Microwave energy-assisted extraction of bioactive polyphenols with green technology based on magnetic ionic liquid and their stability under different conditions

Zhangxing Guo, Chen Chen, Alula Yohannes and Shun Yao*

Sichuan University, School of Chemical Engineering, Department of Pharmaceutical and Biological Engineering, Chengdu 610065, China
Corresponding author’s e-mail address: cusack@scu.edu.cn

Abstract. In recent years microwave-assisted extraction has been proved as a more rapid and energy-efficient technique than conventional ways, here it was employed in this study and a kind of magnetic ionic liquid (C₃MIMFeCl₄) was used as green medium for tea polyphenols (TP). Due to the strong polarity of the ionic liquid, it can quickly absorb microwave energy and increase the temperature; thus the extraction time will be greatly shortened. As the result, it was found when microwave power was set at 200 W, solid-liquid ratio was 1:20 and extraction time was 7 min, the highest extraction efficiency of 0.8 mol/L C₃MIMFeCl₄ could be obtained as 159.8 mg/g. Compared with ultrasonic-assisted mode used in previous study, the extraction time was shortened significantly from 300 min to 7 min. Moreover, magnetic ionic liquid can be easily recovered by external magnetic field. Finally, stability of TP was further investigated under related conditions by spectroscopic characterization meanwhile related intermolecular interactions; it was found IL could provide greater stabilization energy for these extracted objects in this study at some extent. The results of above research are expected to provide useful references for large-scale application of green technology based on ILs and TP production.

1. Introduction

Tea polyphenols (TP) are a kind of bioactive compounds beneficial to human health, which can be extracted from tea leaves and similar raw materials with various solvents. With strong antioxidant activity, TPs are prone to be oxidized during extraction and separation, especially the catechins, which account for 70% of the total amount of tea polyphenols (EGCG is the highest content of monomer among them, generally accounting for 40%) [1,2]. Under pH 3~8 and in approximately 250 °C, TP can remain stable within 1.5 hours, while they are easy to be oxidized and become brown under alkaline condition with pH over 8. So the conditions and ways of TP extraction should be fully concerned by industry and academia. There are various common methods for the extraction and separation of tea polyphenols, including organic solvent extraction, metal ion precipitation and so on [3, 4]. Affected by organic solvents, high temperature heating and co-existing ions during the extraction process, the scientificness, reliability and practicability of related methods together with TP stability have attracted the attention from many researchers. Meanwhile, it takes a very long time to extract these antioxidants in air atmosphere [5]. In order to improve efficiency and reduce the loss of tea polyphenols in the oxygen-containing environment and continuous heating process, it is necessary to investigate the appropriate extraction method of tea polyphenols and their stability in related conditions.
In the preparation process of bioactive constituents, energy and green are two unavoidable problems, which are not mutually exclusive in many old techniques. As a new kind of green solvent, ionic liquids (ILs) are composed of larger organic cations and smaller anions, which are in liquid status near room temperature and also called as molten salts. They have been applied in the extraction of many active substances from natural products successfully in recent years and become the hot point in the field of chemical engineering. Compared with conventional TP extractants (e.g. 40~80 °C water and mixture of ethanol-water), ILs have shown higher selectivity and better recyclability. In the previous research work [6], ultrasonic assisted extraction (UAE) for efficient selective extraction of tea polyphenols has been established by using C3MIMFeCl4, which firstly employed this kind of new magnetic ionic liquid for natural products. So far, the research on other extraction ways and their comparison with UAE have not been carried out, and similar reports for reference are very few. Previous experiments have preliminarily confirmed that the IL will not destroy the structure of tea polyphenols, and the antioxidant activity of tea polyphenols will not reduce, which needs to be further investigated and proved. Meanwhile, different extraction methods and their performance have not been compared; thereby the basic research is urgently needed for further large-scale application of ILs in TP production.

Considering microwave-assisted extraction is a more effective and rapid extraction technique than traditional techniques and its advantages have already been acknowledged in recent years, it was selected by us in the following study. This work aimed to achieve the combination of energy-efficient and green technologies in the preparation process of bioactive TP constituents and developed the optimal extraction method for them, and it was investigated about the appropriate extraction conditions and product stability from the aspects of spectroscopic characteristics and concentration. The study preliminarily explored the influence of relevant factors and their relationship with related results, summarizing the requirements of the involved components on the extraction environment and conditions. The whole research is expected to provide meaningful experimental data and theoretical basis for further scientific and rational application of these methods.

