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Effects of technological parameters of ultrasonic treatment on the protein extraction yield from defatted peanut meal

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Abstract

In the present work, mixture of defatted peanut meal and water was treated with ultrasound for protein extraction. The effects of technological parameters of the ultrasonic treatment including defatted peanut meal/water ratio, ultrasonic power, pH, temperature and time on the protein yield were investigated. The obtained results showed that the ultrasonic treatment reduced the material particle size as well as increased the protein yield by 19% in comparison with the conventional extraction. At the defatted peanut meal/water ratio of 1:20 (w/v), ultrasonic power of 30 W/g, pH of 6.8, temperature of 50°C and sonication time of 15 min, the protein yield achieved maximum of 87.7 ± 0.7%. The use of ultrasound in peanut protein extraction could therefore be a potential technique for improvement in protein yield.

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Introduction

Peanut (*Arachis hypogaea* L.) is a food source which is rich in lipid and protein (Atasie *et al*., 2009). In Vietnam, peanut has mainly been used in edible oil production, and defatted peanut meal is a by-product from this processing. The protein content of defatted peanut meal is around 47 - 55%, and peanut protein contained various essential amino acids (Basha and Pancholy, 1982). Defatted peanut meal is therefore a potential material in the production of protein concentrate and isolate.

In the production of protein concentrate and isolate, extraction is a critical process due to its great impact on the product yield and quality. Alkali is often used as the conventional solvent in peanut protein extraction (Yu *et al*., 2007). However, salt-soluble globulin is the main fraction of peanut protein (Cherry, 1990) which can be extracted by water, which is an eco-friendly and cheap solvent. Recently, the use of ultrasound in protein extraction has attracted great attention. Acoustic cavitation generated from the ultrasonic treatment improved disruption of plant cells and tissues as well as enhanced mass transfer during the extraction (Feng *et al*., 2011). It was reported that ultrasound-assisted extraction significantly increased protein yield from rice bran (Chittapalo and Noomhorm, 2009), soybean (Karki *et al*., 2010), perilla seed (Zhu and Fu, 2012), and pumpkin seed (Tu *et al*., 2015). However, the use of ultrasound in the peanut protein extraction with water solvent has not been reported.

In the present work, mixture of defatted peanut meal and water was treated with ultrasound for protein extraction. The objective of the present work was to investigate the effects of technological parameters of the ultrasonic treatment on the protein yield.

Materials and methods

Materials

Peanut variety of *Arachis hypogaea* VD1 was used in the present work. The peanut was provided by the Research Institute for Oil and Oil Plants (Ho Chi Minh City, Vietnam).

The peanut was soaked in 0.5% NaOH solution for 5 min to remove the silk sheath, followed by drying at 55°C to a moisture content of 7%, then pulverised and passed through a 400 µm sieve. The peanut meal was defatted by the Soxhlet method, and the total lipid content in the meal was less than 2%. Finally, the obtained product was crushed and then passed through a 400 µm sieve. The defatted peanut meal was kept at 4°C for use in all experiments.

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Ultrasound-assisted extraction of protein from defatted peanut meal

Defatted peanut meal and water were mixed in 0.5 L Erlenmeyer flask at the selected ratio. The suspension was then adjusted to the selected pH value by using a 2 N NaOH solution. The mixture was further heated in a thermostatic water bath to the required temperature and then treated with ultrasound by using a horn-type ultrasonic probe with frequency of 20 kHz (Model VC 750, Sonics and Materials Inc, the United States). During the ultrasonic treatment, the temperature of the mixture was kept stable by using a thermostatic water bath (Model SC100-A28; Thermo Fisher Scientific, The United States).

First series:

The material/solvent ratio was changed: 1:5, 1:10, 1:15, 1:20 and 1:25 (w/v). The pH of the suspension, ultrasonic power, temperature and time were fixed as 7.0, 30 W/g, 30°C and 10 min, respectively.

Second series:

The pH of the suspension was varied: 6.8 (natural pH), 7.0, 8.0, 9.0 and 10.0. The material/solvent ratio (w/v) was chosen from the results of the first series. The ultrasonic power, temperature and time were kept at 30 W/g, 30°C and 10 min, respectively.

Third series:

The ultrasonic power was changed: 0, 30, 45 and 60 W/g defatted peanut meal. The material/solvent ratio and pH of the suspension were selected from the results of the first and second series, respectively. The ultrasonic temperature and time were fixed at 30°C and 10 min, respectively.

