Influence of ultrasound treatment on the physicochemical and antioxidant properties of mung bean protein hydrolysate

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\textbf{ABSTRACT}

This study aimed to investigate influence of ultrasonic treatment on physicochemical and antioxidant properties of mung bean protein hydrolysate (MPH). Physicochemical properties of MPH were evaluated by Tricine-SDS-PAGE, particle size distribution, fourier transform infrared spectroscopy (FTIR) and fluorescence spectroscopy, among others. Radicals scavenging activities of ABTS, hydroxyl, superoxide anion, Fe\textsuperscript{2+} chelating ability and reducing power characterized antioxidant activities of MPH. MPH contained four bands of 25.6, 12.8, 10.6 and 4.9 kDa, in which 4.9 kDa was the most abundant. Ultrasonic treatment increased the contents of aromatic and hydrophobic amino acids in MPH. Ultrasonic treatment decreased the content of α-helix of MPH and increased β-sheet and β-turn compared to MPH. MPH-546 W (ultrasonic treatment 546 W, 20 min) had the lowest average particle size (290.13 nm), zeta potential (-36.37 mV) and surface hydrophobicity (367.95 A.U.). Antioxidant activities of ultrasonicated-MPH increased with the ultrasonic power, achieving the lowest IC\textsubscript{50} (mg/mL) of 0.1087 (ABTS), 1.796 (hydroxyl), 1.003 (superoxide anion) and 0.185 (Fe\textsuperscript{2+} chelating ability) in 546 W power. These results indicated ultrasonic treatment would be a promising method to improve the antioxidant properties of MPH, which would broaden the application scope of MPH as bioactive components in the food industry.

1. Introduction

Mung bean protein (MBP) with affluent essential amino acids is deemed to be a vital source of dietary protein [1]. MBP not only has good emulsification, foaming, water absorption and oil absorption properties, but also has many physiological activities, such as antioxidant, antifungal and ACE inhibitory activities [1-3]. In recent years, the hydrolysates of plant-derived proteins have attracted an increasing number of researchers’ attention due to their good biological activities, especially antioxidant activities [4-6]. For example, the rapeseed protein hydrolysates prepared with various proteases (alcalase, proteinase K, pepsin + pancreatin, thermolysin and flavourzyme) and hydrolysates (5–10 kDa) for alcalase and proteinase K had better Fe\textsuperscript{2+} chelating ability than the other three hydrolysates [4]. Zheng et al. [7] demonstrated that the three selected black bean protein hydrolysates for ficin, bromelain and alcalase could scavenge free radicals and chelate ferrous ions, among which alcalase hydrolysate had the best antioxidant activity. Ambigaipalan et al. [8] indicated that alcalase and flavourzyme hydrolysates of date seed protein exhibited high scavenging for ABTS, DPPH and hydroxyl radicals. At present, protein hydrolysates are obtained by protease hydrolysis, which could change the physicochemical and enhance the antioxidant activities without affecting their nutritional value [1].

Some researches focused on integrating various methods to enhance the physicochemical properties of protein hydrolysates, because protein hydrolysates by a single enzymatic treatment had the lower functional and antioxidant properties [9]. Ultrasound is widely used in the food industry due to being a safe, non-toxic and environmental protection technology [10]. Low frequency (20–100 kHz) ultrasound could produce strong shear and mechanical forces due to the thermal and cavitation effect [11]. These forces could change hydrogen bond and hydrophobic interaction as well as molecular structure of protein, so improving the physicochemical and the functional properties of protein [12]. Some studies have been carried out on ultrasonic and enzymatic hydrolysis in order to improve the antioxidant activities of protein hydrolysates. Zhang et al. [9] demonstrated that ultrasound-assisted-
pretreatment improved significantly antioxidant activities of soy protein isolate hydrolysates including DPPH, ABTS and hydroxyl radical scavenging activity. The sweet potato protein hydrolysates hydrolyzed by ultrasonic assisted alcalase showed strong hydroxyl radical scavenging activity and the hydrolysates for ultrasonic assisted flavourzyme had high oxygen radical absorbance capacity in compare with untreated-control [13]. Liu et al. [14] reported that ultrasonic-assisted-heating effected the structure and improved reducing power and ABTS radical scavenging activity of conjugated whey protein peptides.

However, there is no literature on influence of ultrasonic treatment on the physicochemical and antioxidant properties of mung bean protein hydrolysate (MPH). In this study, the effects of different ultrasonic power (114 W, 222 W, 330 W, 438 W and 546 W) on the physicochemical and antioxidant activities of MPH were investigated. The physicochemical properties of MPH were determined by the total amino acid compositions, tricine-sodium dodecyl sulphate–polyacrylamide gel electrophoresis (Tricine-SDS-PAGE), particle size distribution, zeta potential, fourier transform infrared (FTIR) spectroscopy, intrinsic fluorescence spectroscopy and surface hydrophobicity. The antioxidant activities of MPH were assessed using the 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging activity, hydroxyl radical scavenging activity, superoxide anion radical scavenging activity, ferrous chelating ability and reducing power. The aim of this study was to provide support for the application of MPH as antioxidant activities ingredients in the food industry.

2. Materials and methods

2.1. Materials

Peeled mung bean was obtained from Zhanchuang Agricultural Technology Co., Ltd., (Shandong, China). Alcalase (176,743 U/g) was acquired from Novo Co. Ltd., (Beijing, China). 1-anilino-8-naphthalene sulfonate (ANS) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were from Sigma Co., Ltd. (St. Louis, MO USA). Tris-HCl buffer, sodium dodecyl sulphate (SDS), glycerol, bromophenol blue, 2-mercaptoethanol, Coomassie brilliant blue (R-250), trichloroacetic acid (TCA), potassium bromide, pyrogallol, salicylic acid, ferrous sulfate, potassium ferricyanide, ferric chloride, and ferrous chloride were from Beijing Solarbio Technology Co. Ltd., (Beijing, China). All chemicals used in this study were of analytical grade.

2.2. Preparation of mung bean protein (MBP) and its hydrolysate

MBP was prepared using the method depicted by Liu et al. [15]. The ground mung bean flour (1.000 g) was dispersed in 15 mL deionized water and the mixture was adjusted to pH 9.0 with 1.0 M NaOH. The obtained mixture was centrifuged three times at 4000 × g for 10 min at 25 °C after stirred by a magnetic stirrer (S10-3, Shanghai Sile Instrument Co. Ltd., China) at 40 °C for 1 h. The obtained supernatant was adjusted to pH 8.0 with 1.0 M HCl, followed by centrifugation at 4000 × g for 10 min at 25 °C. An aliquot (0.100 g) of obtained precipitate was re-dispersed in 4 mL of deionized water and adjusted to pH 7.0 using 1.0 M NaOH. The dispersion was freeze-dried to gain mung bean protein (MBP). The protein content of MBP was 90.78%, which was determined using the Kjeldahl method (N × 6.25) [16].

