International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:10, No:9, 2016

Applications of High Intensity Ultrasound to Modify Millet Protein Concentrate Functionality

B. Nazari, M. A. Mohammadifar, S. Shojaee-Aliabadi, L. Mirmoghtadaie

***Abstract*—**Millets as a new source of plant protein were not used in food applications due to its poor functional properties. In this study, the effect of high intensity ultrasound (frequency: 20 kHz, with contentious flow) (US) in 100% amplitude for varying times (5, 12.5, and 20 min) on solubility, emulsifying activity index (EAI), emulsion stability (ES), foaming capacity (FC), and foaming stability (FS) of millet protein concentrate (MPC) were evaluated. In addition, the structural properties of best treatments such as molecular weight and surface charge were compared with the control sample to prove the US effect. The US treatments significantly ($P<0.05$) increased the solubility of the native MPC (65.8±0.6%) at all sonicated times with the maximum solubility that is recorded at 12.5 min treatment (96.9±0.82 %). The FC of MPC was also significantly affected by the US treatment. Increase in sonicated time up to 12.5 min significantly increased the FC of native MPC (271.03±4.51 ml), but higher increase reduced it significantly. Minimal improvements were observed in the FS of all sonicated MPC compared to the native MPC. Sonicated time for 12.5 min affected the EAI and ES of the native MPC more markedly than 5 and 20 min that may be attributed to higher increase in proteins tendency to adsorption at the oil and water interfaces after the US treatment at this time. SDS-PAGE analysis showed changes in the molecular weight of MPC that attributed to shearing forces created by cavitation phenomenon. Also, this phenomenon caused an increase in the exposure of more amino acids with negative charge in the surface of US treated MPC, that was demonstrated by Zetasizer data. High intensity ultrasound, as a green technology, can significantly increase the functional properties of MPC and can make this usable for food applications.

***Keywords*—**Millet protein concentrate, Functional properties, Structural properties, High intensity ultrasound.

I. Introduction

PROSOMILLET (*Panicum miliaceum* L.), as a high protein millet with averagely 13.4% protein, shows markedly potential as a new source of plant protein for human nutrition. This plant, despite of their high agricultural, nutritive, and health benefits, was not used in industrial scale of food applications. The main reason of this problem is poor functional properties of this plant protein, and one of the best method for overcoming to this problem is to improve the functional properties of this protein [1].

High intensity ultrasound (high power, low frequency) has disruptive effects on food ingredients, that resulted in change in their functional and structural properties. These effects of ultrasound treatment occur from the cavitation phenomenon. The major advantages of ultrasound for the use in food industry are safety, nontoxicity, and environmentally friendliness. The main effective factors on ultrasound power generally are intensity, velocity, energy, temperature, and pressure [2], [3].

In recent years, considerable research indicates that high intensity ultrasound can lead to improve the functional properties of proteins including solubility, emulsifying, and foam ability [3]-[7].

II. Materials and Methods

Millets were purchased from Seed & Plant Improvement Institute, Karaj, Iran. The seeds were dehulled and ground using a laboratory-scale hammer miller (laboratory Mill 3100, Perten Co.) in the TakMakaron co. Alborz, Iran. All chemicals used in this study were of analytical grade.

A. Preparation of Millet Proteins Concentrate

NaOH (1N) is added to millet flour at a 1:4 (W/V) ratio of flour: solvent to reach pH 9.5. The resulting slurry was stirred at room temperature for 60 min, and then centrifuged at 4000 g for 15 min. Finally, the resulting pellets were freeze dried after the neutralization at pH 6.7-7 [8], [9].

B. High-Intensity Ultrasound Treatment

The MPC dispersions (10% w/v) were prepared in distilled water and then were placed in 80 ml flat bottom conical flask which was maintained at a constant temperature. An ultrasound processor (B03- Ultrasonic Processor, E–Chrom Tech Co., Ltd., Taiwan) equipped with a 3 mm diameter titanium sonotrode probe that provided continuous 20 kHz wave with a total nominal output power of 100 W, was used for sonoprocessing of MPC dispersions. MPC was treated at 20 kHz for 5, 12.5, or 20 min at amplitude of 100% with constant pulse durations. All sonicated and native samples were lyophilized.

C. Solubility

About 100 ml of distilled water were added to 1 g of MPC powder (1% v/v), and the resulted suspension was adjusted to pH 7. Then, the suspension was centrifuged (20000 g for 20

B. Nazari is with the Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, ShahidBeheshti University of Medical Sciences, Tehran, Iran (e-mail: bahmannazari67@gmail.com).

S. Shojaee-Aliabadi is with the Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, ShahidBeheshti University of Medical Sciences, Tehran, Iran (e-mail: saeedeh.shojaee@gmail.com).