Fourth series:

The sonication temperature was varied: 40, 50, 60 and 70°C. The material/solvent ratio, pH of the suspension and ultrasonic power were chosen from the results of the first, second and third series, respectively. The ultrasonic time was kept at 10 min.

Fifth series:

The ultrasonic time was changed: 0, 5, 10, 15 and 20 min. The material/solvent ratio, pH of the suspension, ultrasonic power and temperature were selected from the results of the first, second, third and fourth series, respectively.

At the end of the ultrasonic treatment, the mixture was immediately adjusted to pH 9.0 with 2 N NaOH solutions. Finally, the mixture was centrifuged at 3,000 g and 20°C for 20 min to remove the solid phase; the obtained supernatant was used for protein quantification.

Analytical methods

The total protein content in the defatted peanut meal and the extract was determined by the Kjeldahl method; the conversion factor from nitrogen to protein was 5.46 (Misra, 2001).

The particle size distribution of the material particles at the end of the protein extraction was determined by laser scattering method on the Horiba device, model LA 920 (Japan) according to the procedure proposed by Hong *et al*. (2002).

The protein profile in the extract was analysed by electrophoresis on sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) according to the procedure of Laemmli.

The viscosity was measured at 30 ± 1°C by using Brookfield viscometer DV1 with spindle no.1 and rotation rate of 100 rpm.

Calculation formula

Protein yield was calculated by the following formula:

$$Y = [(C_p \times V)/M] \times 100\% \quad \text{(Eq. 1)}$$

where C_p = protein concentration in the extract (g/L); V = extract volume (L); M = protein content in the defatted peanut meal (g) used in the protein extraction.

Statistical analysis

Each experiment was carried out in triplicate, and the results were presented as mean ± standard deviations. One-way analysis of variance was performed with the Statgraphics plus software (version 3.2). The experimental results were compared by Multiple range tests with $p = 0.05$.

Results and discussion

Effects of material/solvent ratio

The change in peanut protein yield by the material/solvent ratio is shown in Figure 1. It is apparent that the reduction in material/solvent ratio from 1:5 to 1:20 (w/v) increased the protein yield by 10.6%. It can be explained that high solvent ratio improved the mass transfer in solid-liquid extraction as well

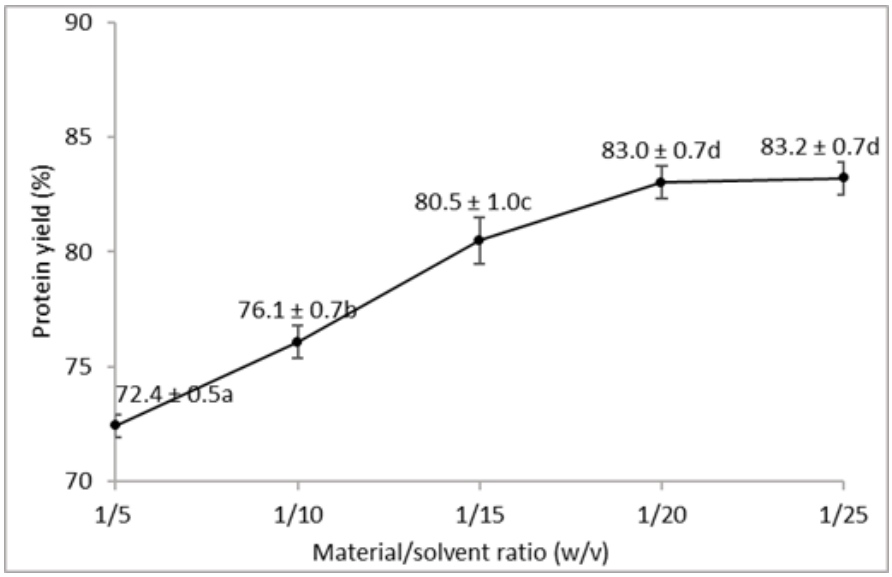

Figure 1. Effects of material/solvent ratio on the peanut protein yield (samples were treated at the ultrasonic power of 30 W/g, pH of 6.8, temperature of 30°C for 10 min; different letters indicate statistical difference ($p < 0.05$).

as the extraction yield (Berk, 1992). However, when the material/solvent ratio was decreased from 1:20 to 1:25 (w/v), the protein yield remained constant. The highest protein yield was 83.0 ± 0.7% when the material/solvent ratio was 1:20 (w/v). Similar material/solvent ratio was previously reported when defatted peanut meal was used in protein extraction without ultrasonic treatment (Yu *et al*., 2007). This ratio was therefore selected for the subsequent experiments.