Freeze-dried MBP was dispersed in deionized water to obtain a 20 mg/mL concentration and hydrolyzed with 3000 U/g (enzyme unit/substrate weight) alcalase activity at the condition of pH 8.0, 60 °C and 4 h. The pH value 8.0 was maintained by adding dropwise 1 M NaOH during the hydrolysis process. After alcalase hydrolysis the hydrolysate dispersion was heated at 100 °C for 10 min to inactivate the enzymes, then cooled to 4 °C and centrifuged at 8000 × g (4 °C) for 20 min. The obtained supernatant was freeze-dried to be regarded as MBP hydrolysate (MPH) [17].

2.3. Ultrasonic treatment of MPH

MPH samples were treated according to the method of Tian et al. [11] with slight modifications. Aliquots of 30 mL of MPH dispersion (100 mg/mL) in a 50 mL glass beaker was treated by ultrasonic homogenizer (JY92-IIDN, NingBo Scientz Biotechnology Co. Ltd., China) with a 6.00 mm diameter titanium probe. Ultrasonic processing was performed at a frequency of 20 kHz with five different outputs power (114 W, 222 W, 330 W, 438 W and 546 W) for 20 min each time (pulse duration was 5 s on time and 1 s off time). The MPH dispersion was immersed in an ice water bath throughout ultrasonic treatment. The treated dispersions by five different outputs power were freeze-dried to be named MPH-114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W, respectively.

2.4. Determination of the physicochemical properties of MPH and ultrasonicated-MPH

2.4.1. Determination of the total amino acid composition

The compositions of the total amino acids were determined using the method of Xie et al. [1] with slight modifications. MPH and ultrasonicated-MPH (0.025 g) were respectively hydrolyzed with 10 mL HCl (6 M) at 110 °C under a nitrogen atmosphere for 24 h. The obtained hydrolyzed solution was filtered with double-layer filter paper and diluted with ultrapure water in a 50 mL volumetric flask. The diluted solution was filtered with a 0.22 μm membrane filter, then analyzed by amino acids automatic analyzer (Biochrom 30°, Biochrom, UK) with Biochrom Na-cation exchange resin column (4.6 × 20 cm).

2.4.2. Tricine-sodium dodecyl sulphate polyacrylamide gel electrophoresis (Tricine-SDS-PAGE)

The Tricine-SDS-PAGES of MPH and ultrasonicated-MPH were performed by the method of Resendiz-Vazquez et al. [18] using 16% separating gel and 4% stacking gel. An aliquot (0.01 g) of MPH and ultrasonicated-MPH, respectively was mixed with 1 mL of reducing sample buffer (0.05 M Tris-HCl in pH 8.0, 1% SDS, 10% glycerol, 0.02% bromophenol blue, and 1% 2-mercaptoethanol) using a vortex (XW-80A, Shanghai Chitang Electronics Co. Ltd., Shanghai, China) and then boiled at 100 °C for 5 min. The following Tris-Tricine running buffer system was used: 0.1 M Tris-HCl, pH 8.9 as an anode buffer and 0.1 M Tris, 0.1 M Tricine, 1 g/L SDS, pH 8.25 as a cathode buffer. Aliquots of the treated samples (10 μL) were respectively loaded into each lane. They were first run at a 30 V electric tension on the stacking gel for 1 h and then at a 100 V electric tension on the separation gel for 2 h. The obtained gel was dyed with Coomassie brilliant blue (R-250) for 1 h and then decolorized with a mixed solution (7.5% glacial acetic acid and 5.0% ethyl alcohol). Prestained protein marker (3.3–31.0 kDa) (Beijing Solarbio Technology Co. Ltd., China) was used as the standard.

2.4.3. Determination of the particle size and zeta potential

The particle sizes and zeta potentials of MPH and ultrasonicated-MPH were determined using the method described by Wang et al. [17] with some modifications. Aliquots of 0.3 mg/mL of MPH and ultrasonicated-MPH dispersion was respectively prepared with sodium phosphate buffer (0.01 M, pH 7.0), and then the particle size distributions and zeta potential of prepared dispersion were determined using a laser particle size analyzer (Zetasizer Nano ZS90, Malvern Instruments Co. Ltd., UK) according to dynamic light scattering and laser doppler.

2.4.4. Determination of the fourier transform infrared spectroscopy (FTIR)

A fourier transform infrared spectrometer (Nicolet iS10, Thermo Fisher Scientific Co. Ltd., USA) was used to analyze the secondary structures of the MPH and ultrasonicated-MPH according to the method described by Xie et al. [1] with some modifications. Aliquots (0.001 g) of MPH and ultrasonicated-MPH were respectively mixed with 0.1 g potassium bromide and ground under an infrared lamp to be pressed into 1
mm slices. Each sample was scanned from 500 to 4000 cm\(^{-1}\) at 25 °C after the background was collected. The measurement resolution was 4 cm\(^{-1}\) with 32 scans. The obtained data were analyzed using Peakfit v4.12.

2.4.5. Determination of the intrinsic fluorescence spectroscopy

The intrinsic fluorescence spectroscopy of MPH and ultrasonicated-MPH was measured using the method of Ai et al. [19] by a fluorescence spectrophotometer (F2700, Hitachi, Japan) with some modifications. Aliquots of 0.03 mg/mL of MPH and ultrasonicated-MPH dispersion were respectively prepared with sodium phosphate buffer (0.01 M, pH 7.0), and then the prepared dispersion were scanned in the range of 300–500 nm wavelength with an excitation wavelength of 280 nm and a slit width of 10 nm.

2.4.6. Determination of the surface hydrophobicity

The surface hydrophobicity of sample was evaluated using the method of Wang et al. [17] with a fluorescence spectrophotometer (F2700, Hitachi, Japan). Aliquots of MPH and ultrasonicated-MPH (4 mL, 0.03 mg/mL) prepared with sodium phosphate buffer (0.01 M, pH 7.0) were respectively mixed with 20 μL of 8 mM ANS (1-anilino-8-naphthalene sulfonate). The obtained mixture was scanned in the range of 390–650 nm at a 375 nm excitation wavelength and a slit width of 10 nm.

2.5. Determination of the antioxidant activities of MPH and ultrasonicated-MPH

2.5.1. Determination of ABTS radical scavenging activity

The ABTS radical scavenging activity of MPH and ultrasonicated-MPH was determined using the method of Shahi et al. [20] with some modifications. The potassium persulphate (2.45 mM, 50 mL) was mixed with 50 mL of 7 mM ABTS and kept in the dark for 12–16 h. The mixture was diluted with 0.2 M phosphate buffer solution (pH 7.4) to obtain an absorbance of 0.70 ± 0.02 at 734 nm, which is called an ABTS\(^+\) radical solution.

MPH and ultrasonicated-MPH (40 μL, 0.1–0.5 mg/mL) were respectively mixed with 4 mL of the ABTS\(^+\) radical solution and kept in a dark environment for 6 min. Then the absorbance of the mixed sample was determined at 734 nm and ABTS radical scavenging activity of the sample was calculated using Eq. (1).

\[
\text{ABTS radical scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \tag{1}
\]

where \(A_0\) is the absorbance of the sample solution and \(A_1\) is the absorbance of water instead of the sample.

