Effect of high intensity ultrasound on physicochemical and functional properties of whey protein isolate and mung bean protein isolate

Danai Charoensuk[1], Wilailuk Chaiyasit[1], Wanlop Chanasattru[2,*]

[1]*Department of Food Science and Technology, Faculty of Science and Technology, Thammasat University, Pathumthani12120, Thailand*
[2]*Food Biotechnology Research Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park, Phahonyothin Road, Khlong Nueng, Khong Luang, Pathumthani 12120, Thailand*
**e-mail: wanlop.cha@biotec.or.th*

Abstract

The aim of this study was to investigate the effects of a high intensity ultrasound (HIU) treatment on the physicochemical and functional properties of whey protein isolate (WPI) and mungbean protein isolate (MPI). MPI and WPI solutions were treated with 0-3400 joules of 20 kHz HIU for 2 min. The results showed that HIU treatment decreased the average particle sizes of both proteins. HIU treatment did not affect on the ζ-potential of MPI but increased the ζ-potential of WPI. HIU treatment had no effect on the molecular weight of MPI and WPI as observed in SDS-PAGE patterns. Treatment with HIU had no impact on the foam capacity and emulsifying activity index (EAI) of both proteins. HIU treatment at energy of 2720 joules or higher increased the foam stability of MPI while WPI treated with HIU did not exhibit significant difference on the foam stability. After treatment with HIU, the emulsifying stability index (ESI) of WPI did not exhibit a difference. The ESI of MPI treated with HIU at energy of 2720 joules or higher decreased compared to that of MPI treated at low energy. However, the ESI of MPI treated with HIU at energy from 2720 – 3400 joules was not different. HIU treatments led to increase the hardness of MPI gel. However, the increasing of the hardness of WPI gel was observed in WPI treated with HIU at the energy of 1071 joules or higher.

Keywords: ultrasound, whey protein isolate, mung bean protein isolate, protein functionality

Introduction

Ultrasound is mechanical waves at a frequency above the threshold of human hearing (>16 kHz). High intensity ultrasound (HIU) of 20 to 100 kHz with 10-100 W/cm^2 can be used to modify the structural and functional properties of globular proteins. Ultrasonic cavitation involves the formation, growth, and violent collapse of small bubbles in liquid during HIU treatment resulting in the beneficial use of ultrasound (Mason et al., 1996). HIU decreases the protein size and changes in the molecular structure, free sulfhydryl groups, molecular size and surface hydrophobicity of proteins resulting in the alteration of the protein functionalities (Arzeni et al, 2012; Hu et al., 2013). Whey protein isolate (WPI) has been widely used in many foods due to its unique properties and ability to improve food quality. Mungbean is an important agricultural produce in Thailand. Production of glass noodle and mungbean flour from mungbean generates protein by-product used as animal feeds. Mungbean protein isolate obtained from glass noodle and mungbean flour productions exhibits poor functionalities such as low gel properties and low foam stability resulting in limited application in food products. Modification of protein could alter the physicochemical and functional properties of MPI and WPI. Protein modifications using HIU could be used to improve the protein functionalities and increase the value added of the WPI and MPI.

Methodology

Materials

Mungbean protein isolate (MPI) was obtained from a local company. Whey protein isolate (WPI) was obtained from Davisco Foods International Inc. (USA). Soy bean oil was purchased from a local supermarket and used without further purification. All chemicals used in this study were analytical grade and used directly without further purification.

High intensity ultrasound treatment

MPI and WPI were dissolved in 5 mM phosphate buffer pH 7.0 at a level of 10% wt. under moderate stirring for 1 h at room temperature. Protein solutions were incubated in an ice bath at least 5 minutes prior to HIU treatment with 20 kHz ultrasonic processors (Vibra cell™ VCX 130, Sonics & Materials Inc., USA) equipped with 6 mm probe for 2 min with cycles of 10 sec work and 5 sec rest. Modified proteins were dried using freeze dryer. The effect of ultrasound intensity levels was studied by varying from 0 to 3400 joules.

Average particle size determination

Average particle size diameter or Z-average (nm) of protein samples was measured using a Zetasizer Nano ZS (Malver Instrument Ltd., Malvern, UK). Protein samples were dissolved in distilled water at a level of 1% wt. Protein solutions were adjusted their pH to 7.0 using 1N of NaOH or 1N of HCl prior to analysis.

