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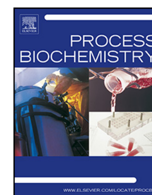

Effects of ultrasound assisted extraction on the physiochemical, structural and functional characteristics of duck liver protein isolate

Ye Zou [a], Li Wang [b], Pengpeng Li [a], Panpan Cai [c], Muhan Zhang [a], Zhilan Sun [a], Chong Sun [a], Zhiming Geng [a], Weimin Xu [a,**], Xinglian Xu [d], Daoying Wang [a,d,*]

[a] Institute of Agricultural Products Processing, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, PR China
[b] College of Food Science and Engineering, Yangzhou University, Yangzhou 225127, PR China
[c] Ginling College, Nanjing Normal University, Nanjing 210024, PR China
[d] Jiangsu Collaborative Innovation Center of Meat Production and Processing, Quality and Safety Control, Nanjing Agricultural University, Nanjing 210095, PR China

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ABSTRACT

This work investigated the impact of ultrasound assisted extraction on the physicochemical, structural and functional properties of duck liver protein isolate (UDLPI). Degreased liver powders were extracted by ultrasound working at a single frequency of 24 kHz and a fixed power of 266 W by a pulsed on-time of 2 s and off-time of 3 s for 42 min in pH11.2 solution. The results revealed that UDLPI yield and the protein content increased by 67.7% and 4.6% respectively compared to that of the conventional alkaline extraction (DLPI). Ultrasound treatment could cause partial protein hydrolysis and unfolding as suggested by differential scanning calorimetry, circular dichroism and fluorescence spectroscopy analysis, leading to increased surface hydrophobicity, surface net charge and gelling property. The particle size reduced from 177.8 nm of DLPI to 156.0 nm of UDLPI. Ultrasound also promoted the storage modulus (G') and solubility of the isolate. Moreover, the foaming expansion was especially strong, compared to DLPI. However, the results of the foam stability, reactive-/total-sulfhydryl groups and sodium-dodecyl sulfate-polyacrylamide gel electrophoresis showed no significant change between UDLPI and DLPI ($P > 0.05$). Therefore, UDLPI with better functional property could be utilized as new materials in the food industry.
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1. Introduction

Animal processing by-products retain a substantial amount of fat and protein [1]. The major by-products of animal processing industry are the spent bones and skin from beef and pork by-products. As an inevitable consequence of incremental duck meat consumption, duck by-products are increasing, such as skin, feet, bones, heads and viscera [2,3]. However, there is litter information on the utilization of poultry. By-products of the poultry processing industry are a readily available potential source of protein [4]. Hence, development of new technologies in search of novel bioactive compounds from duck processing by-products will create value out of what is today considered a waste, and represent unique challenges and opportunities for the livestock farm.

Protein isolate obtained from duck processing by-products represent a growing ingredient market in part due to consumer preferences. Numerous methods for the extraction of protein from plants and animals have been reported [5,6]. Extraction is the first key step to isolate natural bioactive compounds from duck processing by-products. Various extraction method could be selected for different protein types which in turn influences the final composition and functionality of the isolate product [7]. Different extraction methods (i.e. solvent extraction, expelling extraction process, supercritical fluid extraction (SFE) and microwave assisted extraction (MAE)) have been developed for the recovery of bioactive compounds [8]. However, several disadvantages like extra solvent amount in solvent extraction, low yield in expelling process, massive investment in SFE and the requirement for the aqueous phase in microwave assisted extraction indicate the demand of comprehensive extraction method to recover different target compounds in economic condition [9,10].

* Corresponding author at: Institute of Agricultural Products Processing, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, PR China.
** Corresponding author.
E-mail addresses: weiminxu2002@aliyun.com (W. Xu), daoyingwang@yahoo.com (D. Wang).

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Ultrasounds are the high-frequency sound waves (20 kHz) that exceed the frequency hearing capacity of the human ear. Ultrasound is a novel, simple, green, safe, rapid and economically viable technology that is suitable for scale up, extraction, protein digestion, production and drug delivery of food derived bioactive peptides [11,12]. Ultrasound assisted extraction (UAE) is a new simple technique for the recovery of bioactive compounds from different sources [13,14]. The effect of ultrasound in biological medium and efficient alternative to traditional methods is related to cavitation, heating, dynamic agitation, shear stresses, and turbulence [15]. Ultrasonic treatments (20 kHz with amplitude of 20–80%) have used to improve the yield of collagen from seafood by-products by few research groups [16,17]. It facilitates the extraction of heat sensitive compounds with minimal damage and higher yield and may cause chemical and physical changes producing aggregates through covalent and non-covalent bonds by cyclic generation and collapse of cavities. The intensity of ultrasound power creates extra vibration in sample molecules and facilitates the recovery of target compounds from solid material to the liquid solvent phase. Thus, high yield in short extraction time along with the utilization of low solvent amount are remarkable advantages of UAE technique [18]. Moreover, it is possible to obtain a determined aggregate particle size, leading to a defined functional property change [19].

The overall aim of this study was to investigate the yield of duck protein isolate derived from UAE. Moreover, little is known about the physicochemical characteristics and functional properties of the duck liver protein isolate processed by ultrasound assisted alkali (UDLPI) compared with the conventional alkaline extraction (DLPI). The information obtained from this study may be useful in identifying a liver extraction method with high efficiency and low consumption for producing a protein isolate with functionality best suited for a specific end use.

