with different concentration was added into the Erlenmeyer flask. The mixtures respectively were shaken up. Afterward, the mixtures were treated with microwave at 280W power for 10 seconds. Finally, the extracted mixtures were centrifuged at 3000r/min for 15min, and the anthocyanin content of supernatant was determined. As shown in Figure 2, with the increase of [Bmim]Br concentration, the anthocyanin extraction rate of lycium ruthenicum increased accordingly. At 1mol /L of [Bmim]Br, the anthocyanin extraction rate reached to the maximum of 2.46mg/g. With the further increase of [Bmim]Br, the anthocyanin extraction rate began to decrease. Appropriate concentration of [Bmim]Br was favorable for anthocyanin migrating into solution from solid material. Therefore, the extraction rate increased with [Bmim]Br concentration enhancement. However, when [Bmim]Br concentration further enhanced, the viscosity of extraction solution increased. Thus, the efficient migration of anthocyanin was partly hindered and the extraction rate decreased accordingly [19].

Sample to solvent ratio

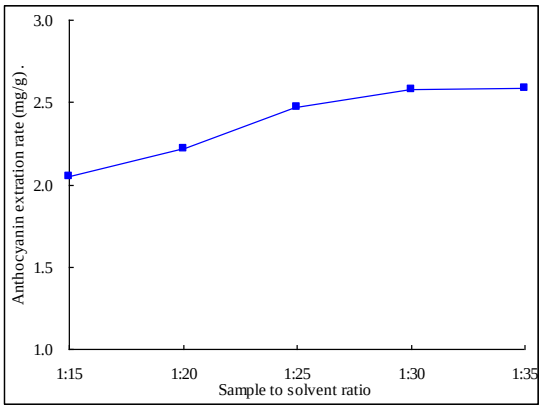

Fig.3 Effect of sample to solvent ratio on the anthocyanin extraction rate of lycium ruthenicum

200 mg of lycium ruthenicum powder was placed into 50 ml-Erlenmeyer flask, and 3, 4, 5, 6 and 7mL of [Bmim]Br with 1.0mol/l concentration were added into the Erlenmeyer flasks. The mixtures respectively were shaken up. Afterward, the mixtures were treated with microwave at 280W power for 10 seconds. Finally, the extracted mixtures were centrifuged at 3000r/min for 15min, and the anthocyanin content of supernatant was determined. As described in Figure 3, with the increase of sample to solvent ratio, the anthocyanin extraction rate of lycium ruthenicum increased gradually. At 1:30 of sample to solvent ratio, the extraction rate was 2.58mg/g, basically reaching to equilibrium. With the further increase of sample to solvent ratio, the extraction rate was almost no increase. Expanding sample to solvent ratio might enlarge the concentration difference of anthocyanin, which prompted more anthocyanin to migrate into solution from solid lycium ruthenicum powder. Therefore, the extraction rate increased with the enhancement of sample to solvent ratio [20].

Microwave power

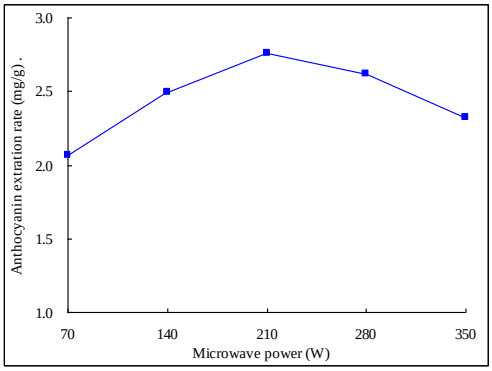

Fig.4 Effect of Microwave power on the anthocyanin extraction rate of lycium ruthenicum

200 mg of lycium ruthenicum powder was placed into 50 ml-Erlenmeyer flask, and 6 mL of [Bmim]Br with 1.0mol/l concentration were added into the Erlenmeyer flasks. The mixtures respectively were shaken up. Afterward, the mixtures were treated with microwave at different power for 10 seconds. Finally, the extracted mixtures were centrifuged at 3000r/min for 15min, and the anthocyanin content of supernatant was determined. As shown in Figure 4, the anthocyanin extraction rate of lycium ruthenicum first increased and then decreased with the enhancement of microwave power. With microwave power from 70W to 210W, the extraction rate increased. And at 210W, it reached to the maximum of 2.76mg/g. Subsequently, the extraction rate decreased with microwave power expansion. Appropriate enhancement of microwave power might accelerate the molecular motion of anthocyanin, and prompt more anthocyanin dissolution from lycium ruthenicum powder. However, anthocyanin was thermal sensitive substance and too high microwave power would lead to the thermal degradation of anthocyanin [21].

Microwave extraction time

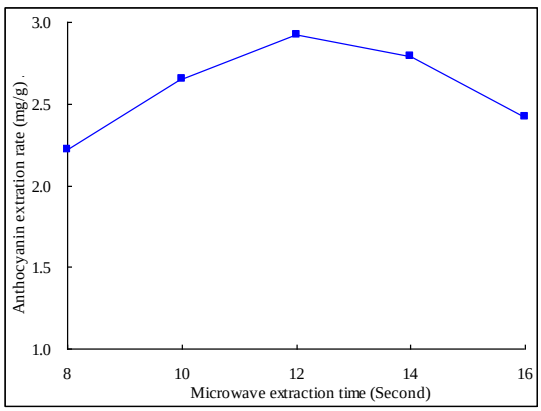

Fig.5 Effect of microwave treatment time on the anthocyanin extraction rate of lycium ruthenicum

200 mg of lycium ruthenicum powder was placed into 50 ml-Erlenmeyer flask, and 6 mL of [Bmim]Br with 1.0mol/l concentration were added into the Erlenmeyer flasks. The mixtures respectively were shaken up. Afterward, the mixtures were treated with microwave at 210W power for different time. Finally, the extracted mixtures were centrifuged at 3000r/min for 15min, and the anthocyanin content of supernatant was determined. As shown in Figure 5, the anthocyanin extraction rate of lycium ruthenicum first increased and then decreased with microwave treatment time extension. And at 12s, the extraction rate was the maximum of 2.92mg/g. subsequently, with the increase of microwave treatment time, the extraction rate decreased. Similar to microwave power, the too long time treatment to lycium ruthenicum with microwave also leads to the thermal degradation of anthocyanin [22].

CONCLUSION

Application of ionic liquid-based microwave-assisted extraction might effectively increase the extraction rate of anthocyanin of lycium ruthenicum. The most suitable conditions were 1.0 mol/L of [Bmim]Br and 210 W of microwave power for 12 seconds with the sample to solvent ratio of 1:30. Under the best condition, the anthocyanin yield of lycium ruthenicum was 2.92mg/g.

Acknowledgements

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