Application of the Microwave-assisted Process to the Fast Extraction of Isoflavone from the Waste Residue of the Soybeans

Li Hua,* Hu Guoqin, and Li Dan

School of Chemical and Energy Engineering, Zhengzhou University, Zhengzhou, Henan 450001, China
**E-mail: Lihua@zzu.edu.cn*
Received April 10, 2009, Accepted September 26, 2009

Due to the importance of isoflavone content in soybean extracts, the microwave-assisted process (MAP) was compared to the conventional extraction methods. For comparison of the three methods, all extraction parameters (solvent, sample to solvent ratio, temperature, *etc*.) were kept the same; the microwave extractor was operated at 187.5 W with an emission frequency of 2450 MHz under atmospheric pressure conditions and the extractions were carried out at 75 °C for 3 min while the conventional reflux was at 75 °C for 3 h. Total yield and crude isoflavone content were determined by ultraviolet spectrophotometric and compared with the three methods. Results indicated that the MAP was comparable to the conventional method in its capability to extract target compounds without causing any degradation; in addition it dramatically reduced the extraction time from 3 h to a few minutes, suggesting that it can be an alternative technique to the time-consuming conventional reflux method.

Key Words: Microwave-assisted process (MAP), Isoflavone, Extraction, Soybean, Waste residue

Introduction

Isoflavone have been known to be the main bioactive components of soybeans. The characteristic isoflavones can be divided into two major groups, according to the chemical properties, namely, the Aglycon and Glucoside.[1] The beneficial effects of isoflavones in the prevention of chronic diseases such as coronary heart disease and osteoporosis, as well as alleviation of postmen-opausal syndrome have been well established.[2-7]

China is a large soybean producer, and much of the soybeans are being processed into bean products and extracted for their oil. But presently, the use of soybeans is only limited to extracting their protein and oil.[8,9] Unfortunately, active compounds in residue and byproduct are often abandoned in great quantities. Therefore, isoflavones can be derived from the waste residue of the soybeans. If one is able to fully utilize the active compounds such as isoflavone in soy waste material, will certainly garner great economic and social benefits.

This paper prescribes the methods for preparing soybean isoflavone. Since the isoflavone content is one of the most important quality indices in soybean products, it is normally a time-consuming process to extract isoflavone using traditional techniques. A conventional 3-hour reflux at 75 °C using 80% ethanol which is then repeated 4 times is the current standard extraction method for isoflavone in the laboratory setting. However, for the mass production of soybean extracts, such as in manufacturing of various soybean products, longer periods of extraction with ethanol are being used, for example 8-hour steps which are repeated 4 times. As a result, the conventional processes are required to be improved in terms of the extraction time and energy consumption.

The microwave-assisted processes make use of microwaves to perform quick extractions of target compounds from various materials. The advantages of this process over the conventional methods include shorter extraction times, less energy consumption, and minimum waste generation.[10,11]

This study intends to confirm the efficacy of MAP as a fast extraction means of isoflavone as compared to the conventional reflux methods in the determination of isoflavone content in soybean.

Experimental Section

Reagents and materials. All reagents including ethanol, acetone, acetic ester, cyclohexane, were of AR grade. The isoflavone standards were purchased from the Sigma-Aldrich Group, the waste residue of the soybeans were purchased from the local food processing factory in China, sample were dried and sieved to select particles of 36 mesh and stored at room temperature until used.

Apparatus. A conventional reflux extractor (250 mL) was used as control against the microwave-assisted extraction. The MAP extractor was operated with a focused irradiation under atmospheric pressure conditions. The basic apparatus consisted of a command box and a microwave module. It operates with an emission frequency of 2450 MHz and a power output varying between 30 W and 700 W in 15 W increments. The apparatus is equipped with a 250-mL quartz vessel, a Graham-type refrigerant column (400-mm length) and a bent extraction tube. The temperature-measuring probe was a digital Megal 500 gas thermometer. Solvents were evaporated using a rotary evaporator.

Extraction procedures. (a) Conventional extraction of isoflavone: A 250-mL round bottom flask fitted with a cooling condenser was used to perform the extractions according to the method described by Li *et al*.[11,12] Extractions were carried out at 75 °C for 3 hours on a mixture consisting of 2.00 g of sample powder and 50 mL of 80% ethanol. When the extraction was complete, the cooled extract was filtered on double layers of filter paper under vacuum and collected in a volumetric flask. The residue was taken back and re-extracted three more times using

fresh solvents each time with the same conditions as above. The condenser was washed upon completion of the extraction with 20 mL of solvent. The washings were collected in the extraction vessel that was further washed with 20 mL of solvents. The washings were added to the extract, and then the flask was filled up to fixed volume. The combined extracts (250 mL) were used for the determination of total extract yield and isoflavone contents.[13,14]