Zeta (ζ)-potential determination

The electrical charge or ζ-potential (mV) of protein samples was measured using a particle electrophoresis instrument (Zetasizer Nano ZS, Malvern Instrument Ltd., UK). Protein samples were dissolved in distilled water at a level of 1% wt. Protein solutions were adjusted their pH to 7.0 using 1N of NaOH or 1N of HCl prior to analysis

Molecular weight determination

Molecular weight of protein samples was determined as described by Zhang (2014). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a discontinuous buffer system with mercaptoethanol using 12.5% separating gel and 4% stacking gel. Protein samples in loading buffer (0.0625 M Tris-HCl, 10% glycerine, 2% SDS, 5% 2-mercaptoethanol and 0.0025% bromophenol blue) were loaded onto the gel (15 µg of protein/well). Gel electrophoresis was performed using AE-6530 Dual mini-slab system (ATTO, Japan) at a constant current of 20 mA for 90 min. Gel was stained and destained using Coomassie Blue G250 and 20% ethanol/80% distilled water mixture, respectively.

Foaming properties determination

Foam capacity and foam stability of protein samples were measured. Briefly, protein samples were dissolved in distilled water at a level of 1% wt. Solutions were adjusted their pH to 7.0 using 1N of NaOH or 1N of HCl. Fifty milliliters of protein solutions were transferred to the 250 ml-graduated glass cylinder with an air-feeder. Air was applied into the cylinder by air pump (Classica®Super X Air Pump, Singapore) at a constant flow rate of 7.5 ml/sec for 20 sec. Volume of foams was measured immediately after turning off the air pump. Initial foam volume and foam volume at 15 min were used to calculate the foaming capacity (FC, %) and stability (FS, %) as follows:

$$FC\ (\%) = [(V_2 - V_1)/V_1] \times 100$$

Where V_1 = volume before foam formation
V_2 = volume after foam formation

$$FS\ (\%) = (V_t/V_0) \times 100$$

Where V_0 = initial volume
V_t = volume at15 min

Emulsifying properties determination

Emulsifying properties of protein samples were determined as described by Pearce and Kinsella (1978). Protein samples were dissolved in 5 mM phosphate buffer pH 7.0 at the level of 1% wt. For emulsion preparation, 10 g of soybean oil and 30 g of protein solution were homogenized using homogenizer (ULTRA TURRAX®T25 basic, IKA® WERKE, Germany) at a speed of 13,500 rpm for 1 min. After that, aliquots of 50 µl of emulsion were collected at 0 and 10 min then mixed with 5 ml of 0.1% SDS solution using vortex mixer for 5 sec. Absorbance was measured at 500 nm using spectrophotometer. The absorbances at 0 (A_0) and 10 min (A_{10}) after emulsion formation were used to calculate the emulsifying activity index (EAI) and emulsion stability index (ESI) as follows:

$$EAI\ (m^2/g\ protein) = (2 \times 2.203 \times A_0 \times \text{dilution factor})/(l \times \Phi \times C \times 10{,}000)$$

Where l = path length of cuvette (cm)
Φ = oil volume fraction of emulsion
C = protein concentration (g/ml) of aqueous phase before emulsion formation

$$ESI\ (min) = (A_0 \times 10)/(A_0 - A_{10})$$

Gelling properties determination

Gel hardness of protein samples was determined. Briefly, protein samples were dissolved in distilled water at the level of 15% wt. and 20% wt. of WPI and MPI, respectively. Solutions were adjusted their pH to 7.0 using 1N of NaOH or 1N of HCl. The protein solutions (20ml) were transferred to 2.5 cm diameter-plastic casing. The plastic casings were sealed on both ends. Samples were boiled for 15 min using boiling bath then immediately cooled down using an ice bath for 5 min prior to store at 4°C for overnight. Protein gels were allowed to stand at room temperature for 1 h then cut into 1.5 x1.5 x 2.0 cm^3 (Width x Length x Height) brick. A texture profile analysis (TPA) of the protein gels was performed using TA-TX2i texture analyzer equipped with a 50 mm diameter aluminum cylinder probe. Gel samples were compressed at constant speed of 5 mm/sec to a compression strain of 50%. Only gel hardness was reported in this study.

Data analysis

All experiments were conducted at least two replicates. Data were analyzed by analysis of variance (ANOVA) using SPSS for window. The Duncan's multiple range test was used to compare the means with a confidence of 95%.

Results

Average particle size

The average particle size diameter (Z-average) of protein isolates treated with HIU at energy level of 0 to 3400 joules were decreased from 1453.57±147.65 to 268.62±5.42 nm and 231.53±12.22 to 147.03±3.79 nm, respectively. There were significantly decreased (p<0.05) in Z-average of both proteins. The Z-average of MPI treated with HIU at energy of 131 joules (356.32±13.62 nm) was one of fourth smaller than that of the MPI without HIU treatment (1453.57±147.65 nm). Z-averages of WPI were decreased (p<0.05) with the increasing energy of HIU as shown in Figure 1.