Effects of pH

Alkaline pH is often used in protein extraction from defatted peanut meal due to an improved protein yield (Yu *et al*., 2007). In the present work, the initial pH of the suspension was varied from 7 to 10 since this pH range did not change functional and nutritional properties of the obtained protein concentrate (Fabian and Ju, 2011). At pH 7.0, 8.0, 9.0 and 10.0, the protein extraction yield was 83.1 ± 0.5%, 81.3 ± 0.4%, 80.8 ± 0.8% and 77.3 ± 0.7%, respectively. It was noted that the increase in pH from 7.0 to 10.0 significantly reduced the protein yield by 7.0% ($p < 0.05$). In ultrasound-assisted extraction, acoustic cavitation was the main phenomenon which contributed to the disintegration of the material cells, thereby improving the extraction efficiency (Vilkhu *et al*., 2008). Our results revealed that the suspension viscosity at pH 7.0 and 10.0 was 0.980 ± 0.015 and 1.083 ± 0.019 cp, respectively; therefore the increase in pH from 7.0 to 10.0 augmented the viscosity of the material/solvent mixture by 10.5%. High viscosity of the material/solvent mixture reduced the acoustic cavitation extent (Mason and Lorimer, 2002) and that led to a decreased protein yield. This result was contrasted to that of Yu *et al*. (2007) who reported a gradual increase in protein yield when the pH value was increased from 7 to 10 in the peanut protein extraction without ultrasonic treatment. According to these authors, NaOH could break hydrogen, amides and disulphide bonds in protein molecules, and that resulted in the improvement in protein yield. However, high pH during the extraction changed functional properties of the obtained protein (Fabian and Ju, 2011).

At the natural pH value (6.8) and pH 7.0, the peanut protein yield was similar and the highest. Consequently, pH adjustment in the ultrasound-assisted extraction of protein from defatted peanut meal was not essential. It was reported that natural pH value of material/solvent mixture was also used in the ultrasound-assisted extraction of protein from defatted soybean meal (Karki *et al*., 2010). The natural pH value (6.8) was selected for the next experiments.

Effects of ultrasonic power

The effects of ultrasonic power on the peanut protein yield are presented in Figure 2. All samples treated with ultrasound had significantly higher protein yield than the control without ultrasonic treatment ($p < 0.05$). Thus, ultrasonic treatment of defatted peanut meal significantly improved the protein yield. The increase in ultrasonic power from 0 to 30 W/g significantly enhanced the protein yield by 6% ($p < 0.05$). Further increase in ultrasonic power from 30 W/g to 60 W/g did not change the protein yield ($p > 0.05$).

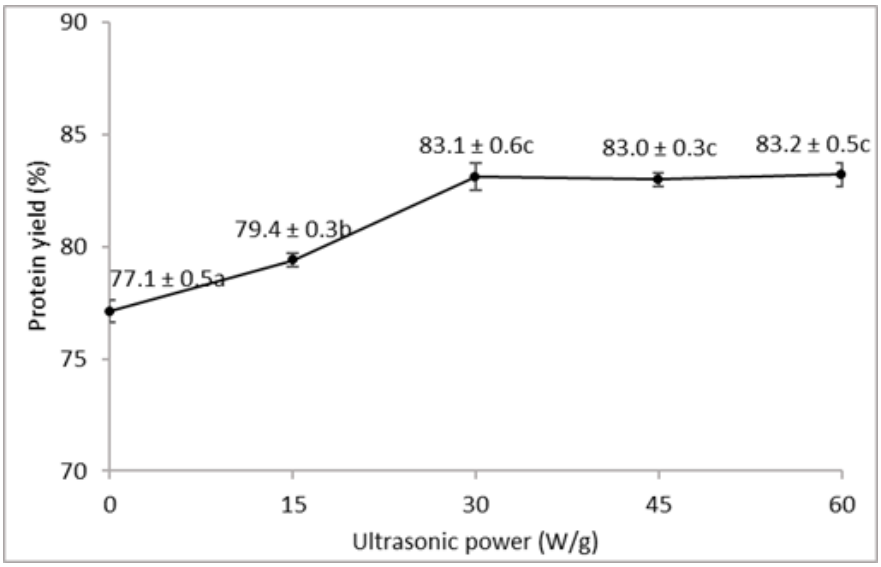

Figure 2. Effects of ultrasonic power on the peanut protein yield (samples were treated at the ultrasonic temperature 30°C, pH of 6.8 for 10 min; material/solvent ratio of 1:20 (w/v); different letters indicate statistical difference ($p < 0.05$).