(b) Microwave-assisted extraction of isoflavone: MAP extractions were performed at 187.5 W power for 3 min on a mixture consisting of different amounts of soy powders (2.00, 2.50, 5.00 and 10.00 g) and 50 mL of 80% ethanol. The effect of irradiation time (1, 2, 3, 4, 5 min) was determined using 2.00 g of the sample. At MAP conditions of 2.00 g and 3 minutes, the effects of variation in microwave power (0.0, 37.5, 112.5, 187.5, 300.0, 375.0 W) was also determined. Based on these conditions, powdered samples (2.00 ± 0.01 g) were weighed and transferred to a 250 mL quartz extraction vessel of the microwave-assisted extraction system. Upon completion of irradiation, the extracts were treated for the reflux method mentioned earlier.

(c) Soxhlet extraction of isoflavone: 2.00 g of sample were weighted in filter paper and placed in a 250 mL Soxhlet glass thimble. The extraction was carried out using 80% ethanol as a solvent at 75 ^{o}C for 3 hours. The crude extract was concentrated using a rotary evaporator under vacuum and collected in a volumetric flask.

Determination of the yield of total-isoflavones. Since there exists several isoflavones in soybeans, total-isoflavones was adopted to describe the characteristics of isoflavones.[15,16] The yield of total isoflavones was determined using a UV spectrophotometer.[8,9] Several soybean-ethanol-water solutions were analyzed at 260 nm using a UV spectrophotometer (TU-1810, Beijing Purkinje General Instrument Co., Ltd, China) to obtain the standard curve $C = 7.9569 \times A + 0.9551$ ($r^2 = 0.9978$), where C is the concentration (mg·L^{-1}) of genistein in the solution and A is the absorbance of the solution. The amount of total-isoflavones in the extract was obtained from the standard curve by measuring the absorbance of its ethanol-water solution. Yields of the total-isoflavones are given on a mass basis related to the dry materials of the soybeans.

The measurement of the yields of the total-isoflavones was described as follows;

2.00 g of sample were weighted and placed in a glass vessel, the extraction was carried out using 80% ethanol as a solvent at certain extraction condition. When the extraction was complete, the solution was leached under vacuum and the solid content (filter cake) was weighed. The liquid, which has passed through a filter was collected in a volumetric flask and diluted to certain multiple, and later was used to determinate the absorbance of the solution using a UV spectrophotometer. The concentration of isoflavone was obtained according to the standard curve equation, and the yields of the total-isoflavones was calculated as follows.

The yields of the total-isoflavones = actual output of isoflavone ÷ the sum of the masses of soybeans × 100% = volume of the extracted liquid × concentration of extracted liquid ÷ the sum of the masses of soybeans × 100%.

The determination of total extract yield and absorptive characteristics of the extract were performed in triplicates. The experimentally determined values for the sample by microwave-assisted extraction were compared to those from the conventional reflux method as well as the method of the Soxhlet extraction for the same samples.

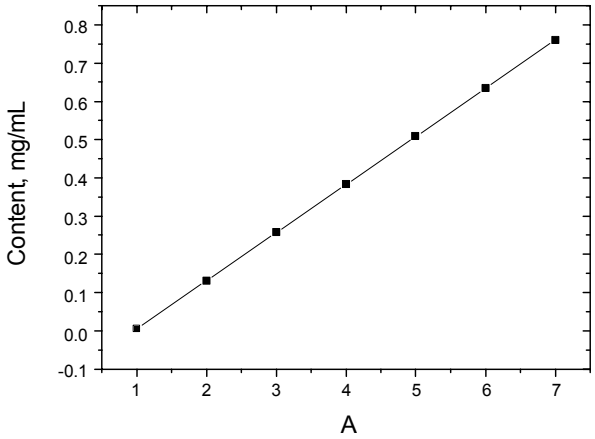

Figure 1. Standard Curve for isoflavone.

Table 1. Effect of the sample to solvent ratio on total yield and isoflavone contents of 80% ethanol extract of soy powder in microwave-assisted process

sample to solvent ratio (w/v)	1:10	1:20	1:25	1:30
yield, %	0.2512	0.2726	0.3282	0.2504

Results and Discussion

The Standard curve for isoflavone. The standard curve of isoflavone contents were analyzed using Genistein standards at 260 nm using a UV spectrophotometer and shown in Figure 1. From Figure 1, the standard curve were obtained, $C = 7.9569 \times A + 0.9551$ ($r^2 = 0.9978$). The amount of total-isoflavones in the extract was obtained from the standard curve by measuring the absorbance of its ethanol-water solution.