The 50% inhibitory concentration values (IC\(_{50}\)) were employed to evaluate the scavenging activity and were calculated using a nonlinear fit to the experimental data [17].

2.5.2. Determination of the hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of MPH and ultrasonicated-MPH was determined using the method of Xie et al. [21] with some modifications. Aliquots of MPH and ultrasonicated-MPH dispersion (3 mL, 1.0–5.0 mg/mL) were respectively mixed with 3 mL Tris-HCl buffer (50 mM, pH 8.2) and incubated at 25 °C for 20 min. The absorbance of mixture was measured every 30 s at 320 nm within 5 min after adding 0.3 mL pyrogallol (3 mM). The superoxide anion radical scavenging activity was calculated using Eq. (3).

\[
\text{Superoxide anion radical scavenging activity (\%)} = \left(1 - \frac{\Delta A}{\Delta A_0}\right) \times 100 \tag{3}
\]

where \(\Delta A_0\) is the difference between the absorbance of water at 5 min and that at 0 min and \(\Delta A\) is the difference between the absorbance of the sample at 5 min and that at 0 min.

2.5.3. Determination of the superoxide anion radical scavenging activity

The superoxide anion radical scavenging activity was calculated by the method of Xie et al. [21] with some modifications. Aliquots of MPH and ultrasonicated-MPH dispersion (3 mL, 1.0–5.0 mg/mL) were respectively mixed with 3 mL Tris-HCl buffer (50 mM, pH 8.2) and incubated at 25 °C for 20 min. The absorbance of mixture was measured every 30 s at 320 nm within 5 min after adding 0.3 mL pyrogallol (3 mM). The superoxide anion radical scavenging activity was calculated using Eq. (3).

\[
\text{Superoxide anion radical scavenging activity (\%)} = \left(1 - \frac{\Delta A}{\Delta A_0}\right) \times 100 \tag{3}
\]

where \(\Delta A_0\) is the difference between the absorbance of water at 5 min and that at 0 min and \(\Delta A\) is the difference between the absorbance of the sample at 5 min and that at 0 min.

2.5.4. Determination of the ferrous (Fe\(^{2+}\)) chelating ability

The ferrous chelating ability was evaluated using the method of Wang et al. [17] with some modifications. An aliquot (1.0 mL) of MPH and ultrasonicated-MPH (0.1–0.5 mg/mL) was respectively added to 2.0 mL of FeCl\(_2\) (0.05 M) and ferrozine (0.5 mM). The absorbance of obtained dispersion was determined at 562 nm after incubation at 25 °C for 15 min. The ferrous chelating ability was calculated using Eq. (4).

\[
\text{Ferrous chelating ability (\%)} = \left(1 - \frac{A_1}{A_0}\right) \times 100 \tag{4}
\]

where \(A_0\) is the absorbance of water instead of sample and \(A_1\) is the absorbance of the sample.

2.5.5. Determination of the reducing power

The reducing powers of MPH and ultrasonicated-MPH were determined by the method of Zhang et al. [9] with some modifications. An aliquot of MPH and ultrasonicated-MPH dispersion (1.0 mL, 1.0–5.0 mg/mL) and 2.5 mL of 1.0% (w/v) potassium ferricyanide solution were incubated at 50 °C for 20 min, then added by 0.5 mL TCA (10%, w/v) to stop the reaction. After centrifuging at 6000 × g for 10 min, the obtained supernatant (2.5 mL) was added to 2.5 mL of deionized water and 0.5 mL of ferric chloride (0.1%, w/v). The absorbance of obtained dispersion was determined at 700 nm after incubation at 25 °C for 10 min and the absorbance value was positively correlated with the reducing power.

2.6. Statistical analysis

All tests were performed in triplicate, and the average value with the standard error was obtained. Origin 8.0 software (OriginLab Corporation, USA) was used for all statistical analyses, and SPSS 13.0 software (SPSS Inc., USA) was used to determine the significant differences. \(P < 0.05\) was defined as a significant difference between samples.

3. Results and discussion

3.1. Physicochemical properties of MPH and ultrasonicated-MPH

3.1.1. The total amino acids composition

Table 1 shows the composition and content of the total amino acids of MPH and ultrasonicated-MPH. The total amino acid contents of MPH, MPH-114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W were 61.11 g/100 g, 64.56 g/100 g, 68.87 g/100 g, 67.65 g/100 g, 67.91 g/100 g and 67.96 g/100 g, respectively, showing that ultrasonic treatment enhanced the total amino acid content of MPH. Similarly, the total amino acid content of grass carp protein hydrolysate increased from 374.68 mg/mL to 441.06 mg/mL after ultrasonic treatment [22]. The higher essential amino acid (EAA) content (47.52%–47.98%) of MPH by ultrasonic treatment than MPH (36.96%) indicated that ultrasonic treatment of MPH could significantly improve the nutritional value of MPH (\(P < 0.05\)). It was reported that the high content of hydrophobic amino acids (HAA; Ala, Val, Ile, Leu, Phe, Pro, Met, Cys) could enhance the antioxidant activity due to the increase in the fat
changes in the molecular weight of wheat gluten (540 W, 720 W, 900 W (50.7 ± 3.1). 3.1.3. Particle size distribution

energy to break the peptide bond of MPH [24]. These might be due to insufficient acoustic energy to break the peptide bond of MPH [24].

Zhang et al. [23] and Sullivan et al. [24] observed that ultrasonic treatment did not cause amide A is approximately 3000 cm⁻¹, which are related to hydrogen bonds on the main chain of polypeptide [17]. Fig. 3 A shows the Tricine-SDS-PAGE profiles of MPH and ultrasonicated-MPH. MPH, MPH-114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W all contained four main bands with 25.6 kDa, 12.8 kDa, 10.6 kDa and 4.9 kDa molecular weights, of which 4.9 kDa was the most abundant. This indicated that ultrasonic treatment improved the absolute value of zeta potential with the results of average particle size [29]. Li et al. [10] reported that ultrasonic treatment could reduce the particle size of MPH, MPH-114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W.

The main peak of amide A is approximately 3000 cm⁻¹, but the peak intensity of MPH-114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W were 41.23%-42.69%, which were higher than that of rapeseed protein alcalase hydrolysates (38.64%), suggesting that MPHs might have a better antioxidant activity than rapeseed protein alcalase hydrolysates.

