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Combination of Microwave Instrumentation and Green Technology Based on Magnetic Ionic Liquid for Effective Extraction of Bioactive Polyphenols

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Keywords: Microwave; Magnetic Ionic Liquid; Tea Polyphenols; Green Technology

ABSTRACT

In this study a kind of magnetic ionic liquid (MIL) was used as extractant for tea polyphenols (TP). Compared with ultrasonic-assisted mode in previous study, the extraction time was shortened significantly from 300 min to 7 min. Moreover, MIL can be easily recovered by external magnetic field. As the result, when microwave power was set at 200 W and solid-liquid ratio was 1:20, 159.8 mg/g could be obtained as the highest extraction efficiency of 0.8 mol/L $C_3MIMFeCl_4$ (*N*-propyl substituted methylimidazole ferric tetrachloride salt). Besides that, stability of TP together with related intermolecular interactions were further investigated under related conditions by spectroscopic analysis; it was proved IL could provide greater stabilization energy for these extracted objects in this study at some extent. Through comparison with current applications and extraction performance, the developed method and related baisc data are expected to promote the industrialization of green effective technologies based on ILs and microwave.

INTRODUCTION

Tea polyphenols (TP) are a kind of bioactive compounds beneficial to human health. Especially, the catechins account for 70% of the total amount of TP, which are easy to be oxidized and become brown in alkaline condition under high temperature. 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. 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. As a useful mass-transfer enhancement technology, ultrasound has been widely used in related engineering fields (Hsu et al., 2013). In the previous research work (Feng et al., 2018), ultrasonic assisted extraction (UAE) for efficient selective extraction of tea polyphenols has been established by using $C_3MIMFeCl_4$, which firstly employed this kind of new magnetic ionic liquid for natural products. After careful screening, it was suggested that ferro-concentration was 0.8 mol/L and ferro-style in the IL was in trivalent ion for satisfied performance. Currently, microwave is widely applied in research and production as a fast, controllable and selective green way (Sun et al., 2010), and the extraction under its assistance is the effective separation of some organic components from the matrix in solid or semi-solid substances by the action of electromagnetic field. On the other hand, as a special kind of green solvent, magnetic ionic liquids (MIL) have been also widely applied in extraction, which can be recovered by external field very conveniently; it make the application process cleaner and efficiently just like water-soluble magnetic nanoparticles (Chen et al., 2008). In summary, the combination of microwave radiation and green solvent can be explored in efficient preparation of natural bioactive products.

EXPERIMENTAL METHODS

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Tea leaf powders were bought from local store, and all of solvents and reagents were of analytical-reagent grade or higher. Relevant microwave extraction equipment was self-made on the basis of household microwave oven. The equipment has a stable power source, accurate temperature measurement and control devices. According to pilot experiment, 0.8 mol/L $C_3MIMFeCl_4$ in its aqueous solution was the most ideal extractant and solid-liquid ratio (1:10~30, g/mL), extraction time (0~10 min) together with microwave power (0~500 W) were investigated as main influential factors. In Figure 1(a), 3 g of tea-leaf powders were placed in a 50 mL conical flask; 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 for quantitation of TP yield (mg/g). The quantitative methods of total polyphenols and EGCG content together with stability investigation of the latter accorded with previous methods (Feng et al., 2018). Because EGCG is the monomer with the highest content (40%) among TP, it is necessary to determine it separately. As the result, the total phenol content was measured at 730 nm and calculated with the linear relationship between absorbance and concentration ($y=0.008x+0.061$, $R^2=0.9999$), and quantitative analysis for EGCG by high performance liquid chromatography was performed by working curve based on peak area and concentration ($y=2.233\times10^4x+26.93\times10^4$, $R^2=0.9999$) at 278 nm.