MAP extraction conditions. The effects of the sample to solvent ratio (w/v) on total yield and isoflavone contents of 80% ethanol extract of soy powder in microwave-assisted process were listed in Table 1. From Table 1, it can be found that the different sample-solvent ratio had different influence on the yield of isoflavones. As can be seen, the total amount of isoflavones extracted increased with the increase on sample-solvent ratio from 1:10 to 1:25. When the sample-solvent ratio was 1:25, the highest yield of isoflavones was obtained. Although the higher sample-solvent ratio can quicken the extraction rate, ratios higher than 1:25 did not increase yield of isoflavones. Most likely, it was due to the volume of extraction solvent was too large; excessive pressure could be generated on the closed extraction cells, which led to the solvent evaporation and even spatter. Comparing the effectiveness of each of sample-solvent ratio on individual yield of each isoflavone, it is clear that 1:25 is the most efficient. Based on these observations, since 1:25 is the most adequate sample-solvent ratio using samples of 2.00 g, the

ratio will be used for further optimization of extraction conditions.

The effect of irradiation time (1, 2, 3, 4, 5 min) on the yield was performed using 2.00 g samples and the results were shown in Table 2. From Table 2, it can be found that increasing the irradiation time from 1 to 3 minutes gradually increased the yield of the extracted isoflavones and the irradiation time longer than 3 minutes did not increase yield of isoflavones. This was an indication that quantitative recoveries were achieved and that longer extraction time was unnecessary and also may increase variation and degradation of isoflavones, which will lead to a lower extraction yield because of decreased interaction between solvent and sample. Thus it was considered that 3 minutes of irradiation time was the optimal time to ensure the achievement of quantitative extraction.

From Table 3, one can see that the yield of isoflavones increased with the increase of the irradiation power. However, as the power reached levels higher than 187.5 W, the yield of isoflavones decreased with the increase of the irradiation power. The main reason was due to the fact that by increasing the microwave power from 37.5 to 187.5 W, the energy obtained from microwave irradiation of the fixed material gradually increased in unit time, this aggravated the cells to break down of the soybeans and random thermal motion between active ingredient of soybean isoflavones in soybean cake and solvent molecules, leading to a higher extraction yield because of increased interaction between solvent and sample. However, the solvent used, ethanol, had relatively high dielectric properties ($\varepsilon = 24$) to absorb microwaves, and thus easily reached its boiling point when irradiated. When the irradiation power was higher than 187.5 W, it was enough to reach the boiling point (78 ^{o}C) for an 80% ethanol solution with or without the sample and caused rapid boiling of the extraction mixture, Due to the intensity of localized temperature, degradation of the isoflavones was inevitable. Thus it was considered that 187.5 W of the power output was the optimum power for the extraction by MAP.

Table 2. Effect of irradiation time on total yield and isoflavone content of 80% ethanol extract of soy powder in microwave-assisted process (MAP)

irradiation time, min	1	2	3	4	5
yield, %	0.2399	0.3013	0.4584	0.3375	0.3143

Table 3. Effect of irradiation power on total yield and isoflavone content of 80% ethanol extract of soy powder in microwave-assisted process (MAP)

irradiation power, W	37.5	112.5	187.5	300.0	375.0
yield, %	0.3263	0.3669	0.4627	0.3073	0.3115

Table 4. Orthogonal experiment in microwave-assisted process

	irradiation power, W	irradiation time, min	sample to solvent ratio	yield, %
1	112.5	2	1:20	0.3201
2	187.5	2	1:25	0.3782
3	112.5	3	1:25	0.4504
4	187.5	3	1:20	0.4126
K_1 (sum of grade I)	0.7705	0.6983	0.7327	
K_2 (sum of grade II)	0.7908	0.8630	0.8286	
R (extreme difference)	0.0203	0.1647	0.0959	

The Orthogonal experiment in microwave-assisted process was arranged, the result was listed in Table 4. From Table 4, the optimum yield was obtained at 3 minutes of irradiation, 187.5 W of irradiation power, and 1:25 of sample to solvent ratio.

The efficacy of extraction steps in MAP. The effect of repeated and successive extractions of the residue was compared between MAP and conventional reflux (see Table 5). In the initial extraction step of both MAP (3 minutes, 80% ethanol) and the reflux (3 hours) methods, the relative yield and isoflavone content were higher in the MAP (77.23%, 81.55%) than in the conventional method (69.82%, 68.89%). The second extraction of the residue made it possible to extract 94.91% and 90.34% in total extract yield and 98.31% and 90.69% in isoflavone contents, respectively, by the MAP and the conventional reflux. By the fourth extraction, similar amounts of total yield and crude isoflavone were obtained by both methods.