Table 1

| Table 1 | The total amino acid compositions of MPH, MPH-114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W. |
|---------|---------------------------------------------------------------------------------------------------|
| Amount (g/100 g) dry basis | MPH | MPH-114 W | MPH-222 W | MPH-330 W | MPH-438 W | MPH-546 W |
| Asp | 6.34 | 5.62 | 5.76 | 5.65 | 5.62 | 5.70 |
| Thr | 1.62 | 2.50 | 2.70 | 2.64 | 2.64 | 2.65 |
| Ser | 3.12 | 3.39 | 3.60 | 3.55 | 3.55 | 3.55 |
| Glu | 10.73 | 8.12 | 8.64 | 8.52 | 8.47 | 8.50 |
| Gly | 2.63 | 2.47 | 2.63 | 2.60 | 2.63 | 2.61 |
| Ala | 2.74 | 3.06 | 3.31 | 3.22 | 3.17 | 3.19 |
| Cys | 0.12 | 0.07 | 0.08 | 0.06 | 0.06 | 0.07 |
| Val | 2.90 | 3.22 | 3.33 | 3.34 | 3.35 | 3.39 |
| Met | 1.31 | 1.50 | 1.84 | 1.74 | 1.84 | 1.80 |
| Ile | 2.78 | 3.53 | 3.81 | 3.67 | 3.78 | 3.77 |
| Leu | 3.34 | 4.92 | 5.23 | 5.14 | 5.22 | 5.26 |
| Tyr | 3.11 | 2.95 | 3.15 | 3.11 | 3.09 | 3.11 |
| Phe | 3.58 | 4.70 | 4.97 | 4.92 | 4.93 | 4.89 |
| Lys | 1.85 | 2.78 | 2.98 | 2.94 | 2.92 | 2.94 |
| His | 1.98 | 4.51 | 4.80 | 4.77 | 4.73 | 4.73 |
| Arg | 4.53 | 5.40 | 5.67 | 5.67 | 5.64 | 5.64 |
| Pro | 8.43 | 5.82 | 6.32 | 6.11 | 6.25 | 6.21 |
| TAs | 61.11 | 64.56 | 68.87 | 67.65 | 67.91 | 67.96 |
| AAAs | 6.69 | 7.65 | 8.12 | 8.03 | 8.02 | 8.00 |
| BCAs | 9.02 | 11.67 | 12.42 | 12.15 | 12.35 | 12.42 |
| HAs | 25.20 | 26.82 | 28.94 | 28.20 | 28.60 | 28.58 |
| PAs | 8.36 | 12.69 | 13.45 | 13.38 | 13.31 | 13.26 |
| NCAAs | 17.07 | 13.74 | 14.40 | 14.17 | 14.09 | 14.20 |
| EAs | 22.59 | 30.68 | 32.94 | 32.33 | 32.58 | 32.61 |

Total amino acids (TAs): Phe, Tyr, Branch chain amino acids (BCAAs): Leu, Ile, Val. Hydrophobic amino acids (HAs): Ala, Val, Ile, Leu, Phe, Pro, Met, Cys. Positively charged amino acids (PAs): Arg, His, Lys. Negatively charged amino acids (NCAAs): Asp, Glu. Essential amino acid (EAs): Thr, Cys, Val, Met, Ile, Leu, Tyr, Phe, Lys, His.

The average particle sizes of MPH, MPH-114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W were 326.30 nm, 479.67 nm, 333.80 nm, 319.17 nm, 296.37 nm and 290.13 nm, respectively.

The ultrasonic treatment improved the absolute value of zeta potential than MPH. Wang et al. [27] and Malik et al. [28] also reported that high ultrasonic power could reduce the particle size of rice protein hydrolysates (800 W) from about 1400 nm to 800 nm and peanut protein (500 W) from 114.6 nm to 89.5 nm.

3.1.4. Zeta potential

The zeta potential could reflect the surface electrical properties of particles in dispersion. The higher the absolute value of zeta potential of particles in disperse system, the stronger the repulsion among particles, which was conducive to dispersion of particles [10]. The zeta potentials of MPH, MPH-114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W were −33.30 mV, −25.00 mV, −31.50 mV, −33.87 mV, −35.40 mV and −36.37 mV, respectively (Fig. 2). These data displayed that MPH-330 W, MPH-438 W and MPH-546 W dispersion had higher zeta potentials than MPH, while MPH-114 W, MPH-222 W had lower zeta potentials than MPH. This indicated that the higher ultrasonic powers could enhance zeta potentials of MPH and its dispersibility in disperse system. However, low ultrasonic power could cause particles aggregation of MPH due to the weak particles repulsion, which was consistent with the results of average particle size [29]. Li et al. [10] reported that ultrasonic treatment improved the absolute value of zeta potential (about −16 mV to −25 mV) to make rapeseed protein to disperse easily.

And the increase of zeta potential of MPH was due to the destruction of protein structure after ultrasonic treatment to make more charges accumulate on the molecular chain [30].

3.1.5. Fourier transform infrared spectroscopy (FTIR)

FTIR, an important tool to reflect the secondary structure of proteins, is related to the vibrational states of chemical bonds in proteins [11,19]. The main peak of amide A is approximately 3000–3500 cm⁻¹ caused by N–H bending and O–H stretching vibrations, which are related to hydrogen bonds on the main chain of polypeptide [17]. Fig. 3A shows the FTIR spectra of MPH and ultrasonicated-MPH in the range of 4000–400 cm⁻¹. The peaks range of amide A of MPH and ultrasonicated-MPH all were in 3000–3500 cm⁻¹, but the peak intensity of ultrasonicated-MPH (16.51%-52.73%) were higher than that of MPH (70.53%), suggesting that ultrasonic treatment enhanced the strength of hydrogen bonding among MPH molecules [19]. The hydrophobic region of the protein could be reflected in the range of 3000–2800 cm⁻¹ caused by C–H stretching vibrations [17]. The peak values of MPH and ultrasonicated-MPH for hydrophobic region were 2929 cm⁻¹ and 2933 cm⁻¹, showing that ultrasonic treatment led to blue-shift of MPH.
Moreover, the peak intensities (30.28%-66.50%) of ultrasonicated-MPH were stronger than that of MPH (81.85%), indicating that ultrasonic treatment destroyed the hydrophobic regions of MPH. The main peak of amide I of MPH and ultrasonicated-MPH appeared at approximately 1600 – 1700 cm$^{-1}$ on behalf of complex secondary structures due to the stretching of C=O and the bending of N=H [19,21]. The characteristic spectra of α-helices were the range of 1650–1660 cm$^{-1}$, β-sheets 1600–1640 cm$^{-1}$, random coils 1640–1650 cm$^{-1}$, and β-turns 1660–1700 cm$^{-1}$ [19]. The contents of α-helix, β-sheet, β-turn and the random coil of MPH, MPH-114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W were 17.04%, 20.86%, 41.61%, 20.79%; 12.42%, 21.81%, 46.21%, 19.56%; 12.43%, 22.27%, 45.12%, 20.18%; 12.83%, 21.90%, 45.03%, 20.24%; 14.12%, 22.62%, 42.40%, 20.86%; 12.81%, 22.02%, 44.67%, 20.49%, respectively (in Table 2) by simulating the peak area of the amide I band according to PeakFit software (Systat Software Corporation, USA). These data showed that the α-helix structures of ultrasonicated-MPH were significantly lower than those of MPH, while the β-sheet and β-turn of ultrasonicated-MPH were higher than those of MPH. And the random coil structure of ultrasonicated-MPH changed hardly compared with MPH. The decrease of the α-helix content for ultrasonicated-MPH might be that ultrasonic cavitation caused the partial unfold of α-helix [31]. The increase of the β-sheet and β-turn content for ultrasonicated-MPH could be attributed to the transformation of partial unfold the α-helix.
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into β-sheet and β-turn. Similarly, ultrasonic treatment (200 W-600 W) made the content of the α-helix decrease from 21.02% to 19.06% and the β-sheet increase from 36.85% to 44.92% in soybean protein isolate [32]. However, Zhang et al. [33] reported that ultrasonic treatment reduced the contents of β-turn (from 29.52% to 27.06%) in wheat gluten and increased β-sheet content (from 42.48% to 44.65%). These different changes might arise from the varied protein types, concentrations and sonication parameters [34]. The main peak of amide II is about 1500–1600 cm⁻¹ due to the N–H stretching. The stronger peak intensity (15.04%-53.87%) of ultrasonicated-MPH than that of MPH (71.55%) with range of 1500–1600 cm⁻¹ showed that ultrasonicated-MPH had a greater number of amino groups than MPH [35].