The higher the ultrasonic power, the more intensive the acoustic cavitation, the greater the disintegration of the material particles and the higher the extraction efficiency. Similar observation was previously reported when ultrasound was applied to protein extraction from soybean meal (Moulton and Wang, 1982), from defatted soybean meal (Karki *et al*., 2010) and from defatted rice bran meal (Chittapalo and Noomhorm, 2009). The ultrasonic power of 30 W/g was selected for further experiments.

Effects of sonication temperature

The ultrasonic treatment at 30°C and 40°C resulted in similar protein extraction yield (83.1 ± 0.3%). The increase in sonication temperature from 40°C to 50°C enhanced the protein yield from 83.1% to 85.2%. Nevertheless, further increase in sonication temperature from 50°C to 60°C and 70°C reduced the protein yield from 85.2% to 83.0% and 80.9%, respectively. It can be explained that high temperature reduced the intensity of bubble collapse due to high vapour pressure. However, increased temperature augmented number of cavitation bubbles as well as reduced viscosity in the extraction system and that resulted in a more violent bubble collapse (Patist and Bates, 2008). As a consequence, there is an appropriate temperature at which the viscosity is low enough to produce violent cavitation bubbles, yet the temperature is low enough to avoid the dampening effect on collapse by a high vapour pressure. In addition, high temperature promoted irreversible denaturation of protein molecules in the extract (Rustom *et al*., 1991) leading to a lower protein yield. In this study, the highest protein yield was recorded at the sonication temperature of 50°C.

Previous studies showed that temperature had great impact on the protein yield when ultrasound was not used in protein extraction (Rickert *et al*., 2004; Kain *et al*., 2009). Response surface methodology was previously applied to optimise peanut protein extraction without ultrasonic treatment, and the optimal extraction temperature found was 50°C (Rustom *et al*., 1991). The appropriate temperature of ultrasonic and conventional extraction of protein from defatted peanut meal was therefore similar.

Effects of sonication time

The effects of sonication time on the protein yield is illustrated in Figure 3. Increase in sonication time from 0 to 15 min augmented the protein yield by 10.6% while longer treatment time significantly reduced the protein yield. This observation is in agreement with the findings in the ultrasound-assisted extraction of pumpkin seed protein (Tu *et al*., 2015). It can be explained that short cavitation time was not enough for complete disintegration of material particles resulting in low extraction yield. Nevertheless, prolonged sonication time led to extensive accumulation of hydroxyl-free radicals in the extract, which might react with several functional groups within the protein molecules and coagulate proteins (Jambrak *et al*., 2009).

Peanut protein extraction without ultrasonic treatment was previously investigated by various researchers. Yu *et al*. (2007) extracted peanut protein with alkaline solvent under stirring conditions for 1 h for the production of protein concentrate. In the production of protein isolate, Kain *et al*. (2009) also extracted peanut protein with alkaline solvent for 1 h.

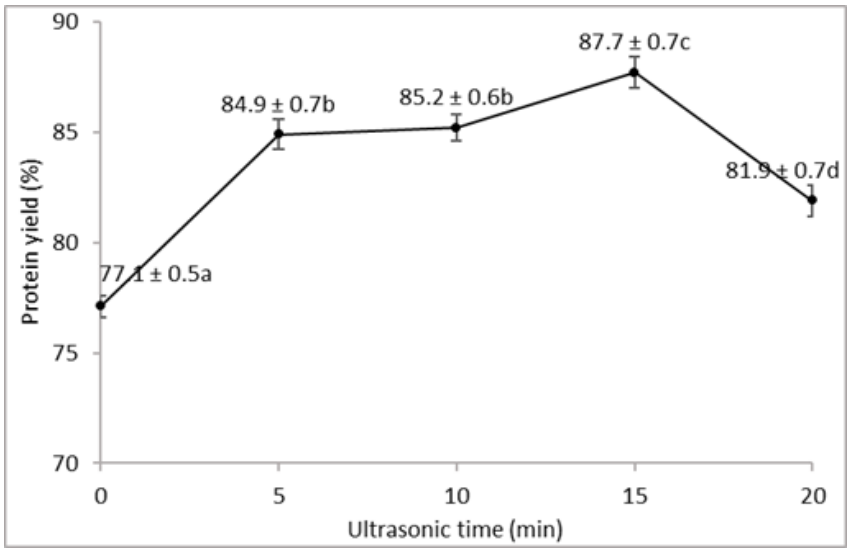

Figure 3. Effect of sonication time on the peanut protein yield (samples were treated at the ultrasonic power of 30 W/g, pH of 6.8, temperature of 50°C; material/solvent ratio of 1:20 (w/v); different letters indicate statistical difference ($p < 0.05$).