RESULTS AND DISCUSSION

In the extraction process, the high-frequency microwave 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. With extraction efficiency as evaluation index, the optimized conditions were determined by orthogonal experiments of three factors and three levels for main factors, which include solid-liquid ratio (1:10, 1:20, 1:30), microwave power (0 W, low- medium heat power: 200 W, medium heat power: 400 W) and time (4, 7, 10 min). As the result, the highest extraction efficiency with 0.8 mol/L $C_3MIMFeCl_4$ 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. Moreover, the influence of related conditons was microwave power > time > solid-liquid ratio, which also can be found in Fig.1(b). Over high energy can decompose tratget constituents, and long extraction time is also harmful. Compared with the method of Feng et al., 2018, the extraction time was shortened significantly from 300 min to 7 min, which proved microwave radiation was effective to promote organic components separated from the matrix. For its strong polarity, when the MIL is used in the microwave assisted process, it can quickly absorb radiation energy and increase the temperature, thus the extraction time is greatly shortened. For comprehensive comparison, Table 1 summarizes the difference among various extraction methods, and it can be found about their respective characteristics and conditions. Furthermore, Figure 2 shows the comparison on extraction efficiency of these methods. Compared with other reports (Wang et al., 2017) for the extraction of similar natural products with phenolic hydroxyl groups, the microwave power was lower and extraction time was shortened at different extent in this study.

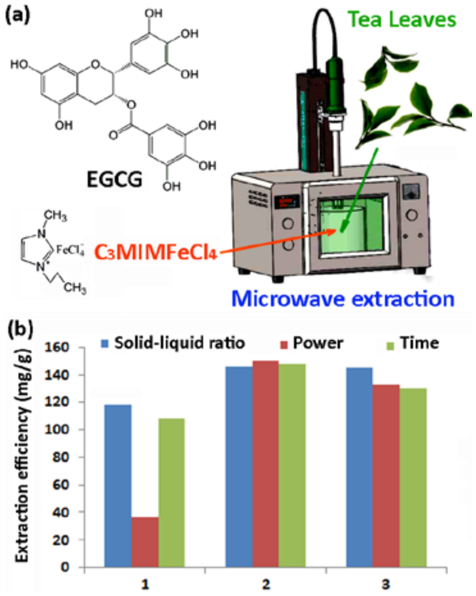

Fig. 1. Ionic liquid and EGCG (main constituent in tea leaves) in microwave extraction[a] and comparative analysis for different conditions at low, medium and high levels (1~3)[b]

On the other hand, excessive microwave radiation may be harmful to the TP stability together with its thermal effect; part of radiation influence on extraction efficiency is acutally exerted by its impact on the stability of TP. Moreover, the effect of Fe^{3+} from the anion of $C_3MIMFeCl_4$ on extracted TP molecules also should be explored when the MIL is used as as a new kind of special extractant. Here $C_3MIMPro$ was selected as a compared solvent, which was composed of the same cation with $C_3MIMFeCl_4$ and nonmagnetic proline anion. The latter ($[Pro]^-$) has been proved to have strong recognition ability for TP in previous study. Generally, the duration of both production and experimental research of TP is mostly controlled within 24 h, so the period of stability investigation for TPs was determined as one day in this study. The UV full-wavelength spectra of the tea polyphenols solution are shown in Figure 3 (a-c) before and after the addition of different molar ratios of Fe^{3+} and the magnetic or common ionic liquid. The results demonstrate that the ultraviolet full-wavelength spectrum of the tea polyphenol mixed with Fe^{3+} 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 at 273 nm when the ionic liquid of C_3MIMPro with the equimolar amount of $FeCl_3$ was added to the mixed solution of the TP-iron ions. It probably result from the preferential interaction between Fe(III) ions and proline anion in IL, which shows the latter has a protective effect on TP. Finally, $C_3MIMFeCl_4$ replaced $FeCl_3$ 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 $FeCl_3$, and the maximum absorption wavelength existed near 273 nm. Compared with the ordinary ferric salt, the ionic liquids with the $FeCl_4^-$ anion has a less destructive impact on the conjugated structure of TP, which is beneficial to the stability of whole system.

Table 1. Comparison on different TP extraction ways

Solvent	Solid-liquid ratio	Time	External conditions	Ref.
50% DMF	1g:10mL×3	60min×3	room temperature	[1]Nihal et al., 2006
50% DMF	1g:10mL×3	60min×3	room temperature	[2]Nihal et al., 2006
70% EtOH	1g:40mL	165 min	75ºC electric heating	Zhang et al., 2016
80% EtOH	1g:10mL×2	25min×2	260 W ultrasound	Zhang, 2010
Water	1g:20mL	30min	80ºC+ 200 W microwave	[1]Ezzohra et al., 2009
Water	1g:20mL	20min	100ºC+ 200 W microwave	[2]Ezzohra et al., 2009
Water	1g:16mL	40min	96ºC electric heating	Zhang et al., 2012
Super critical CO_2 + 65% EtOH	10g:4mL	60min	50ºC + 35 MPa	Wang et al., 2009