3.1.6. Intrinsic fluorescence spectroscopy

Intrinsic fluorescence can reflect the characteristics of aromatic amino acids (Tyr and Trp) in proteins and characterize tertiary structures of protein [11]. Trp residues play a major role in fluorescence peak wavelength from 320 to 350 nm, because the emission intensity of the Tyr residues is weak [19]. Fig. 3B shows the intrinsic fluorescence spectra of MPH and ultrasonicated-MPH in the range of 325–500 nm. The λmax value of ultrasonicated-MPH (336.5 nm-337.0 nm) changed scarcely in comparison with MPH (337.5 nm). The intrinsic fluorescence intensity was mainly affected by the energy transfer between Trp and Tyr as well as the fluorescence quenching of adjacent groups [17]. The intrinsic fluorescence intensities (3647–3771 A.U.) of ultrasonicated-MPH were lower than that (3928 A.U.) of MPH, indicating that ultrasonic treatment could reduce the intrinsic fluorescence intensity of MPH and change the tertiary structure of MPH. This might be due to a reduction of energy transfer from Trp to Tyr or an increase in the number of fluorescence quenching groups [17]. Similarly, Xiong et al. [26] reported that ultrasonic treatment (amplitude 60% and 90%) decreased

| Table 2 | Secondary structure of MPH, MPH-114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W. |
|---------|---------------------------------------------------|
| α-helix (%) | β-sheet (%) | β-turn (%) | Random structure (%) |
| MPH | 17.04 ± 0.21a | 20.86 ± 1.03b | 41.61 ± 0.55d | 20.79 ± 0.38a |
| MPH-114 W | 12.42 ± 0.11ab | 21.81 ± 0.98b | 46.21 ± 0.34c | 19.56 ± 0.98a |
| MPH-222 W | 12.43 ± 0.05c | 22.27 ± 0.66b | 45.12 ± 0.25c | 20.18 ± 0.54a |
| MPH-330 W | 12.83 ± 0.52c | 21.90 ± 0.81ab | 45.03 ± 0.41c | 20.24 ± 1.01a |
| MPH-438 W | 14.12 ± 0.22c | 22.62 ± 0.65a | 42.40 ± 0.28c | 20.86 ± 0.56a |
| MPH-546 W | 12.82 ± 0.12ab | 22.02 ± 0.65a | 44.67 ± 0.28c | 20.49 ± 0.76a |

Different letters in the same column indicate a significant difference (P < 0.05). The results are expressed as the mean ± standard deviation (n = 3).
intrinsic fluorescence intensity from 950 to 650 of ovalbumin, which might contribute to the change of tertiary structure and partial unfold of ovalbumin.

3.1.7. Surface hydrophobicity

ANS, an exogenous fluorescent marker, binds with exposed hydrophobic regions in some unfolded proteins to produce fluorescence, which is positively correlated with hydrophobicity [7]. The fluorescence emission spectra of MPH and ultrasonicated-MPH bound with ANS are depicted in Fig. 3C. The surface hydrophobicity of MPH-114 W, MPH-222 W, MPH-330 W and MPH-438 W (386.91–422.62 A.U.), except MPH-546 W (367.95 A.U.), were higher than MPH (368.75 A.U.), showing that ultrasonic treatment made more hydrophobic groups expose. The decrease of surface hydrophobicity of MPH-546 W might be due to proteins re-polymerization through hydrophobic binding at high ultrasonic power [32]. It was reported that ultrasonic cavitation promoted molecular expansion and disrupted hydrophobic interactions [10]. Xiong et al. [26] also reported that ultrasonic treatment changed tertiary structure of ovalbumin and caused unfolding of molecules to increase the surface hydrophobicity of ovalbumin. Similarly, the surface hydrophobicity of soybean protein isolate increased from 700 to 850 at ultrasonic power (from 200 W to 400 W) and decreased from 850 to 750 at 600 W [32].

3.2. Antioxidant activity of MPH and ultrasonicated-MPH

3.2.1. ABTS radical scavenging activity

ABTS, a chemically stable chromophore compound, produces stable water-soluble free radicals after oxidation. Therefore, ABTS radical scavenging ability could be used to evaluate the antioxidant activity of proteins or peptides [36]. Fig. 4A displays that the ABTS radical scavenging activities of MPH and ultrasonicated-MPH increased from 39.19% to 99.10% as the concentration increased from 0.1 to 0.5 mg/mL. The ABTS radical scavenging activities of 0.5 mg/mL of MPH, MPH-
114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W reached 95.60%, 95.46%, 96.53%, 98.22% and 99.10%, respectively, and the IC$_{50}$ values of these treated samples were 0.1209, 0.1219, 0.1273, 0.1169, 0.1140 and 0.1087 mg/mL, respectively. These results showed that ultrasonic treatment could significantly ($P < 0.05$) improve the ABTS radical scavenging activities of MPH. Similarly, the ABTS radical scavenging activities of soybean protein isolate and Chlorella pyrenoidosa protein were significantly enhanced after ultrasonic treatment [36,37].

The IC$_{50}$ values of MPH and ultrasonicated-MPH were 84.34%, 86.52%, 87.01%, 88.16%, 89.41% and 90.69%, respectively, and the IC$_{50}$ acids (11.77%-11.87%) of ultrasonicated-MPH were higher than that of untreated MPH suggested that ultrasonic treatment was beneficial for enhancing the ABTS radical scavenging activities of MPH. Moreover, the high aromatic amino acids could improve antioxidant capacity by converting free radicals into stable molecules through electron doners [1]. The higher ABTS free radical scavenging activity of ultrasonicated-MPH than MPH might be that aromatic amino acids (11.77%-11.87%) of ultrasonicated-MPH were higher than that of MPH (10.95%). The IC$_{50}$ (0.1081–0.1219 mg/mL) of the ABTS radical scavenging activities of MPH and ultrasonicated-MPH were lower than those of tree peony seed proteins hydrolysates (1.57–2.21 mg/mL) and soy protein hydrolysates (0.827–1.055 mg/mL), indicating that the ABTS radical scavenging activity of MPH was superior to tree peony seed proteins hydrolysates and soy protein hydrolysates [9,17].

