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Effect of ultrasound treatments on functional properties and structure of millet protein concentrate

Bahman Nazari[a], Mohammad Amin Mohammadifar[b], Saeedeh Shojaee-Aliabadi[a], Ehsan Feizollahi[a], Leila Mirmoghtadaie[a,*]

[a] Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Food Production Engineering, DTU Food, Technical University Of Denmark, Søltofts Plads227, Dk-2800 Lyngby, Denmark

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ABSTRACT

In this study, the effect of high power ultrasound (US) probe in varying intensities and times (18.4, 29.58, and 73.95 W/cm^2 for 5, 12.5 and 20 min respectively) on functional properties of millet protein concentrate (MPC) was investigated, and also the structural properties of best modified treatment were evaluated by FTIR, DSC, Zeta potential and SDS-PAGE techniques. The results showed the solubility in all US treated MPC was significantly ($p < .05$) higher than those of the native MPC. Foaming capacity of native MPC (271.03 ± 4.51 ml) was reduced after US treatments at low intensities (82.37 ± 5.51 ml), but increased upon US treatments at high intensities (749.7 ± 2 ml). In addition, EAI and ES increased after US treatments. One of the best US treatments that can improve the functional properties of MPC was 73.95 W/cm^2 for 12.5 min that resulted in reduction of molecular weight and increase nearly 36% in the negative surface charge that was confirmed by SDS-page and Zeta potential results, respectively.

1. Introduction

Proso millet (*Panicum miliaceum* L.), a comparatively short-season crop, requires little water and is able to grow at a wide range of altitudes. This cereal has considered in food production because of its advantages including high yield, affluence and low cost.[1]. In addition, proso millet has higher (13.4%) protein content than many common cereals such as wheat (10.5%) and rice (6.8–7.4%) [2–4]. It contains considerable quantity of essential amino acids especially the Sulphur containing amino acids (methionine and cysteine). On the other hand, millets because of their agricultural advantages, health benefits and nutritive values have been received specific alterations as a good food source from developing countries. Health benefits such as, decreasing tumor incidence, reducing blood pressure, cholesterol absorption, preventing cardiovascular diseases and cancer, also nutritive values including provide a variety of nutrients and antioxidants needed for human health, have been reported for millets [5,6]. They are a gluten free cereal and thus is appropriate for people with wheat/gluten allergies [7].

Proteins play different roles in food matrix that named functional properties. According to Kinsella [8], the functional properties are "those physical and chemical properties that influence the behavior of proteins in food systems during processing, storage, cooking and consumption" and which are affected by multiple factors such as pH, drying, heating, ionic strength, storage conditions, presence of reducing agents, and physical, chemical or enzymatic modifications [8].

Among the different physical methods for proteins modifications, ultrasound, which is defined as sound waves having frequency that exceeds the hearing limit of the human ear (~20 kHz), has simple, cost-effective, energy saving and environment friendly advantages [9,10]. Generally, ultrasound power is affected by pressure, temperature, intensity, energy and velocity, that based on frequency range it can be divided into high and low energy ultrasound. Frequency between 20 and 100 kHz with high intensity (10–1000 W/cm^2) used in high energy ultrasound which caused alterations in mechanical, physical, or chemical/biochemical attributes of proteins because of creation of high pressure (1000 atm) and temperature (5000 K) during cavitation phenomenon. In contrast, frequency between 5 and 10 MHz with low intensity (1 W/cm^2) has non-destructive effects and is used to ensure high quality and safety of foods applications [10–12]. Generation of high power ultrasound could be done with sonication bath and/or transportable cheap ultrasonic immersion probes due to various goals in food manufacturing [13].

In recent years, many researchers have investigated the impact of

* Corresponding author.
E-mail address: le_mirmoghtadaie@yahoo.com (L. Mirmoghtadaie).

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ultrasound on functional properties of vegetable and animal protein sources specially soy proteins. In this case, the researchers showed strong effect of ultrasound treatment on foaming, solubility, emulsifying and other functional properties of these proteins [11,14–19].

Several studies showed that emulsifying performance of egg white protein (20 kHz, 4.27 W, for 20 min) and dairy proteins (20 kHz, 34 W/cm^2, for 2 min) improved after high power ultrasound treatment [17,20]. In a study conducted on animal and vegetable proteins, it was found out that solubility of pea protein concentrate and emulsifying performance of bovine gelatin, egg white protein and pea protein concentrate improved after ultrasound treatment (20 kHz, acoustic intensity of ~34 W/cm^2 for 2 min) [17]. In another study, different ultrasound power (200 W, 400 W, 600 W) were used to modify emulsifying properties of Soy protein isolates (SPI). They found increase in emulsifying properties of SPI after using ultrasound. The results showed the middle power ultrasound (400 W) treated protein had a lower saturation surface load and a higher protein adsorption fraction that can explain its better emulsifying capability [15]. Hu et al. [18] studied the effects of 20 kHz (low- frequency) ultrasound at different time (15 or 30 min) and power (200, 400 or 600 W) on soy protein isolate structural and functional properties. They did not find significant change in the protein electrophoretic patterns. The surface hydrophobicity and protein solubility of SPI were increased with increase in both of time and power of ultrasonic treatment [18] that could lead to increase in emulsifying and foaming activity.

