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Application of ultrasound treatment for improving the physicochemical, functional and rheological properties of myofibrillar proteins

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ABSTRACT

The aim of this study was to evaluate the impact of duration (10, 20 and 30 min) and power (100 and 300 W) of high-intensity ultrasound (20 kHz) on physicochemical properties of beef myofibrillar proteins in order to investigate novel process for modification of its functional characteristics. Results showed that augmentation of duration and power of ultrasound led to enhance pH. Also, the water holding capacity and gel strength were improved by increasing pH. The highest value in pH, reactive sulfhydryl content, water holding capacity and gel strength was obtained in sample subjected to 30 min of ultrasound at 300 W. The particle size distribution of the proteins was decreased after ultrasound treatment because of the cavitation force of ultrasound waves. In this circumstance, an improvement of emulsifying properties can be obtained. Ultrasonic waves had significant effects on the rheological properties of myofibrillar proteins. Treated samples were more elastic and stiffer than control, although the inverse trend was observed after 30 min treatment at each power. Finally, a reducing trend in viscosity was observed by increasing time and power of sonication. Ultrasonic treatment could successfully improve functional properties with effect on physicochemical properties of myofibrillar proteins.

1. Introduction

Proteins are the main structural and functional compounds of processed meat and thus are considered as the determinant factor of texture and appearance of these products. Evaluating the functional characteristics of meat proteins is necessary for less use of expensive meats, producing new products and improving the quality of existing products [21]. Myofibrillar proteins (especially actin and myosin) play an important role in functional properties of meat. These proteins are the largest part of proteins in muscle tissue and have a moderate solubility compared to the sarcoplasmic and stroma proteins [33]. For example, it is estimated that 97% of water holding capacity in meat is only due to the presence of these proteins. Although it is difficult to determine the exact amount of myofibrillar proteins participating in the emulsifying capacity of meat, it seems that these proteins are responsible for >75% of the meat emulsifying capability, and in fact they can form >90% of the emulsifying capacity of total meat [9,12,45]. Obviously, the myofibrillar proteins have the important effects on the quality and application of meat. Myofibrillar proteins usually are able to form the gel network and increase the water holding capacity due to entrapping the moisture. Among all myofibrillar proteins the impact of actin and myosin, which constitute the majority of myofibrillar proteins, on the functional properties of meat have been widely studied. It seems that the rest of the myofibrillar proteins do not play a role in this context [35].

High-intensity ultrasound (HUS) is a new effective technology for the food processing and is considered as safe, non-toxic, and environment-friendly technology. Ultrasound is based on mechanical waves at a frequency higher than the human hearing threshold and can be divided into two frequency ranges of high (100 kHz–1 MHz, power < 1 W cm^{-2}) and low (16–100 KHz, power in the range of 10–100 W cm^{-2}). Low-intensity ultrasound (high frequency) is more commonly used as a technique for providing information about physicochemical properties of food such as firmness, ripeness, sugar content, acidity. In contrast, high intensity ultrasound (low frequency) can be used to change the food properties physically or chemically [5,36].

The effects of ultrasound on liquid systems are mainly due to the cavitation phenomenon. The sonic cavitation produces intense physical forces that include shear forces, shock waves, and turbulences, thereby potentially changes the functional properties of proteins by physical or chemical methods. Also, during the sonication, the bubbles are formed quickly and collapsed intensely that it can be one of the reasons of chemical and physical changes. In addition the increased pressure and temperature in the vicinity of these cavities are the basis of changes in medium exposed to ultrasound. The periodic production and collapse of cavities generates the shear forces around the bulk liquid, which these shear forces are strong enough to break the covalent bonds in the polymeric materials [5,15]. Furthermore, ultrasound can produce

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very active free radicals from water molecules ($H_2O \rightarrow H + OH$), which results in reactions with other molecules. Ultrasound is widely used in many processes, such as inactivation of microorganisms and enzymes, extraction and homogenization [6]. In particular, the aim of conventional use of ultrasound for meat and meat products mainly is tenderness and curing of the meat [7,21,29,37]. High-intensity ultrasound creates a physical breakdown in meat tissues, increases the proteolytic activity and accelerates the mass transfer through cavitation [7,16,37]. Several studies have reported that ultrasound influences the structural properties and improves the functional properties of food proteins, such as whey protein [8,19,20], soy protein isolates [17,18] and egg white proteins [3,4]. Other studies have used high intensity ultrasound to modify the physical and functional properties of the protein, such as solubility, coagulation, emulsification and foaming ability. For example, Madadlou, Emam-Djomeh, Mousavi, Mohamadifar and Ehsani [27] found that ultrasonic treatment of casein could delay the coagulation point and increase the firmness of the casein gel. Also, Madadlou, Mousavi, Emam-Djomeh, Ehsani and Sheehan [28] reported a decrease in opacity of casein solutions by ultrasonic radiation [27,28]. Kresic, Lelas, Jambrak, Herceg and Brncic [23] showed that the ultrasonic radiation of whey proteins significantly increased the solubility and apparent viscosity, and flow behavior was changed by shear thickening [23]. Zisu et al. reported that sonication reactions at a frequency of 20 kHz could be used to reduce the viscosity and improve the gelation of casein and whey protein in dairy products [46]. High intensity ultrasound not only is a quick, efficient and reliable procedure to improve the quality of food protein, but also has the potential for the development of new products in the food industry [36]. The study aims to evaluate the effects of high intensity ultrasound treatment as a function of ultrasound power and treatment time on the functional and structural properties of myofibrillar proteins in order to obtain a better understanding of the high intensity ultrasound physical effects on these type of proteins that may improve their applications in the food industry.