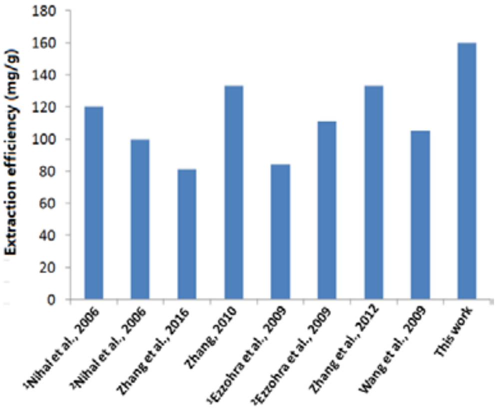

Fig. 2. Comparison on extraction efficiency of above methods in Table 1

Besides that, the IR spectra in Fig. 4 indicate that O-H characteristic stretching vibration exists at 3422~3300 cm^{-1}, proving that phenol hydroxyls exist in all molecules. Generally, the IR characteristic range of phenolic hydroxyl peaks is between 3400

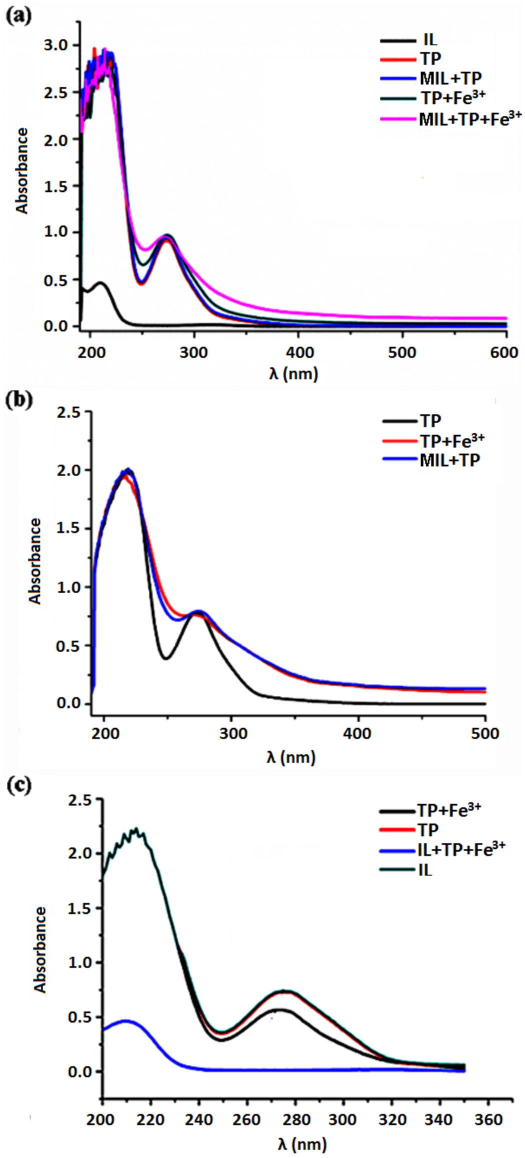

Fig. 3. UV spectra of TP with and without MIL/IL (the molar ratio of Fe^{3+}: TP = 1:1^a, 2:1^b, 3:1^c)

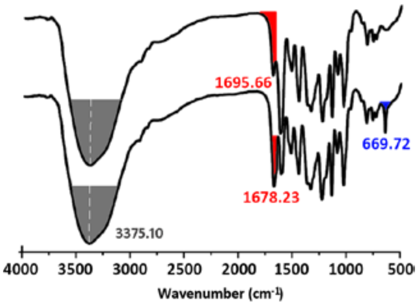

Fig. 4. FT-IR spectra (KBr disc) of tea polyphenols (upper) and $TP\text{-}FeCl_3$ complex (lower)