Validation of MAP extraction for soybean. The total ethanol soluble extract of soybean is normally used for the commercial production of soy products. This is required to contain more isoflavone contents. The validation of the microwave-assisted extraction technique was performed by comparing the values obtained for isoflavone samples. Table 5 shows that the values obtained for the total yield and the crude isoflavone by the MAP extraction were similar to the values of conventional reflux. Table 6 shows the comparison result for the soybean isoflavones using three different methods: Soxhlet extraction, conventional reflux and MAP extraction methods. These results confirmed

Table 5. The experimental result between microwave-assisted process and the conventional reflux at 75 ^{o}C

No. of extraction step	MAP		conventional reflux	
	yield, % (Rel.%)	content (μg/g) (Rel.%)	yield, % (Rel.%)	content (μg/g) (Rel.%)
1	0.3862 (77.23)	1187(81.55)	0.3491 (69.82)	1003 (68.89)
2	0.0884 (17.68)	244 (16.76)	0.1026 (20.52)	317 (21.80)
3	0.0215 (4.3)	16.5 (1.13)	0.0415 (8.30)	122 (8.35)
4	0.00395 (0.79)	8 (0.56)	0.0068 (1.36)	14 (0.96)
Total	0.5000 (100)	1456 (100)	0.5000 (100)	1456 (100)

Table 6. Comparison of total yield and isoflavone contents of 80% ethanol extract from soy powder by different extraction methods

extraction methods	Soxhlet extraction	Conventional reflux	MAP
yield, % (Rel. %)	0.23 (46)	0.45 (90)	0.46 (91.4)

the possibility of microwave-assisted extraction as an alternative method to the time-consuming conventional reflux in the extraction of isoflavone compounds in soybean.

Conclusions

In summary, the main limitation of the current extraction techniques for isoflavone was the long extraction period (totaling up to 3 hours) using 80% ethanol. Since extraction time and energy consumption associated with the conventional process are not desirable, therefore need to be improved. In this study, the advantage of MAP over the conventional reflux was validated in that the extraction time was dramatically reduced from more than 3 hours to a few minutes, thereby saving considerable amounts of energy used for the process.

Due to the abundance and low price of soybeans in China, the extraction isoflavone from the waste residue of the soybeans can not only fully utilize the active compounds in waste residue, but may also be used as an alternative resource. By developing and applying the multitudes of valuable resources such as isoflavone contained in soybeans, it becomes clear that the develop ments in techniques such as MAP will achieve many social and economic benefits.

References

1. Kao, T. H.; Chen, J. T.; Chen, B. H. *Food Chemistry* **2008**, *107*, 1728.
2. Rostagno, M. A.; Palma, M.; Barroso, C. G. *J. Chromatogr. A* **2005**, *1076*, 110.
3. Xu, H. N.; He, C. H. *Separation and Purification Technology* **2007**, *56*, 85.
4. Fritz, K. L.; Seppanen, C. M.; Kurzer, M. S.; Csallany, A. S. *Nutrition Research* **2003**, *23*, 479.
5. Lydeking-Olsen, E.; Jensen, J. B. E.; Setchell, K. D. R.; Damhus, M.; Jensen, T. H. *J. of Nutrition* **2002**, *132*, 581S.
6. Setchell, K. D.; Cassidy, A. *J. of Nutrition* **1999**, *129*, 758S.
7. Zubik, L.; Meydani, M. *American of Journal of Clinical Nutrition* **2003**, *77*, 1459.
8. Kao, T. H.; Chen, B. H. *J. of Agricultural and Food Chemistry* **2006**, *54*, 7544.
9. Kao, T. H.; Lu, Y. F; Chen, B. H. *European Food Research and Technology* **2005**, *221*, 459.
10. Mauricio, A. R.; Miguel, P.; Carmelo, G. B. *Analytica Chimica Acta* **2007**, *588*, 274.
11. Li, H. *Food Science and Technology* **2007**, *33*, 230.
12. Li, H. *Science and Technology of Food Industry* **2007**, *29*, 168.
13. Li, H. *Food Science and Technology* **2008**, *34*, 122.
14. Gao, J. Y.; Xu, J. L. *China food Additives* **2003**, *14*, 16.
15. Zhang, Z. T.; Wang, X. L.; Liu, Q. G.; Chen, Z. G.; Gao, Z. W. *Chin. Pharm. J.* **1999**, *34*, 301.
16. Li, J. J.; Li, W. H.; Gao, X.; Li, D. W.; Qin, W. N.; Huang, W. *J. Northwest Univ.* **1998**, *28*, 131.