Thus, 1 h was considered to be the appropriate time for peanut protein extraction. In the present work, ultrasound was applied to peanut protein extraction. The mixture of defatted peanut meal and water was treated with ultrasound for 15 min, then adjusted the pH to 9.0 and immediately centrifuged for recovery of the protein extract. The extraction time in the ultrasound-assisted method was much shorter than that in the conventional method. Short time was a great advantage of the ultrasound-assisted method in peanut protein extraction.

Figure 4 presents particle size distribution of defatted peanut meal in the extraction system at the end of the operation. The particle size range in the conventional method (from 0 to 900 μm) was larger than that in the ultrasonic method (from 0 to 800 μm). The mean size of the material particles in the conventional extraction (151 μm) was significantly higher than that in the ultrasonic extraction (124 μm). This result confirmed the reduction of particle size of the defatted peanut meal due to acoustic cavitation during the ultrasound-assisted extraction.

Figure 5 reveals that the protein profile from both ultrasonic and conventional extraction was similar. The molecular weight of peanut protein varied from 10 to 70 KDa. It can be concluded that the use of ultrasound only improved the protein extraction yield but did not change the protein composition.

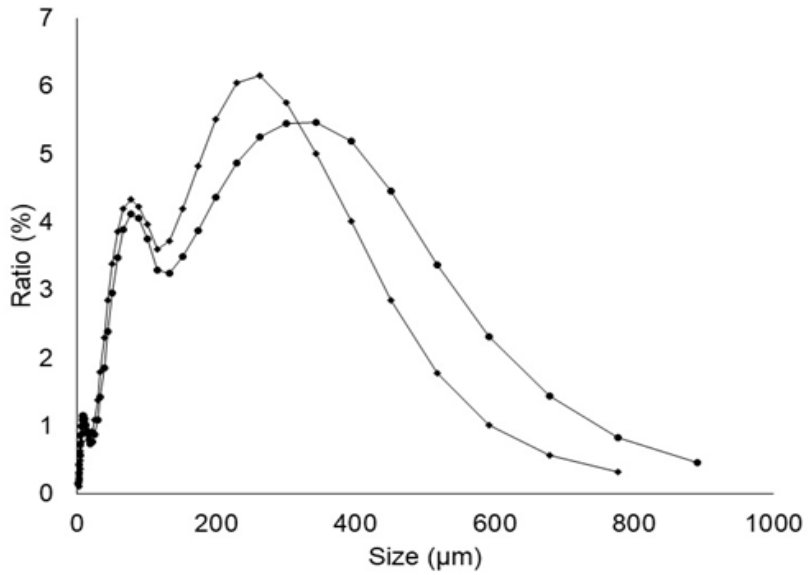

Figure 4. Particle size distribution of the defatted peanut meal at the end of the extraction (round symbol: conventional method; diamond symbol: ultrasound-assisted method).

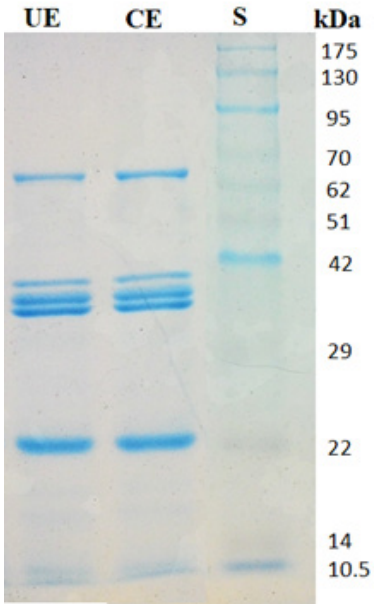

Figure 5. Electrophoresis of peanut protein extracts (UE: ultrasonic extraction; CE: conventional extraction; S: standard proteins).

Conclusions

Ultrasonic treatment of defatted peanut meal significantly reduced the particle size and highly improved the protein extraction yield. In addition, ultrasound-assisted method significantly reduced the extraction time than the conventional method. The application of ultrasound to peanut protein extraction therefore has potentials for industrial application.