M. A. Mohammadifar is with the National Food Institute, Technical University of Denmark (e-mail: mohamadif@sbmu.ac.ir).

L. Mirmoghtadaie is with the Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, ShahidBeheshti University of Medical Sciences, Tehran, Iran (corresponding author, e-mail: mirmoghtadaie@sbmu.ac.ir).

min at room temperature), and the supernatant was aliquot diluted with 1:10 (v/v) in dissociating buffer (50 mM EDTA, 8 M urea at pH 10). Finally, absorbance was measured at 280 nm on the prepared sample [10].

$$\text{Solubility (\%)} = \frac{\text{Absorbance of the supernatant}}{\text{Absorbance of the dispersion before centrifugation}} \times 100$$

D.Foaming Properties

Foam capacity (FC) and FS of the MPC at pH 7 were evaluated with slight modification [11].

MPC suspension (3% w/v) was prepared and mixed by using a magnetic stirrer for 45 min after adjusting to pH 7. The sample was then mixed in a rotor-stator homogenizer (IKA T25-Digital Ultra Turrax, Staufen, Germany) at speed 4 for 6 min. The total foam volume was recorded as FC and for measure the FS, the foam volumes were read at 0, 10, 30, 60, and 120 min after mixing [11].

E. Emulsifying Properties

10 ml of MPC suspension (1% w/v) was homogenized with 10 ml vegetable oil by using a rotor-stator homogenizer (IKA T25-Digital Ultra Turrax, Staufen, Germany) at speed 5 after centrifuge the prepared emulsions at 1000 g for 5 min and the EAI was measured as [12]:

$$\text{EAI (\%)} = \frac{Height\ of\ emulsified\ layer\ in\ the\ tube}{Height\ of\ the\ total\ content\ in\ the\ tube} \times 100$$

For the evaluation of the ES, before centrifuging the emulsion at 1000 g for 5 min, it was heated at 80 °C for 30 min and calculated as;

$$\text{ES (\%)} = \frac{Height\ of\ emulsified\ layer\ after\ heating}{Height\ of\ total\ content\ in\ the\ tube} \times 100$$

F. SDS-PAGE

SDS-PAGE was registered by using Sambrook procedure [13]. Mix of 0.001 g of samples and 200 microliters of reducing SDS loading buffer were heated to 90 °C for 10 min. For each sample, 25-microgram protein was loaded into a 12% gel.

G.Zeta Potential

The Zeta potential of native and US treated MPC was measured by using a Zetasizer Nano ZS (Malver Instrument Ltd., Malvern, Worcestershire, UK). Solutions of 2 mg/ml MPC samples were prepared in deionized water and adjusted to pH 7. Then, the solutions were filtered through a 0.45 µ HA Millipore membrane prior to analysis.

H.Fourier-Transform Infrared Spectra (FTIR)

FTIR spectra of native and US treated were recorded from wave number 450–4000 cm^{-1} by using a FT-IR spectrophotometer (Perkin Elmer Spectrum RX I, USA).

I. Differential Scanning Calorimetry (DSC)

A Shimadzu DSC-60 differential scanning calorimeter (Shimadzu, Kyoto, Japan) was used to determine the DSC thermograms. The thermograms parameters were registered by heating 2.88 mg sample from 25 to 135°C at 10 °C/min under a nitrogen atmosphere, and an empty pan was used as reference.

III. Statistical Analysis

All treatments were performed in triplicate. The data were statistically analyzed with SPSS vs.21 software. One-way analysis of variance (ANOVA) with a 95% confidence interval was used to assess the significance of the obtained results. The ANOVA data with $P<0.05$ were considered as statistically significant.

IV. Results and Discussions

A. Solubility

As suggested in Fig. 1, solubility of MPC in all of treated times is significantly higher than the native MPC; however, the solubility declined significantly in the highest US time (20 min) in this study.

The increase in solubility of MPC upon the US treatment may be due to the created breakage in intramolecular interactions by cavitation phenomenon that resulted in decline in molecular weight. This is confirmed by SDS-PAGE results [14]. Also, increase in hydrophobic groups upon the US treatment that buried in native MPC, caused higher increase in negative charge on MPC surface that confirmed by Zeta potential results, and subsequently increased electrostatic repulsions and protein solubility [15].

Hydrophobic interactions created by high intensity ultrasound, consequently formed small aggregations and declined solubility, previously pointed out by [3] and [16].