2. Materials and methods

2.1 Chemicals and instruments

Tea polyphenols were obtained from Aikeda chemical reagent factory (Chengdu, China). Gallic acid (GA) and epigallocatechin gallate (EGCG, shown in Figure 1) were purchased from Mansite Biotechnology Co., Ltd (Chengdu, China), and all of their purities were above 98.5%.

![Figure 1. Structures of ionic liquid and EGCG (main constituent) in tea leaves extracted by microwave reactor](image-url)
Folin-Ciocalteu’s phenol reagent was purchased from Nanjing Ordovician Biotechnology Co., Ltd. Ferric chloride and sodium carbonate were provided by Kelong chemical reagent Co. Ltd (Chengdu, China). C₂₅MIMFeCl₄ (also shown in Figure 1) and C₂₅MIMPro were synthesized in advance and their purities were both above 98%, which was determined by A LC-20AT high-performance liquid chromatography system with a SPD-M20A diode array detector (Shimadzu, Kyoto, Japan). The total TP content was determined on a TU-1810 ultraviolet–visible (UV–Vis) spectrometer (Purkinje General Instrument Co. Ltd., Beijing, China). IR spectra were recorded with a Spectrum II FT-IR Instrument (PerkinElmer, USA). Tea leaf powders were bought from local store. All of the solvents and reagents used in the following research were of analytical-reagent grade or higher.

2.2 Quantitative analysis and stability investigation of total polyphenols

The determination method of total polyphenols content was developed as following: firstly, the standard curve of total phenol content was founded based on Foline-phenol method [7] and GA was used as standard compound, and then the total phenol content was measured at 730 nm and calculated in the tested product according to the standard curve equation (y=0.008x+0.061, R²=0.9999). Generally, the time length of preparation and experimental research of TP is mostly controlled within 24 hours, so the period of stability investigation for TPs was determined as one day in this study. Respectively, the TP aqueous solutions with concentrations of 10 g/L, 20 g/L and 30 g/L were prepared and settled in different sealed transparent glass bottles for 1 h–24 h at 25 °C, and then they were sampled and analyzed by UV spectroscopy according to above equation. It was observed about the variance of the UV full-wavelength spectrum of different concentrations of TP solution after various durations. Moreover, the change of total phenol content with time was investigated. 30 g/L TP aqueous solution was prepared, sealed and investigated at different temperature. Finally, it was sampled at 2 h intervals and analyzed with ultraviolet-visible spectrophotometer using standard curve.

Considering the potential effect of ionic liquids and metal ions on TP stability in new extraction way, the UV spectrum stability of a mixture solution of TP and iron ions in the presence or absence of IL was investigated. 3 g/L aqueous solution of tea polyphenols was prepared and placed in nine test tubes respectively, which were divided into three groups. Both two groups among them were mixed with Fe³⁺ (FeCl₃) with three different mole ratios (1:1, 1:2 and 1:3) and one of them was further mixed with C₂₅MIMPro which had the same molar of Fe³⁺. Meanwhile, the third group was the mixture of TP and C₂₅MIMFeCl₄ with three mole ratios. After a period of time, TP solution was used as a control to observe the change of the UV full wavelength spectrum of the solution in nine test tubes.

2.3 Quantitation and stability investigation of EGCG in tea polyphenols

Firstly, the EGCG standard curve was developed and the specific experimental steps are as follows: (1) 25 mg of EGCG standard compound was weighed and dissolved with 50 mL water; (2) the obtained solution was then diluted to 30, 60, 90, 120, 150, 180, 210, 240, 300 μg/mL, respectively; (3) quantitative analysis by HPLC was used to establish an EGCG standard curve based on peak area and concentration (y=22332.96x+269349.51, R²=0.9999). HPLC conditions were as following: detection wavelength was 278 nm, Waters C₁₈ liquid chromatographic column (4.6 × 250 mm, 5 μm) was used and mobile phase was methanol-water-glacial acetic acid (35: 64.5: 0.5, V/V/V); the flow rate was 0.4 mL/min, the column temperature was set at 25 °C, and the injection volume was 10 μL. Secondly, the stability of EGCG content was studied. Secondly, the stability of EGCG under various temperature and time conditions was further investigated, because it is the most main compound in TP. Besides that, 30 g/L TP aqueous solution was prepared, sealed and observed at 20 °C, 30 °C, 40 °C, 60 °C, and 80 °C respectively. It was sampled every 2 hours and diluted, and finally quantified by HPLC. Taking the initial concentration of EGCG as the reference standard, the relative content of EGCG and its change with time at different temperatures were observed.