and 3200 cm^{-1} (peak position near 3375 cm^{-1} in Fig. 4). If there is hydrogen bond association, the peak will broaden; the more intermolecular and intramolecular hydrogen bonds, the wider the peak becomes. Taking EGCG as an example, after interacting with Fe^{3+}, its phenolic hydroxyl peaks has become wider. Moreover, within the range of 1715~1670 cm^{-1}, there is characteristic absorption of carbonyl. Both its peak position and intensity are sensitive to surrounding chemical environment; as long as adjacent groups exert the influence on it through steric effect, electronic effect or H-bond, the wave number of its absorption peak will undergo blue shift or red shift. After interaction with iron ions, the carbonyl stretching vibration peak of TP is red shifted from 1695.66 cm^{-1} to 1678.23 cm^{-1}. Iron ions form coordination bond with phenolic hydroxyls, which affects the wavenumber of C=O stretching of carbonyl, resulting in the shift to lower wave number. Furthermore, after formation of the complex, the vibaration peak of $Fe\cdots O$ will appear in the range of 550~700 cm^{-1}. Accordingly, a strong absorption signal can be found near 669 cm^{-1}, which is a characteristic peak of the Fe-TP complex. In addition, the spectra are similar under the presence or absence of Fe^{3+}, and many medium or weak peaks resulting from the vibrations of related groups in TP structure can also be observed in IR spetra of the complex, which indicate the TP structure can be well remained.

CONCLUSIONS

After highly efficient extraction within short time, TP can be obtained with less consumption of ionic liquids and energy in the microwave-assisted mode, which shows unique advantages and features by comparison with current reports. For these unstable constituents, IL also can provide obvious stabilization effect through molecular interactions; the TP structure can be well remained. This study is expected to lay the foundation for industrialization of green technologies based on ILs and microwave.

REFERENCES

Chen, C.J., and Lai, H.Y., “Production control and characterization of bilayer-surfactant coated stable water-soluble monodisperse magnetic iron oxide nanoparticle”, *J. Chin. Soc. Mech. Eng.*, Vol. 29, No. 6, pp. 457-465 (2008).

Ezzohra, N., Valerie, T., Hakima E.H., Es-Seddik E.B., Farid C., and Olivier D., “Microwave-assisted water extraction of green tea polyphenols”, *Phytochem. Anal.*, Vol. 20, pp. 408-415 (2009).

Feng, X.T., Zhang, W., Zhang, T.H., and Yao, S., “Systematic investigation for extraction and separation of polyphenols in tea leaves by magnetic ionic liquids”, *J. Sci. Food Agric.*, Vol. 98, No. 12, pp. 4550-4560 (2018).

Hsu, I.C., and Tsao, C.C., “Analysis of Burr Height in Ultrasonic-Assisted Drilling of Ti-6Al-4V Alloy”, *J. Chin. Soc. Mech. Eng.*, Vol. 34, No. 1, pp. 31-38 (2013).

Nihal, T., Ferda, S., and Sedat, V., “Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods”, *Food Chem.*, Vol. 99, No. 4, pp. 835-841 (2006).

Sun, M.T., and Wu, C.P., “A numerical study on the evenness of gas flow field in a vacuum chamber for microwave ECR PE-CVD process on large sized substrates”, *J. Chin. Soc. Mech. Eng.*, Vol. 31, No. 5, pp. 435-442 (2010).

Wang, F., Sun, Q., Hou, Y.Y., Han, F.Y., and Li, H.Z., “Optimization of the extraction process of total coumarin from Cortex Qinpi by ionic liquid combined with ultrasound and microwave”, *Chin. Trad. Pat. Med.*, Vol. 39, No. 9, pp. 1947-1950 (2017).

Zhang, S., “Study on extraction process of tea polyphenols”, *Food Sci. & Tech.*, Vol. 35, No. 8, pp. 267-270 (2010).

Zhang, X., Cheng, J., Yao, L., and Pan, Y., “Extraction and antioxidant activity of tea polyphenols from baiyaqilan tea”, *Chin. J. Bioproc. Eng.*, Vol. 14, No. 4, pp. 48-54 (2016).

Zhang, X., Xu, F., Gao, Y., Wu, J., Sun, Y., and Zeng, X., “Optimising the extraction of tea polyphenols, (-)-epigallocatechin gallate and theanine from summer green tea by using response surface methodology”, *Int. J. Food Sci & Tech.*, Vol. 47, No. 10, pp. 2151-2157 (2012).