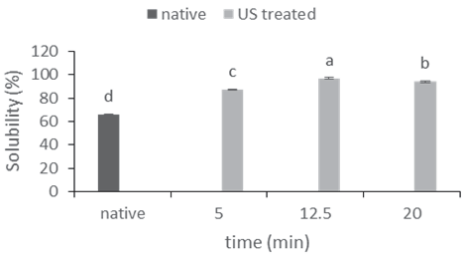

Fig. 1 Solubility of native and US treated MPC at varying times

B. Foaming Properties

The effect of US treatment on the FC and FS of the MPC is shown in Fig. 2 and Table I, respectively. The foam capacity of US treated MPC significantly increased, which could be due to the MPC molecules unfolded during the US treatment and enhanced in the exposure to the hydrophobic regions. Indeed, the protein molecules penetration in the air/water interface was accelerated by the created shearing force upon US treatment [17], [18]. On the other hand, the improvement of FS was not considerable.

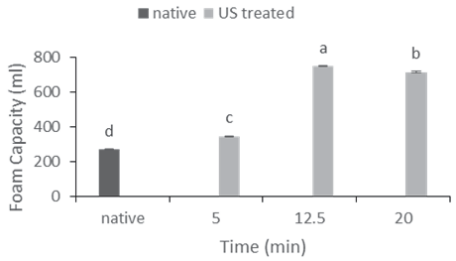

Fig. 2 Foam Capacity of native and US treated MPC at varying times

TABLE I
EFFECT OF US TREATMENTS ON FS OF MPC

US treated time (min)	Stability Time (min)		
	0	10	30
native	271.03±4.5^{e}	4.37±1.52^{e}	-
5	346.37±2.08^{d}	14.70±03^{cd}	-
12.5	749.70±2^{a}	95.70±6.56^{a}	14.37±0.58^{a}
20	716.03±3.51^{b}	25.70±3^{b}	-

Results are the mean values of triplicate determinations, ± standard deviation. Means in each column followed by different letters are significantly different (P<0.05): small letters show statistical differences for data in column, respectively.

A (native), B1 (5min), B2 (12.5min), B3 (20min)

C. Emulsifying Properties

The US treatment of MPC significantly (P>0.05) increased both EAI and ES, compared to the native MPC. With the increase in US treatment time up to 12.5 min, both EAI and ES significantly increased, but the highest US treatment time (20 min) markedly decreased those.

Our findings provide more evidence for the other studies. Arzeni et al. reported that decrease and increase in the droplet size and hydrophobic groups number, respectively, upon the US treatment would improve the EAI. These two events are confirmed by our SDS-PAGE and Zeta potential results. Also, Jambrak et al. reported that the turbulent flow created by the US treatment could change in protein orientation in a manner that resulted to better oil bubbles integration in the emulsion and subsequently increase ES [7], [5], [19]-[22].

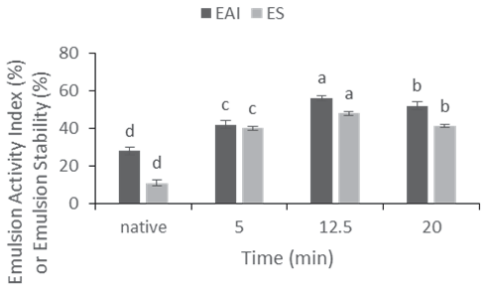

Fig. 3 EAI and ES of native and US treated MPC at varying times

D. SDS-PAGE

Comparison of SDS-PAGE patterns presented in Fig. 4 showed difference in bonds revealed in the 40-50 kDa specially, after Us treatment time for 12.5 as best treatment time for improve functional properties in our study.

Decrease observed in the molecular size of MPC after US treatment may be due to cavitation phenomenon and bubble collapse in surroundings protein, upon US treatment [17].

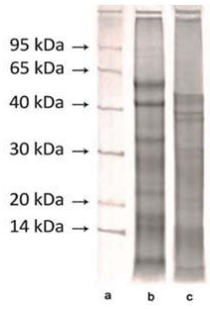

Fig. 4 SDS-PAGE electrophoretic profiles of ladder (a), native (b) and US treated (c) MPC for 12.5 min

E. Zeta Potential

The Zeta potential of native and US treated for 12.5 MPC, were measured to determine alter in MPC amino acids surface change after US treatment.

The Zeta potential of native MPC was -32.9 but increase to -42.2 for its US treated for 12.5 min. Jiang et al. [15] also point out that the Zeta potential of black bean protein isolate increases negatively after the high intensity US treatment (20 kHz). They reported that this increase was due to the conformational change in protein and increase charge residues exposure in the protein surface after the US treatment.

REFERENCES

[1] S.M. Ahmed, Zh. Qing, Ch. Jing and Sh. Qun, "Millet Grains: Nutritional Quality, Processing, and Potential Health Benefits" Comprehensive Reviews in Food Science and Food Safety, Vol.12. 2013.

[2] T. Awad, H. Moharram, O. Shaltout, D. Asker, and M. Youssef, "Applications of ultrasound in analysis, processing and quality control of food: A review". Food Research International, 48(2), 410-427. 2012

[3] C. Arzeni, K. Martínez, P. Zema, A. Arias, O. Pérez, and A.M.R. Pilosof. "Comparative study of high intensity ultrasound effects on food proteins functionality". Journal of Food Engineering, 108(3), 463-472. 2012.