3.2.2. Hydroxyl radical scavenging activity

Hydroxyl radical, the most active free radical in biological tissue proteins, is easy to react with DNA, proteins and lipids [20]. So hydroxyl radical scavenging ability could also estimate the antioxidant property of proteins or peptides. Hydroxyl radical scavenging rates of 5.0 mg/mL of MPH-114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W were 86.52%, 87.01%, 88.16%, 89.41% and 90.69%, respectively, which were higher than that of MPH (84.34%). And MPH-546 W had the lowest IC$_{50}$ value (1.796 mg/mL), followed by 1.970, 2.189, 2.502, 2.710 and 2.704 mg/mL for MPH-438 W, MPH-330 W, MPH-222 W, MPH-114 W and MPH, respectively (in Fig. 4B). These data displayed that hydroxyl radical scavenging activity of ultrasonicated-MPH became stronger with the increase of ultrasonic power from 114 W to 546 W. The IC$_{50}$ of ultrasonicated soybean protein (1.34 mg/mL) for scavenging hydroxyl radical was lower than that of untreated soybean protein (1.45 mg/mL), indicating that ultrasonic treatment improved antioxidant activity of soybean protein [9]. Ultrasonic treatment destroyed the dense structure of protein to release more electron or hydrogen, so improving hydroxyl radical scavenging activity of ultrasonicated-MPH [9,38].

3.2.3. Superoxide anion radical scavenging activity

To further confirm the antioxidant activity, the superoxide anion radical scavenging activities of MPH and ultrasonicated-MPH were determined at 1.0–5.0 mg/mL (Fig. 4C). The superoxide anion radical scavenging activities of 5.0 mg/mL of MPH, MPH-114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W were 61.44%, 63.84%, 67.30%, 67.32%, 68.00%, and 68.69%, respectively. And the IC$_{50}$ of these treated samples were 2.427, 1.127, 1.416, 1.478, 1.113, and 1.003 mg/mL, respectively. The lower IC$_{50}$ of ultrasonicated-MPH than untreated MPH suggested that ultrasonic treatment was beneficial for enhancing superoxide anion radical scavenging activity of MPH.

3.2.4. Ferrous (Fe$^{2+}$) chelating ability

Fe$^{2+}$ chelating ability could evaluate the degree of lipid oxidation, cell and tissue damage, so as to reflect the antioxidant properties of samples [5]. The Fe$^{2+}$ chelating abilities at 0.5 mg/mL of MPH, MPH-114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W were 82.86%, 82.96%, 83.93%, 88.34%, 88.04% and 90.99%, respectively (Fig. 4D). And the IC$_{50}$ values of MPH, MPH-114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W were 0.218, 0.215, 0.208, 0.192, 0.196, and 0.185 mg/mL, respectively. These data indicated the IC$_{50}$ values of ultrasonicated-MPH were lower than that of MPH, especially MPH-438 W and MPH-546 W, demonstrating ultrasonic treatment could improve Fe$^{2+}$ chelating ability of MPH. Ultrasonic treatment could increase the number of carboxyl and amino groups of acidic and basic amino acid branches, especially Phe, Tyr and Lys (in Table 1), to produce more negative charges, which was conducive to Fe$^{2+}$ binding [7,8]. Similarly, Fe$^{2+}$ chelating activity (34.37%) of ultrasonicated chlorella pyrenoidosa protein at 6.0 mg/mL was higher than that of untreated chlorella pyrenoidosa protein (19.97%) [36]. Wang et al. [17] and He et al. [4] reported IC$_{50}$ to Fe$^{2+}$ chelating abilities of tree peony seed protein hydrolysates and rapeseed protein alcalase hydrolysate were 0.99–2.70 mg/mL and 3.93 mg/mL. The IC$_{50}$ (0.185–0.218 mg/mL) values of MPH and ultrasonicated-MPH were lower than that tree peony seed and rapeseed protein hydrolysates, displaying that MPH had a stronger Fe$^{2+}$ chelating ability than tree peony seed and rapeseed protein hydrolysates.

3.2.5. Reducing power

The reducing power, a method to assess antioxidant activity of sample, was measured by reducing ferric iron (Fe$^{3+}$) to ferrous iron
(Fe²⁺) [14,17]. The reducing powers of MPH, MPH-114 W, MPH-222 W, MPH-330 W, MPH-438 W and MPH-546 W at 5.0 mg/mL were 0.5190, 0.5310, 0.5585, 0.5815, 0.5946 and 0.6377, respectively (in Fig. 4E), displaying that ultrasonicated-MPH had stronger reducing powers than that of MPH at the same concentration. Similarly, the reducing power of whey protein isolate-Ga increased from 0.02 to 0.61 when being treated that of MPH at the same concentration. Similarly, the reducing power of whey protein isolate-Ga increased from 0.02 to 0.61 when being treated

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[38x733](Fe²⁺) produce more negative charges, so improving hydroxyl radical scavenging activity of MPH. Meanwhile, the high aromatic and hydrophobic amino acids of MPH due to ultrasonic treatment also improved antioxidant capacity of MPH by converting free radicals into stable molecules [11]. Ultrasonic treatment increased the contents of Phe, Tyr and HAAas (Table 1) and improved the antioxidant activity of MPH.

4. Conclusion

This paper studied the physicochemical properties and the antioxidant activities of MPH treated by different ultrasonic power (114 W, 222 W, 330 W, 438 W and 546 W). MPH and ultrasonicated-MPH consisted of four main subunits, showing that ultrasonic treatment had no effect on subunits of MPH. Ultrasonic treatment reduced the proportion of α-helix structure of MPH and increased the proportions of β-sheet and β-turn. Ultrasonic treatment destroyed the dense structure of protein and hydrophobic region and released more carboxyl and amino groups of acidic and basic amino acid branches, especially Phe, Tyr and Lys, to produce more negative charges, so improving hydroxyl radical scavenging activity and Fe²⁺ chelating ability of MPH. Moreover, the stronger ultrasonic power could produce high turbulence and shear energy to increase the collision between particles and cavitation bubble collapse, thus reducing the particle size and exposing more hydrophobic ends of MPH. And the smaller particle size and the more hydrophobic ends of MPH conferred a benefit on ABTS free radical scavenging activity of MPH. Meanwhile, the high aromatic and hydrophobic amino acids of MPH due to ultrasonic treatment also improved antioxidant capacity of MPH by converting free radicals into stable molecules through electron donors. Therefore, ultrasonic treatment could be used to enhance the biological activity of MPH, making it as potential antioxidant ingredients in the food industry.