Zeta (ζ)-potential

The ζ-potentials of all samples were negative. After HIU treatment at energy of 0 to 3400 joules, the ζ-potentials of MPI were not significantly difference (p≥0.05) while the ζ-potentials of WPI increased from -28.85±3.33 to -22.42±0.65 mV as shown in Figure 2.

Molecular weight

Molecular weights of MPI and WPI without and with HIU treatment at energy level of 0 to 3400 joules were analyzed using SDS-PAGE technique. The results showed that SDS-PAGE patterns of MPI and WPI were not different.

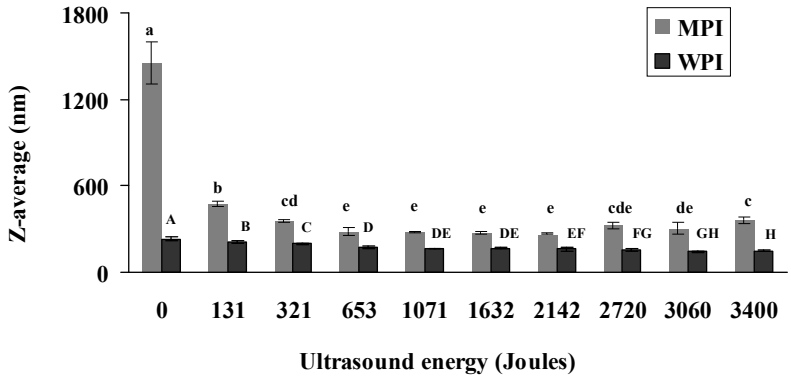

Figure 1 Effect of HIU treatment at 0-3400 joules on the Z-average (nm) of MPI and WPI. Different letters or capital letters in the same protein indicate significant difference (p<0.05).

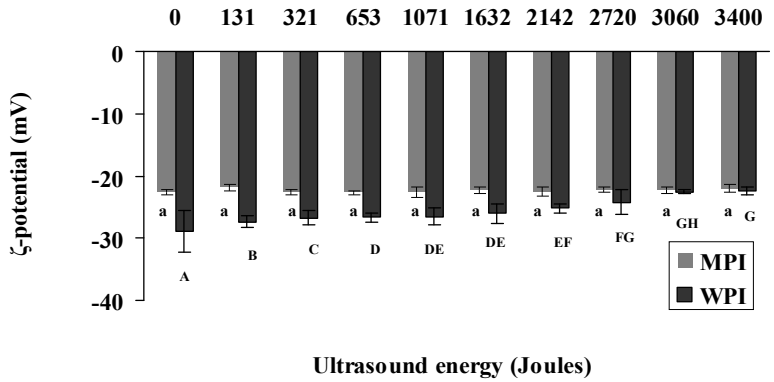

Figure 2 Effect of HIU treatment at 0-3400 joules on the ζ-potential (mV) of MPI and WPI. Different letters or capital letters in the same protein indicate significant difference (p<0.05).

Foaming properties

HIU treatment at energy level from 0 to 3400 joules had no impact on the foam capacities of MPI and WPI. Foam capacity of MPI and WPI was in the range from 355.00±9.90 to 311.00±35.36% and 266.00±33.94 to 257.00±21.21%, respectively. Foam stability of WPI was not affected by the HIU treatment while foam stability of MPI increased (p<0.05) after treated with the HIU at energy of 2720 joules or higher as shown in Figure 3.

Emulsifying properties

HIU treatment at energy level of 0 to 3400 joules of MPI and WPI had no effect on the EAI. However, the ESI of MPI were significantly decreased (p<0.05) from 19.01±0.80 to 16.13±1.25 m^2/g protein at energy level of 2720 joules. In addition, further increasing HIU energy from 2070 joules had no impact on the ESI of the MPI as shown in Figure 4. HIU treatment at energy of 0 to 3400 joules had no effect on the ESI of WPI.

Gelling properties

The gel hardness of protein isolates treated with HIU at the level of 0 to 3400 joules increased from 4.60±0.43 to 9.62±0.62 N and 19.90±0.06 to 26.50±1.08 N for MPI and WPI, respectively. The increasing of the gel hardness of MPI and WPI depended on the energy used in HIU treatment. Gel hardness of MPI treated with HIU at energy of 321 - 1071 joules was not significant difference (p≥0.05) but higher than that of control. Increasing the energy of HIU from 1071 to 3400 joules caused an increased of the gel hardness of MPI. The hardness of WPI gel increased when treated with the HIU at energy of 1071 joules or higher. However, gel hardness of WPI treated with HIU at energy of 1071 joules and higher was not significant difference (p≥0.05) as shown in Figure 5.