2. Materials and methods

2.1. Preparation of degreased duck liver powder

After slaughtering, duck livers were treated with the method described by Yang et al. [20] with a slight modification. Prior to an experiment, the smaller blocks were tempered overnight in a refrigerator at 4 °C. Distilled and deionised water was added to unfrozen duck livers at a 1:2 ratio (duck liver/water, w/v), followed by a homogenisation in a laboratory blender with a high speed (Model T25 D S-25, IKA, Germany) for 3 min and heated at 95 °C for 10 min to denature the endogenous enzymes in the livers, and then extracted thoroughly with ethanol to obtain degreased duck liver powders from our previous report [21]. At last, the degreased powders were dried at 60 °C in air-drying oven to a constant weight, and the dry powders were stored in plastic bags at 4 °C until further analysis.

2.2. Alkaline extraction of liver protein

For alkali extraction of liver protein, Degreased liver powders were mixed with a known amount of sodium hydroxide solution (pH11.2) and stirred continuously for 5 h at 50 °C using a vibrator (Model HH-8, Guohua electric appliance co., LTD, China). The mixture was then centrifuged at 5000*g* for 15 min using a TDL–40 B Centrifuge (Vnicen MR, Herolab, Germany). The supernatants of alkaline extraction were adjusted to pH 4.3 with 0.5 mol/L HCl, left overnight at 4 °C to precipitate the protein, and then the mixture was centrifuged (13400*g* for 30 min) to precipitate the extract. The supernatant of duck liver protein isolate (DLPI) was carefully collected for further treatment.

2.3. Ultrasonic assisted alkaline extraction of liver protein

Degreased liver powders were weighed into a glass container containing a known amount of sodium hydroxide solution (pH11.2) and temperature-controlled ultrasonic generator of 40 °C (The recovery yield of the protein isolate reached the peak value at 40 °C from our single-factor test in ultrasound treatment). They were extracted by ultrasound with a 1.5 cm flat tip probe operating in a pulsed on-time of 2 s and off-time of 3 s for 42 min. A probe ultrasonic reactor (SC-II, Chengdu Jiuzhou ultrasonic technology co., LTD.) working with a single frequency of 24 kHz and a fixed power of 266 W was used in the ultrasonication experiments. The supernatant phase was carefully collected for further treatment. The supernatants of ultrasonic assisted alkaline extraction were treated as the above section. The precipitate (duck liver protein isolate by ultrasonication (UDLPI)) was then freeze dried, and the dry powders were stored in plastic bags at −20 °C until further analysis.

2.4. Total protein content

The total protein content of both DLPI and UDLPI was estimated by the Bradford procedure [22]. The sample was dispersed in an amount of NaOH (pH11.2), heated in the boiling water for 10 min and cooled in an ice-water bath. After cooling the solution was filtered through Whatman No. 1 filter paper. Then, 15 mL of the filtrate was centrifuged with 15 mL of anhydrous ether at 3000*g* for 10 min. After centrifugation, 0.05 mL of the lower phase was taken and mixed with 3.0 mL of Bradford reagent and the absorbance was measured at 595 nm (UV-6100, Meipuda of instrument co., LTD, Shanghai, China). Bovine serum albumin (Sigma-Aldrich, St Louis, MO, USA) was used as a standard.

2.5. Surface hydrophobicity

The surface hydrophobicity was determined through fluorescence spectroscopy with an ANS probe in accordance with the method described by Dodero and Messina [23] with slight modifications. The relative fluorescence intensity was obtained using an FL-4600 fluorescence spectrophotometer (Hitachi, Ibaraki, Japan). Fluorescence intensity was recorded at excitation and emission wavelengths of 340 and 470 nm, respectively. The index of surface hydrophobicity was determined from the initial slope of the plot of fluorescence intensity versus protein concentration.

2.6. Reactive and total sulfhydryl groups determination

The estimation of reactive (R-SH) and total (T-SH) sulfhydryl groups were performed using protocols of Jimenez-Guzman and Cruz-Guerrero [24] and Coan [25], respectively. Samples were prepared by homogenizing (4500 *g* for 1 min) 250 mg of recovered protein in 25 mL of Tris-glycine buffer (pH 8.0) containing 5 mM of EDTA. The homogenate was filtered before use. For R-SH, 1 mL of filtrate was mixed with 4 mL of Tris-glycine–8 M Urea buffer (pH 8.0) and 50 μL of Ellman's reagent. The mixture was kept for 1 h at 4 °C with intermittent stirring. The absorbance of the solutions was measured at 412 nm against a blank of Ellman's reagent at the same concentration without samples using a spectrophotometer. In case of T-SH estimation, to 1 mL of the filtrate, 4 mL of Tris-glycine–10 M Urea and 50 μL of Ellman's reagent (10 mM 5, 5-dithiobis (2-nitrobenzoic acid)) were added and mixed well by vortex mixer (Fisher, Scientific, On, Canada). The SH content was calculated by using molar extinction coefficient of 13, 600 M^{-1} cm^{-1} and expressed as μM.

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