CRediT authorship contribution statement

Fen-Fang Liu: Methodology, Writing - original draft, Writing - review & editing. Ying-Qu Li: Conceptualization, Data curation, Formal analysis, Funding acquisition. Gui-Jin Sun: Investigation. Chen-Ying Wang: Investigation, Formal analysis. Yan Liang: Investigation. Xiang-Zhong Zhao: Investigation. Jin-Xing He: Investigation. Hai-Zhen Mo: Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] J.H. Xie, M.X. Du, M.Y. Shen, T. Wu, L.H. Lin, Physico-chemical properties, antioxidant activities and angiotensin I converting enzyme inhibitory of protein hydrolysates from mung bean (Vigna radiata), Food Chemistry 270 (2019) 243-250, https://doi.org/10.1016/j.foodchem.2018.07.103.
[2] M.X. Du, J.H. Xie, B. Gong, X. Xu, W. Tang, X. Li, C. Li, M.Y. Xie, Extraction, physicochemical characteristics and functional properties of mung bean protein, Food Hydrocolloids 76 (2017) 131-140, https://doi.org/10.1016/j.foodhyd.2017.01.003.
[3] M. Li, Y. Zhang, S. Xia, X. Ding, Finding and isolation of novel peptides with anti-proliferation ability of hepatocellular carcinoma cells from mung bean protein hydrolysates, Journal of Functional Foods 62 (2019) 103557, https://doi.org/10.1016/j.jff.2019.103557.
[4] R. He, A.T. Girgih, S.A. Malomo, X. Ju, R.E. Ahko, Antioxidant activities of enzymatic rapspey protein hydrolysates and the membrane ultrafiltration fractions, Journal of Functional Foods 5 (1) (2013) 219-227, https://doi.org/10.1016/j.jff.2012.10.008.
[5] M. Nikoo, J.M. Regenstein, F. Noori, S. Péri Gheghlaghi, Autolysis of rainbow trout (Oncorhynchus mykiss) by-products: Enzymatic activities, lipid and protein oxidation, and antioxidant activity of protein hydrolysates, LWT-Food Science and Technology 140 (2021) 110702, https://doi.org/10.1016/j.lwt.2020.110702.
[6] Z. Wang, X. Liu, H. Xie, Z. Liu, K. Rakarizham, C. Yu, F. Shahidi, D. Zhou, Antioxidant activity and functional properties of Alcalase-hydrolyzed scallop protein hydrolysate and its role in the inhibition of cytotoxicity in vitro, Food Chemistry 344 (2021) 128566, https://doi.org/10.1016/j.foodchem.2020.128566.
[7] Z. Zheng, J. Li, J. Li, H. Sun, Y. Liu, Physicochemical and antioxidative characteristics of black bean protein hydrolysates obtained from different enzymes, Food Hydrocolloids 97 (2019) 105222, https://doi.org/10.1016/j.foodhyd.2019.105222.
[8] P. Ambigaipalan, A.S. Al-Khalifa, F. Shahidi, Antioxidant and angiotensin I converting enzyme (ACE) inhibitory activities of date seed protein hydrolysates prepared using Alcalase, Flavourzyme and Thermolysin, Journal of Functional Foods 18 (2015) 1125-1137, https://doi.org/10.1016/j.jff.2015.01.021.
[9] W. Zhang, L. Huang, W. Chen, J. Wang, S. Wang, Influence of ionic liquid pretreatments on the functional properties of soy protein hydrolysates, Ultrasonics Sonochemistry 73 (2021) 105546, https://doi.org/10.1016/j.ultsonch.2021.105546.
[10] Y. Li, Y.X. Cheng, Z. Zhang, Y. Wang, B.K. Mintah, M. Dabbour, H. Jiang, R. He, H. Ma, Modification of rapspey protein by ultrasound-assisted pH shift treatment: Ultrasonic mode and frequency screening, changes in protein solubility and structural characteristics, Ultrasonics Sonochemistry 69 (2020) 105240, https://doi.org/10.1016/j.ultsonch.2020.105240.
[11] R. Tian, J. Feng, G. Huang, B.o. Tian, Y. Zhang, L. Jiang, X. Sui, Ultrasound driven conformational and physicochemical changes of soy protein hydrolysates, Ultrasonics Sonochemistry 68 (2020) 105202, https://doi.org/10.1016/j.ultsonch.2020.105202.
[12] Y. Zou, H. Yang, P.P. Li, M.H. Zhang, X.X. Zhang, W.M. Xu, D.Y. Wang, Effect of different time of ultrasonic treatment on physicochemical, thermal, and antioxidant properties of chicken plasma proteins, Poultry Science 98 (2019) 1925-1933, https://doi.org/10.3934/ps.pey502.
[13] I. Habinesshuti, T.-H. Mu, M. Zhang, Ultrasound microwave-assisted enzymatic production and characterization of antioxidant peptides from sweet potato protein, Ultrasonics Sonochemistry 69 (2020) 105262, https://doi.org/10.1016/j.ultsonch.2020.105262.
[14] L. Liu, X.D. Li, L.L. Du, X.X. Zhang, W.S. Yang, H.D. Zhang, Effect of ultrasound assisted heating on structure and antioxidant activity of whey protein peptide gradiented with galactose, LWT- Food Science and Technology 109 (2019) 130-136, https://doi.org/10.1016/j.lwt.2019.04.015.
[15] F.F. Liu, Y.Q. Li, L.-Y. Wang, X.-Z. Zhao, Y. Liang, J.X. He, H.-Z. Mo, Impact of pH on the physicochemical and rheological properties of mung bean (Vigna radiata l.) protein, Process Biochem 111 (2021) 274-284, https://doi.org/10.1016/j.pbi.2020.10.008.
[16] Gb 5009.5, Determination of protein in foods, National Standard, China, 2016.
[17] Y.-F. Wang, C.-Y. Wang, S.-T. Wang, Y.-Q. Li, H.-L. Mo, J.-X. He, Physicochemical properties and antioxidant activities of tree peony (Paeonia suffruticosa Andr.) seed protein hydrolysates obtained with different proteases, Food Chemistry 345 345 (2021) 128765, https://doi.org/10.1016/j.foodchem.2020.128765.
[18] A.E. Rosendal-Vazquez, J.A. Ulloa, J.E. Ustas-Silvas, P.U. Bautista-Rosales, J. C. Ramirez-Ramirez, R. Rosas Ulloa, L. Gonzalez-Torres, Effect of high-intensity ultrasound on the technofunctional properties and structure of jackfruit (Artocarpus heterophyllus) seed protein isolate, Ultrasonics Sonochemistry 37 (2017) 436-444, https://doi.org/10.1016/j.ultsonch.2017.01.042.
[19] M. Ai, T. Tang, L. Zhou, Z. Ling, S. Guo, A. Jiang, Jiang, Effects of different proteases on the emulsifying capacity, rheological and structure characteristics of preserved egg white hydrolysates, Food Hydrocolloids 87 (2019) 933-942, https://doi.org/10.1016/j.foodhyd.2018.09.025.
[20] Z. Shahi, S.Z. Sayed-Alangi, L. Najafian, Effects of enzyme type and process time on hydrolysis degree, electrophoresis bands and antioxidant properties of
hydrolyzed proteins derived from defatted Bunium persicum bios. press cake, Heliyon 6 (2) (2020) 03365, https://doi.org/10.1016/j.heliyon.2020.e03365.