References

Atasie, V. N., Akinhanmi, T. F. and Ojiodu, C. C. 2009. Proximate analysis and physico-chemical properties of groundnut (*Arachis hypogaea* L.). Pakistan Journal of Nutrition 8(2): 194–197.

Basha, S. M. and Pancholy, S. K. 1982. Composition and characteristics of basic proteins from peanut (*Arachis hypogaea* L.) seed. Journal of Agricultural and Food Chemistry 30(6): 1176–1179.

Berk, Z. 1992. Technology of production of edible flours and protein products from soybeans. In FAO Agricultural Services Bulletin No. 97. Rome, Italy: Food and Agriculture Organization of the United Nations.

Cherry, J. P. 1990. Peanut protein and product functionality. Journal of the American Oil Chemists' Society 67(5): 293–301.

Chittapalo, T. and Noomhorm, A. 2009. Ultrasonic assisted alkali extraction of protein from defatted rice bran and properties of the protein concentrates. International Journal of Food Science and Technology 44(9): 1843–1849.

Fabian, C. and Ju, Y. H. 2011. A review on rice bran protein: its properties and extraction methods. Critical Reviews in Food Science and Nutrition 51(9): 816–827.

Feng, H., Barbosa-Cánovas, G. V. and Weiss, J. 2011. Ultrasound Technologies for Food and Bioprocessing. 1st Ed. New York: Springer-Verlag.

Hong, K., Nakayama, K. and Park, S. 2002. Effects of protective colloids on the preparation of poly (l-lactide)/poly (butylene succinate) microcapsules. European Polymer Journal 38(2): 305–311.

Jambrak, A. R., Lelas, V., Mason, T. J., Krešić, G. and Badanjak, M. 2009. Physical properties of ultrasound treated soy proteins. Journal of Food Engineering 93(4): 386–393.

Kain, R. J., Chen, Z., Sonda, T. S. and Abu-Kpawoh, J. C. 2009. Study on the effect of control variables on the extraction of peanut protein isolates from peanut meal (*Arachis hypogaea* L.). American Journal of Food Technology 4(1): 47–55.

Karki, B., Lamsal, B. P., Jung, S., van Leeuwen, J., Pometto III, A. L., Grewell, D. and Khanal, S. K. 2010. Enhancing protein and sugar release from defatted soy flakes using ultrasound technology. Journal of Food Engineering 96(2): 270–278.

Mason, T. J. and Lorimer, J. P. 2002. Applied Sonochemistry: Uses of Power Ultrasound in Chemistry and Processing. Germany: Wiley-VCH.

Misra, J. B. 2001. Variation in nitrogen-to-protein conversion factor for peanut. Peanut Science 28(2): 48–51.

Moulton, K. J. and Wang, L. C. 1982. A pilot-plant study of continuous ultrasonic extraction of soybean protein. Journal of Food Science 47(4): 1127–1129.

Patist, A. and Bates, D. 2008. Ultrasonic innovations in the food industry: From the laboratory to commercial production. Innovative Food Science and Emerging Technologies 9(2): 147–154.

Rickert, D. A., Meyer, M. A., Hu, J. and Murphy, P. A. 2004. Effect of extraction pH and temperature on isoflavone and saponin partitioning and profile during soy protein isolate production. Journal of Food Science 69(8): C623–C631.

Rustom, I. Y. S., López-Leiva, M. H. and Nair, B. M. 1991. Optimization of extraction of peanut proteins with water by response surface methodology. Journal of Food Science 56(6): 1660–1663.

Tu, G. L., Bui, T. H., Tran, T. T., Ton, N. M. and Man Le, V. V. 2015. Comparison of enzymatic and ultrasonic extraction of albumin from defatted pumpkin (Cucurbita pepo) seed powder. Food Technology and Biotechnology 53(4): 479–487.

Vilkhu, K., Mawson, R., Simons, L. and Bates, D. 2008. Applications and opportunities for ultrasound assisted extraction in the food industry - A review. Innovative Food Science and Emerging Technologies 9(2): 161–169.

Yu, J., Ahmedna, M. and Goktepe, I. 2007. Peanut protein concentrate: Production and functional properties as affected by processing. Food Chemistry 103(1): 121–129.

Zhu, J. and Fu, Q. 2012. Optimization of ultrasound-assisted extraction process of perilla seed meal proteins. Food Science and Biotechnology 21(6): 1701–1706.