3. Results and discussion

3.1 Optimization of extraction conditions
Microwave-assisted extraction was performed in XH-300UL type multifunctional reactor (2450 MHz, 10–1000 mL, Xianghu Sci & Tech Development Co. Ltd, Beijing, China). According to pilot experiment, 0.8 mol/L C$_3$MIMFeCl$_4$ in its aqueous solution was the most ideal extractant and solid-liquid ratio (1:10–1:50, g/mL), extraction time (0–30 min) together with microwave power (100–300 W) were main influential factors in microwave-assisted extraction. 3 g of tea-leaf powders were placed in a 50 mL conical flask and then were mixed with above IL aqueous solution at a preset concentration. After extraction in microwave at 720 r/min for certain duration, the extract solution was adjusted to pH 3 and settled for a while. At last, 1 mL of supernatant was taken out of the system and diluted to 30 mL, and its UV-Vis absorbance was measured at 730 nm according to the spectroscopic method in section 2.2; the TP amount in the extract could be obtained through the standard curves. In the extraction process, the high-frequency electromagnetic wave penetrates the extractant medium, reaches the inside of the extracted raw material, and then rapidly transforms into heat energy and makes the temperature inside the cells rapidly increase. In addition to generating thermal effects, it can also produce biological effect (non thermal effect). When the pressure inside the cells exceeds the endurance capacity of the cells, the cells will break up; meanwhile the permeation of solvent molecules and salvation of target constituents are accelerated. As the result, the active ingredients can be quickly extracted from the cells and dissolved in the extraction medium. The characteristics of microwave-assisted extraction were proved by the fact that the heating speed was very fast and the heating time of raw materials was short. As one of important factors, the solid-liquid ratio should be very crucial to improve the extraction efficiency of TP. Excessive solvent is unnecessary and will result in the greater burden of solvent recovery, on the other hand less extractant will make the extraction of target constituents incomplete. Moreover, if the extraction time was too long or microwave power was too high, carbonization would begin to occur on the surface of tea powders at different extent and extraction efficiency would decrease because the thermal instability of TP. The optimized conditions were determined by orthogonal design of above three factors. As the result, the highest extraction efficiency of 0.8 mol/L C$_3$MIMFeCl$_4$ in microwave assisted mode could be obtained as 159.8 mg/g when solid-liquid ratio was 1:20, microwave power was 200 W and extraction time was 7 min. Compared with the result of ultrasonic assisted extraction (185.4 mg/g), it was a little lower. However, the extraction time was shortened significantly from 300 min to 7 min, which was an obvious advantage of microwave assisted extraction. Due to the strong polarity of the ionic liquid, when it is used in the microwave assisted process, it can quickly absorb microwave energy and increase the temperature; the other organic solvent is no longer needed, thus the extraction time is greatly shortened. Finally, in order to explore actual performance of above extraction conditions in real applications, the amplification experiment was carried on the PZ-TQ-50L type industrial microwave extraction unit (0-6kW, 2450±50MHz, 30–90 °C, total volume: 50 L) and 3 kg raw material of tea leaves was used. Final extraction efficiency indicated nearly 82.8% TP could be extracted and MIL could be easily recovered by external magnetic field and then reused.

3.2 Stability and interaction study

The investigation results indicates that at the temperature lower than 80 °C, the UV absorption spectrum and content of total tea polyphenols were more stable than those of single compound EGCG. The complex system composed of many compounds with high value of entropy was indeed more stable, and other co-existing constituents could protect EGCG effectively in the solution system. Fe$^{3+}$ ion can originate from the anion of MIL (C$_3$MIMFeCl$_4$) when the latter is used as extraction medium, or result from the metal instruments and pipes in industrial production. So its effect on extracted TP molecules should be explored. Besides the potential influence from metal ions, it should be also studied about the other effects of IL as a new kind of special extractant. Here C$_3$MIMPro was selected as comparison, which was composed of the same cation with C$_3$MIMFeCl$_4$ and nonmagnetic L-proline anion. The latter has been proved to have strong recognition ability for TP in previous study [8]. The UV full-wavelength spectra of the tea polyphenols solution are shown in Figure 2 (a-c) before and after the addition of different molar ratios of Fe$^{3+}$ and the magnetic/common ionic liquid. The results
demonstrate that the ultraviolet full-wavelength spectrum of the tea polyphenol mixed with Fe³⁺ solution has no obvious change on the comparison with the tea polyphenol solution when the molar ratio of tea polyphenols to iron ions was equal to or less than 1:2. It is also found that the maximum absorption wavelength of the solution was still 273 nm when the ionic liquid of [C₃MIM][Pro] with the equimolar amount of FeCl₃ was added to mixed solution of the tea polyphenol-iron ion. It probably results from the preferential interaction between iron ions and amino acid anions in IL, which shows the latter has a protective effect on tea polyphenols. Finally, C₃MIMFeCl₄ replaced FeCl₃ and it was mixed with tea polyphenol solution. Under conditions of the above three molar ratios, the change in content and UV spectrum is much smaller than that in the direct use of FeCl₃, and the maximum absorption wavelength existed near 273 nm. It shows that comparing to the ordinary ferric salt, the imidazolium type ionic liquids with the FeCl₄⁻ anion has a less destructive impact on the conjugated structure of TPs. Most ionic liquids have high stability and also can provide higher stabilization energy for those reaction intermediates or extraction objects in previous studies, so it plays a crucial role in the stabilization for the whole system.