[4] B. Karki, B. P. Lamsal, D. Grewell, A. L. Pometto, J. van Leeuwen, S. K. Khanal, and S. Jung, "Functional Properties of Soy Protein Isolates Produced from Ultrasonicated Defatted Soy Flakes". *Journal of the American Oil Chemists' Society, 86*, 1021-1028, 2009.

[5] A. R Jambrak, V. Lelas, T. J. Mason, G. KrešićandM.Badanjak, "Physical properties of ultrasound treated soy proteins". *Journal of Food Engineering, 93*(4), 386-393. 2009.

[6] L. Chen, J. S. Chen, L. Yu, K. G. Wu, X. L. Liu, and X. H. Chai "Modifications of Soy Protein Isolates Using Ultrasound Treatment for Improved Emulsifying Properties". In *Advanced Materials Research.* Vol. 554, pp. 944-948: Trans Tech Publ. 2012.

[7] C. Arzeni, O. E. Pérez, and A. M. Pilosof, "Functionality of egg white proteins as affected by high intensity ultrasound". *Food Hydrocolloids, 29*(2), 308-316. 2012.

[8] Mirmoghtadaie, L., Kadivar, M., &Shahedi, M. "Effect of Modified Oat Starch and Protein on Batter Properties and Quality of Cake". *Cereal chemistry, 86*(6), 685-691. 2009.

[9] AOAC. "Association of Official Analytical Chemists". In *Official methods of analysis*. Washington, DC: 14th ed. 1985.

[10] M. Britten, H. J. Giroux, and V. Gaudin, "Effect of pH during heat processing of partially hydrolyzed whey protein". *Journal of dairy science, 77*(3), 676-684. 1994.

[11] L. Mirmoghtadaie, M. Kadivar, and M. Shahedi, "Effects of succinylation and deamidation on functional properties of oat protein isolate". *Food Chemistry, 114*(1), 127-131. 2009.
[12] O. S. Lawal, "Functionality of native and succinylated Lablab bean (Lablab purpureus) protein concentrate". *Food Hydrocolloids, 19*(1), 63-72. 2005.
[13] J. Sambrook, and D. W. Russell Molecular cloning. A laboratory manual. Third. *Cold pring Harbor Laboratory Press, New York*. 2001.
[14] A. R. Jambrak, T. J. Mason, V. Lelas, Z. Herceg, and I. L. Herceg "Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions". *Journal of Food Engineering, 86*(2), 281-287. 2008.
[15] L. Jiang, J. Wang, Y. Li, Z. Wang. J. Liang, R. Wang, Y. Chen, and M. Zhang, "Effects of ultrasound on the structure and physical properties of black bean protein isolates". *Food Research International, 62*, 595-601. 2014.
[16] N. A. Camino, O. E.Pérez, and A. M. Pilosof, "Molecular and functional modification of hydroxy propyl methyl cellulose by high-intensity ultrasound". *Food Hydrocolloids, 23*(4), 1089-1095. 2009.
[17] A. R. Jambrak, V. Lelas, T. J. Mason, and G. Krešic, "Ultrasonic effect on physicochemical and functional properties of a-lactalbumin". *Food Science and Technology, 43*, 254-262. 2010.
[18] H. Zhang, I.P. Claver, K.X. Zhu, and Z. H., "The Effect of Ultrasound on the Functional Properties of Wheat Gluten". Molecules, 16, 4231-4240. 2011.
[19] L. Chen, J. S. Chen, L. Yu, K. G. Wu, X. L. Liu, and X. H. Chai, "Modifications of Soy Protein Isolates Using Ultrasound Treatment for Improved Emulsifying Properties". In *Advanced Materials Research* (Vol. 554, pp. 944-948): Trans Tech Publ. 2012.
[20] J. O'Sullivan, B. Murray, C. Flynn, and I. Norton, "The effect of ultrasound treatment on the structural, physical and emulsifying properties of animal and vegetable proteins". Food Hydrocolloids. 2015.
[21] J. O'Sullivan, M. Arellano, R. Pichot, and I. Norton, "The effect of ultrasound treatment on the structural, physical and emulsifying properties of dairy proteins". *Food Hydrocolloids, 42*, 386-396. 2014.
[22] S. Yanjun, C. Jianhang, Z. Shuwen, L. Hongjuan, L. Jing, L. Lu, H. Uluko, S. Yanling, C. Wenming, and G. Wupeng, "Effect of power ultrasound pre-treatment on the physical and functional properties of reconstituted milk protein concentrate". *Journal of Food Engineering, 124*, 11-18. 2014.