Due to the burgeoning world population and on the other hand enhancing cost and confined supply of animal proteins, new sources of plant proteins for use in food applications, will need to be developed [8]. The main novelty of this work is choosing millet protein concentrate as low cost and nutritious protein source. It can be show different behavior from other proteins mentioned in literature in different intensity and time. Therefore, the purpose of the present study was to examine the impact of high power ultrasound (20 kHz) on improvement of emulsifying, foaming and solubility of millet protein concentrates to determine the possibility of using these proteins in different food applications such as emulsifier and egg replacer. In addition, FTIR, DSC, Zeta potential and SDS-Page methods was used to evaluate the relationship between physicochemical and structural properties of native and modified protein.

2. Materials and methods

2.1. Materials

Proso millet seeds were purchased from Seed & Plant Improvement Institute, Karaj, Iran. The seeds were cleaned by hand, sieved to remove the foreign materials, and milled using a laboratory-scale hammer miller (laboratory Mill 3100, Perten co.) in the quality control lab of Ard daran Co belonging to Tak Makaron co. Alborz, Iran. The resulting millet flour were packed in polyethylene bags and stored in a refrigerator and used during one week after milling. All chemicals used in this study were of analytical grade. NaOH was purchased from Merck (Darmstat, Germany). gel electrophoresis protein markers Tris base, Glycine, sodium dodecyl Sulfate (SDS) were obtained from Sigma Chemical Co. (Tehran, Iran). The water used in all experiments was passed through a distillation unit (A4000D, Aquatron, UK).

2.2. Methods

2.2.1. Preparation of millet protein concentrates

Millet flour was dispersed in distilled water at a flour: solvent ratio of 1:4 (w/v); the pH was adjusted to 9.5 with 1 N NaOH to enhance protein solubilisation and it was stirred at room temperature for 60 min. The pH of the supernatant obtained after centrifuging at 4000*g* for 30 min was adjusted to 4.0 with 1 N HCl. Then it recentrifuged at 4000*g* for 15 min, and the protein concentrate (pellet) was recovered [21].

2.2.2. Ultrasound treatment of millet proteins

50 ml of MPC dispersions (10% w/w) [18] were prepared by adding MPC powder into distilled water and placed in 100 ml flat bottom conical flask. An ultrasonic 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. The ultrasound probe was submerged in a depth of 10 mm in the sample and protein concentrate dispersions were sonicated for 5, 12.5 and 20 min at amplitudes of 20%, 60% and 100% with constant pulse durations. Sample was placed in a bottle of ice during sonication and its temperature was constant at ambient temperature (20–30 °C). After ultrasound treatment, all samples were lyophilized and then stored at refrigerator temperature (4 °C) in airtight containers separately until they were analyzed. The percentage protein content (total basis) of the concentrate was 73% using Kjeldahl method [22].

2.2.3. Acoustic energy determination

Actual ultrasound energy determination is necessary to ascertain the influence of ultrasound intensity in a treatment and also to be able to compare different treatments [11], since the ultrasound energy is partly lost in the form of heat when ultrasound passes though the medium [23]. The dissipated acoustic power applied to the solution was calculated with calorimetric procedure according to Margulis et al. [24]. This method involves determining the temperature increase during the first 30 s of the experiment. Acoustic power (P) was then calculated using the equation:

$$P_a = MC_p(dT/dt)$$

where Pa (W) is the acoustic power, M is the mass of ultrasound treated solution (g), Cp is the specific heat of the medium (kJ/gK) and dT/dt is the rate of temperature change with respect to time. Then, the acoustic power intensity, Ia (W/cm^2), was calculated as follows:

$$I_a = P_a/S_A$$

where Ia (W/cm^2) is acoustic power intensity, Pa (W) is the acoustic power, SA is the surface area of the ultrasound emitting surface (cm^2).

It is expressed in watts per unit area of the emitting surface (W/cm^2), or in watts per unit volume of the sonicated solution (W/cm^3). Ultrasonic treatment with the 20-kHz probe at amplitudes of 20, 60, and 100% generated power outputs of 18.4, 29.58, and 73.95 W/cm^2, respectively.

Samples were marked as: A (native), B1 (18.4 W/cm^2 – 5 min), B2 (18.4 W/cm^2 – 12.5 min), B3 (18.4 W/cm^2 – 20 min), C1 (29.58 W/cm^2 – 5 min), C2 (29.58 W/cm^2 – 12.5 min), C3 (29.58 W/cm^2 – 20 min), D1 (73.95 W/cm^2 – 5 min), D2 (73.95 W/cm^2 – 12.5 min) and D3 (73.95 W/cm^2 – 20 min).

2.2.4.1. Solubility. For this test, 1 g of the protein concentrate powder was dispersed in 100 ml of distilled water (1% w/ w) and adjusted to pH 7 with either 1 N NaOH or 1 N HCl. The suspension was centrifuged at 20,000*g* for 15 min in 23 °C and absorbance was measured at 280 nm for a sample aliquot diluted 1:10 (vol/ vol) with dissociating buffer (50 mM EDTA, 8 M urea at pH 10). The same procedure was performed for suspensions treated with ultrasound [25].

$$Solubility\,(\%) = (absorbance\ of\ the\ supernatant / absorbance\ of\ the\ dispersion\ before\ centrifugation) \times 100$$

2.2.4.2. Foaming capacity (FC) and foam stability (FS). Foam capacity and foam stability of the millet protein concentrates at pH 7 were determined according to the slightly modified method of Mirmoghtadaie et al. [26]. It was observed that 33.3 ml of protein concentrate dispersion (3%, w/v in distilled water) were adjusted to pH 7 and mixed using a magnetic stirrer for 45 min, followed by mixing in