As a supplement and comparison, here infrared spectroscopy (IR) was also used to analyze the stability together with intermolecular interaction under complex conditions. In detail, Fe³⁺ and TP were firstly mixed and dissolved completely in the volatile solvent (e.g. methanol, ethanol, etc), and then the solvent was removed to obtain solid powders of their uniform mixture. After the sample was dried and pressed into KBr tablets, the change in infrared spectrum was analyzed by LWR300 FT-IR Fourier transform infrared spectroscopy in the mode of light transmittance analysis, and the spectral scanning range was 4000–400 cm⁻¹. The infrared spectra of tea polyphenols before and after Fe³⁺ treatment are shown in Figure 3.

![Figure 2. UV spectra of TP with and without ionic liquid (the molar ratio of Fe³⁺: polyphenols = 1:1, 2:1, 3:1)](image)

![Figure 3. FT-IR spectra of tea polyphenols and TP-FeCl₃ complex (KBr disc)](image)
The spectrum indicates that O-H characteristic stretching vibration absorbance can be found at 3422–3300 cm\(^{-1}\), proving that phenol hydroxyl exists in all molecules. Moreover, within the range of 1715–1670 cm\(^{-1}\), there is characteristic absorption of carbonyl, and the peak position (wave number, cm\(^{-1}\)) and intensity are sensitive to the chemical environment of the carbonyl group; e.g. as long as its chemical environment changes, the wave number of its absorption peak will undergo blue shift or red shift. After interaction with iron ions, the carbonyl stretching vibration peak is red shifted from 1695.66 cm\(^{-1}\) of tea polyphenols to 1678.23 cm\(^{-1}\). This is because iron ions form coordination bond with phenolic hydroxyl groups, which affects the vibration frequency of C=O stretching of carbonyl, resulting in the shift to lower wave number. Furthermore, a strong absorption peak appeared near 669 cm\(^{-1}\), which is a characteristic peak of the Fe-TP complex. In addition, the spectrums are similar under the presence or absence of Fe\(^{3+}\), and the absorption peak resulting from the vibrations of many groups in TP structure can also be observed on the infrared spectrum of the treated samples.

4. Conclusions

As the result, TP extraction can be achieved in a very short time without too much consumption of ionic liquids and microwave; the highest extraction efficiency of 0.8 mol/L C\(_3\)MIMFeCl\(_4\) in microwave assisted mode could be obtained as 159.8 mg/g when solid-liquid ratio was 1:20, microwave power was 200 W and extraction time was 7 min. Compared with ultrasonic assisted extraction, its extraction efficiency was a little lower meanwhile extraction time was shorten significantly, which was an obvious advantage of microwave assisted extraction. Due to the strong polarity of the magnetic ionic liquid, when it is used in the microwave assisted process, it can quickly absorb microwave energy and increase the temperature; the other organic solvent is no longer needed, thus the extraction time is greatly shortened. At the temperature lower than 80 °C, the UV absorption spectrum and content of TP total phenol were more stable than those of single compound EGCG. IR spectra proved the interaction between TP and Fe (III) together with the influence of the latter. The complex system composed of many compounds with high value of entropy was indeed more stable, and other co-existing constituents could protect EGCG effectively in the solution system. It was also proved that FeCl\(_3\) could reduce the stability of tea polyphenol solution. On the contrary, IL can provide greater stabilization energy for these extraction objects in this study at some extent, so it plays a crucial role in the stability of the whole system. According to the infrared spectrum of tea polyphenols and its mixture with Fe\(^{3+}\), it can be found that iron ions would cause the carbonyl stretching vibration peak to lower wave number. Moreover, the characteristic peak of metal ion complex appeared, indicating that Fe\(^{3+}\) under certain conditions would have complexation with tea polyphenols.

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