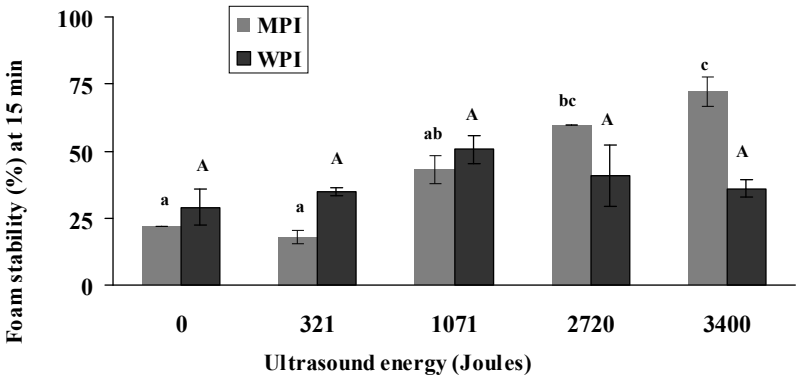

Figure 3 Effect of HIU treatment at 0-3400 joules on the foam stability (%) at 15 min of MPI and WPI. Different letters or capital letters in the same protein indicate significant difference (p<0.05).

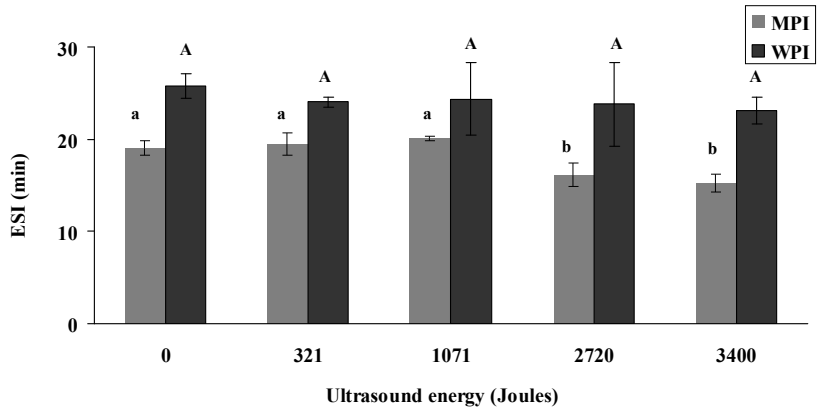

Figure 4 Effect of HIU treatment at 0-3400 joules on the ESI (min) of MPI and WPI. Different letters or capital letters in the same protein indicate significant difference (p<0.05).

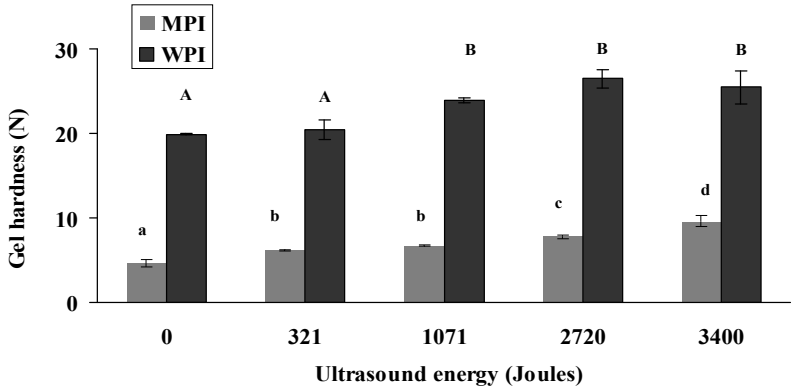

Figure 5 Effect of HIU treatment at 0-3400 joules on the gel hardness (N) of MPI and WPI. Different letters or capital letters in the same protein indicate significant difference (p<0.05).