[21] H. Xie, J. Huang, M.W. Woo, J. Hu, H. Xiong, Q. Zhao, Effect of cold and hot enzyme deactivation on the structural and functional properties of rice dreg protein hydrolysates, Food Chemistry 345 (2021) 128784, https://doi.org/10.1016/j.foodchem.2020.128784.

[22] X. Yang, Y. Li, S. Li, X. Ren, A. Olayemi Oladejo, F. Lu, H. Ma, Effects and mechanism of ultrasound pretreatment of protein on the Maillard reaction of protein hydrolysate from grass carp (Ctenopharyngodon idella), Ultrasonics Sonochemistry 64 (2020) 104964, https://doi.org/10.1016/j.ultsonch.2020.104964.

[23] H. Zhang, L.P. Claver, K.X. Zhu, H. Zhou, The effect of ultrasound on the functional properties of wheat gluten, Molecules 16 (12) (2011) 4231–4240, https://doi.org/10.3390/molecules16124231.

[24] J. O’Sullivan, M. Park, J. Beevers, The effect of ultrasound upon the physicochemical and emulsifying properties of wheat and soy protein isolates, Journal of Cereal Science 69 (2016) 77–84, https://doi.org/10.1016/j.jcs.2016.02.013.

[25] L.Z. Jiang, J. Wang, Y. Li, Z. Wang, J. Liang, R. Wang, Y. Chen, W. Ma, B. Qi, M. Zhang, Effects of ultrasound on the structure and physical properties of black bean protein isolates, Food Research International 62 (2014) 595–601, https://doi.org/10.1016/j.foodres.2014.04.022.

[26] W.F. Xiong, Y.T. Wang, C.L. Zhang, J.W. Wan, B.R. Shah, Y.Q. Pei, B. Zhou, J. Li, B. Li, High intensity ultrasound modified ovalbumin: Structure, interface and gelation properties, Ultrasonics Sonochemistry 31 (2016) 302–309, https://doi.org/10.1016/j.ultsonch.2016.03.026.

[27] L. Wang, J. Ding, Y. Fang, X. Pan, F. Fan, P. Li, Q. Hu, Effect of ultrasound power on properties of edible composite films based on rice protein hydrolysates and chitosan, Ultrasonics Sonochemistry 65 (2020) 105049, https://doi.org/10.1016/j.ultsonch.2020.105049.

[28] M.A. Malik, H.K. Sharma, C.S. Saini, High intensity ultrasonic treatment of protein-hydrolysate from grass carp (Ctenopharyngodon idella), Ultrasonics Sonochemistry 39 (2017) 511–519, https://doi.org/10.1016/j.ultsonch.2017.05.026.

[29] L.T. Zhang, Z. Pan, K.Q. Shen, X.H. Cai, B.D. Zheng, S. Miao, Influence of ultrasound-assisted alkali treatment on the structural properties and functional properties of rice protein, Journal of Cereal Science 79 (2019) 204–209, https://doi.org/10.1016/j.jcs.2019.10.013.

[30] Y. Zhao, F. Li, M.T. Carvajal, M.T. Harris, Interactions between bovine serum albumin and alginate: an evaluation of alginate as protein carrier, Journal of Colloid and Interface Science 332 (2) (2009) 345–353, https://doi.org/10.1016/j.jcis.2008.12.046.

[31] D.C. Kang, Y.H. Zou, Y.P. Cheng, L.J. Xing, G.H. Zhou, W.G. Zhang, Effects of power ultrasound on oxidation and structure of beef proteins during curing processing, Ultrasonics Sonochemistry 33 (2016) 47–53, https://doi.org/10.1016/j.ultsonch.2016.04.024.

[32] S. Yan, J. Xu, S. Zhang, Y. Li, Effects of flexibility and surface hydrophobicity on emulsifying properties: Ultrasound-treated soybean protein isolate, LWT-Food Science and Technology 142 (2021) 110881, https://doi.org/10.1016/j.lwt.2021.110881.

[33] J. Zhang, D.L. Luo, J.L. Xiang, W. Xu, B.C. Xu, P.Y. Li, J.H. Huang, Structural Variations of Wheat Proteins under ultrasonic treatment, Journal of Cereal Science 99 (2021), 103219, https://doi.org/10.1016/j.jcs.2021.103219.

[34] C. Letang, M. Piau, C. Verdier, L. Lefebvre, Characterization of wheat-flour-water doughs: a new method using ultrasound, Ultrasonics 39 (2) (2001) 133–141, https://doi.org/10.1016/S0041-624X(00)00058-5.

[35] A.B. Stefanovic, J.R. Jovanovic, B.D. Balanec, N.Z. Sekuljica, S.M.J. Tanaskovic, M. B. Djoricinovic, Z.D. Knezevic-Jugovic, Influence of ultrasound probe treatment time and protease type on functional and physicochemical characteristics of egg white protein hydrolysates, Poultry Science 97 (6) (2018) 2218–2229.

[36] H. Lian, C. Wen, J. Zhang, Y. Feng, Y. Duan, J. Zhou, Y. He, H. Zhang, H. Ma, Effects of simultaneous dual-frequency divergent ultrasound-assisted extraction on the structure, thermal and antioxidant properties of protein from Chlorella pyrenoidosa, Algal Research 56 (2021) 102294, https://doi.org/10.1016/j.algal.2021.102294.

[37] X. Tong, J. Cao, T. Tian, B.o. Lyu, N. Cui, S. Liu, H. Wang, L. Jiang, Changes in structure, rheological property and antioxidant activity of soy protein isolate fibrils by ultrasound pretreatment and EGCG, Food Hydrocolloids 122 (2022) 107884, https://doi.org/10.1016/j.foodhyd.2021.107884.

[38] X. Chen, D.L. Fang, R.Q. Zhao, J. Gao, B.M. Kimatu, Q.H. Hu, G.T. Chen, L.Y. Zhao, Effects of ultrasound-assisted extraction on antioxidant activity and bidirectional immunomodulatory activity of Flammulina velutipes polysaccharide, International Journal of Biological Macromolecules 140 (2019) 505–514, https://doi.org/10.1016/j.ijbiomac.2019.08.163.

[39] C. Wen, J. Zhang, J. Zhou, Y. Feng, Y. Duan, H. Zhang, H. Ma, Slit divergent ultrasound pretreatment assisted watermelon seed protein enzymolysis and the antioxidant activity of its hydrolysates in vitro and in vivo, Food Chemistry 328 (2022) 127135, https://doi.org/10.1016/j.foodchem.2020.127135.

[40] B.H. Sarmadi, A. Ismail, Antioxidative peptides from food proteins: A review, Peptides 31 (10) (2010) 1949–1956, https://doi.org/10.1016/j.peptides.2010.06.020.