2. Materials and methods

2.1. Materials

Longissimus dorsi muscle from five Holestein bulls with approximate weight of 550 ± 50 kg and age of ~3 years was separated immediately after slaughter. The muscles were transferred to the laboratory and after 12 h storage at 16 °C, they were cut to small pieces and stored at −18 °C until use.

2.2. Myofibrillar protein extraction

After thawing of meat samples at 4 °C, they were grounded. One part of grounded meat was mixed with four volumes cold extracting medium consisting 100 mM KCl, 25 Mm K_2HPO_4/KH_2PO_4 and 2 mM EDTA (pH = 7.5). The mixture was homogenized for the 30 s at 16,000 rpm (Ultra-Turrax t 18 IKA, German). Then, the homogenate was centrifuged at 16,000*g* for 15 min. After separating the supernatant containing the sarcoplasmic proteins, the resulting sediments were again homogenized by four volumes the extracting buffer and then passed through polyethylene strainer (18 mesh) for separation of stromal proteins. The resulting solution was centrifuged at 16,000*g* for 15 min and the sediments were then homogenized with four volumes extracting buffer containing 10% sodium chloride. The protein concentration of the solution was determined using biuret method and adjusted to 3% by adding the sodium chloride-extracting buffer [13].

2.3. High-intensity ultrasound

Ultrasound treatment was performed using an ultrasonic processor (APU500a, Adeeco Co., Iran) with a maximum net power output of 500 W in a frequency of 20 kHz. Twenty ml of the extracted myofibrillar proteins with 3% concentration were sonicated in a 50 ml glass double-walled beaker equipped with a cooling jacket for either 0, 10, 20 or 30 min at two powers of 100 W and 300 W. During ultrasound treatment, the probe (12 mm diameter) was immersed in the liquid to a depth of 1.5 cm from the bottom and the ice water was circulated around the vessel. An ultrasound pulse mode of on-time 2 s and off-time 4 s was used. After ultrasound treatment, the samples were stored at 4 °C for analyses. All samples preparations and treatments were carried out with five replicates.

2.4. pH measurement

pH of the myofibrillar protein solution was determined before and after ultrasound treatment at room temperature. pH of treated and control samples was measured by immersion of pH probe (Jenoy 3330, USA) into a mixture of 10% meat in water.

2.5. Particle size analysis

The particle size was determined immediately after the ultrasound treatment when the particles were still dispersed. Particle size distribution was performed using a Static Laser Particle Analyser (Horiba, LA-930, Japan) according to the multimodal light-scattering method. The samples were diluted with distilled water before measuring the particle size to prevent the multiple scattering effects.

2.6. Myofibrillar protein solubility

To determine myofibrillar protein solubility, 1 ml of 3% protein solution was mixed with 2 ml of 10% saline phosphate buffer (pH = 7.3). The solution was stirred for 30 min and then centrifuged at 10,000 × *g* for 15 min. The supernatant was then extracted and its protein concentration was determined using the biuret method [13]. The absorption of protein solution was read at 540 nm (Unico UV2100, UK) and expressed as mg protein/ml solution. Bovin serum albumin (Sigma Chemical Co., St. Louis, MO) was used for drawing of standard curve.

2.7. Water holding capacity (WHC)

WHC of the myofibrillar proteins was determined with some modifications in a method described by Kocher and Foegeding (1993) [22]. From each sample, 1 g was transferred to a test tube, and heated in a water bath at 80 °C for 20 min. It was cooled at 4 °C for 24 h. The test tube was centrifuged at 10,000 × *g* for 10 min (Z36HK, Hermle, Germany). After centrifugation, the supernatant was discarded and weight of the sample before and after centrifuge were subtracted. WHC is expressed as the percentage of retained water divided by the initial gel weight before centrifugation.

2.8. Emulsifying properties

The emulsifying properties of myofibrillar protein were determined with a few modification of turbidometric technique described by Pearce and Kinsella (1978) [32]. For preparation of the emulsion, 2 ml of sesame oil and 0.8 ml of protein solution in 0.1 M phosphate buffer (pH 6.5, 1.0% protein) were shaken and homogenized by a homogenizer (Ultra-Turrax t18 IKA, Germany) at 10,000 rpm for 2 min at 20 °C. An aliquot from a newly prepared emulsion (50 μl) was taken from 0.5 cm end of a beaker and dispersed within 5 ml of 0.1% sodium dodecyl sulfate (SDS), and the absorption was measured at a wavelength of 500 nm against a 0.1% SDS as the blank solution by a spectrophotometer (Unico UV2100, UK). The emulsions were stored undisturbed for 10 min at 20 °C, and then 50 μl aliquot was taken from 0.5 cm end of the beaker and dispersed within 5 ml of 0.1% SDS solution. The absorption at