Discussion

This study showed that HIU treatment was able to reduce the average particle size of MPI and WPI with consistence to previous study in sodium caseinate, whey protein isolate and milk protein isolate (O'Sullivan et al., 2014). The decrease in the protein size might be due to breaking up of the protein aggregates by shear force and partial cleavage the intermolecular hydrophobic interaction (Jambrak et al., 2014). The ζ-potential of MPI and WPI were negative due to the pH of the protein solutions was above the isoelectric point (PI). The ζ-potential of MPI was not altered by HIU treatment while the ζ-potential of WPI increased after HIU treatment. It has been reported that ζ-potential of black bean protein isolate treated with HIU decreased with the increasing of the power of HIU treatment at the energy of 1800 to 7200 joules but increased for the black bean protein isolate treated at high energy of HIU (10800 joules) (Jiang et al., 2014). HIU had no influence on molecular weight pattern of MPI and WPI, indicating that HIU treatment did not alter tertiary structure of the proteins with consistence to previous study in black bean protein isolate (Jiang et al., 2014). Similar results were also reported in the molecular weight studies of dairy proteins (O'Sullivan et al., 2014). HIU treatment did not affect the foam capacity of MPI and WPI while Jambrak et al., (2008) found that the foam capacity and stability of WPI were improved with HIU treatment. This difference might be due to the difference in protein samples or difference in the levels of HIU treatment. In our study, foam stability of WPI treated with HIU (0-3400 joules) did not exhibit significant difference while the foam stability of MPI increased after HIU treatment with energy of 2720 joules or higher. In addition, Jambrak et al., (2008) suggested that the foam properties of protein could be improved as well as damaged by HIU treatments depending on its conditions such as power level and treatment time. Our results showed that the emulsion properties of WPI did not change by HIU treatment. The ESI of MPI decreased after treated with the HIU at high energy level while the results of Yanjun et al., (2014) showed that the EAI and ESI of milk protein concentrate increased after ultrasound treatment. They suggested that HIU treatment could change the structure of protein, the surface hydrophobicity, and the molecular flexibility resulting in improvement of emulsion properties. Our study, MPI and WPI were treated with HIU at short time thus negligible change of protein structure. Hardness of gels prepared from MPI and WPI treated with HIU increased with the increasing of the energy of HIU treatments. Similar results were also observed by Azeni et al., (2012). Whey protein concentrate treated with ultrasound exhibited higher elastic gel and storage modulus (G') than that of the control, which can be contributed to a higher protein aggregation promoted by hydrophobicity.

Conclusion

HIU treatment had effect on the physicochemical and functional properties of MPI and WPI. In MPI, HIU treatment had effect on average particle size, foam stability, ESI and gel hardness while in, it had effect only on average particle size, ζ-potential, and gel hardness. However, HIU treatment did not alter molecular weight, foam capacity, EAI of both MPI and WPI. Therefore, this method could be used to improve foam stability of MPI, gel hardness of both MPI and WPI and would be able to apply to the other protein applications.

Acknowledgements

This research has been performed with the support of Food Biotechnology Research Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand.

References

Arzeni C., Perez O. E. and PilosofM. R. (2012) Functionality of egg white proteins as affected high intensity ultrasound. Food Hydrocolloids 29:308-316.

Hu H., Wu J., Li-ChanE. C. Y., Zhu L., Zhang F., Xu X., Fan G., Wang L., Huang X. and Pan S. (2013)Effects of ultrasound on structural and physical properties of soy protein isolate (SPI) dispersions. Food Hydrocolloids 30:647-655.

Jambrak A. R., Mason T. J., Lelas V., Paniwnyk L. and Herceg Z. (2014) Effect of ultrasound treatment on particle size and molecular weight of whey protein. J Food Eng 121:15-23.

Jambrak A. R., Mason T. J., Lelas V., Herceg Z. and Herceg I. L. (2008) Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. J Food Eng 86:281-287.

Jiang L., Wang J., Li Y., Liang J., Wang R., Chen Y., Ma W., Qi B. and Zhang M. (2014) Effect of ultrasound on the structure and physical properties of black bean protein isolates. Food Res Int 62:595-601.

Mason T. J., Paniwnyk, L., and Lorimer, J. P. (1996) The uses of ultrasound in food technology. Ultrason Sonochem 3:253–260.

O'Sullivan J., Arellano M., Pichot R. and Norton I. (2014) The effect of ultrasound treatment on the structural, physical and emulsifying properties of dairy proteins. Food Hydrocolloids (in press).

Pearce K. N. and Kinsella J. E. (1978) Emulsifying properties of protein: Evaluation of a tubidometric technique. J Agric Food Chem 26:716-723.

Yanjun S., Jianhang C., Shuwen Z., Hongjuan L., Jing L., Lu L., luko H., Yanling S., Wenming., Wupeng G. and Jiaping L. (2014) Effect of power ultrasound pre-treatment on the physical and functional properties of reconstituted milk protein concentrate. J Food Eng 124:11–18.

Zhang Q., Tu Z., Xiao H, Wang H., Huang X., Liu G., Liu C., ShiY., Fan L., and Lin D. (2014) Influence of ultrasonic treatment on the structure and emulsifying properties of peanut protein isolate. Food Bioprod Process 92:30-37