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Improvement of meat tenderness by simultaneous application of high-intensity ultrasonic radiation and papain treatment☆

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ABSTRACT

The present study aims to develop a novel process for improving meat tenderness. Three muscles were selected among five young Holstein bulls *Longissimus lumborum* muscles and were cut into 3 × 3 × 3 cm pieces. Samples were sonicated by ultrasonic probe (20 kHz) at power of 100 and 300 W for 10, 20, and 30 min both in the presence and absence of 0.1% papain enzyme solution. The effects of treatments were investigated on proteolytic activity, filtering residues, Warner–Bratzler shear force (WBSF), meat textural profile (TPA), and muscle microstructure. Application of enzyme, either singly or coupled with ultrasound significantly ($P < 0.05$) decreased the filtering residue, WBSF and textural parameters. The most proteolytic activity and the highest tenderness were obtained when the combined treatment was applied at ultrasonic power of 100 W for 20 min. Finally, the results showed that, combined treatment can be employed as a useful tool for the meat tenderization.

1. Introduction

Tenderness is known as one of the most important attributes of meat that greatly influences its consumer acceptability (Istrati, 2008). Post-slaughter tenderization is the result of the proteolysis of myofibrillar and cytoskeletal proteins by proteases as well as the loss of connective tissue compounds, especially the collagen (Lawrie & Ledward, 2006). As tenderness accounts as a major meat-eating satisfaction, food scientists have always sought efficient tenderization processes that are capable of improving meat quality (Got et al., 1999). So far, mechanical, chemical, biochemical, physical, and enzymatic methods have been employed by the meat processing industry to achieve desirable tenderness in meat products (Istrati, 2008).

Application of enzyme for tenderization of meat has been considered for years. Exogenous enzymes such as papain, bromelain, and ficin have been widely used as meat tenderizers (Gerelt, Ikeuchi, & Suzuki, 2000; Ashie, Sorensen, & Nielsen, 2002). Papain is extracted from papaya latex (EC 3.4.22.2) and is one of the commonest plant enzymes used for artificial tenderization of meat due to its ability to break down both myofibrillar proteins and connective tissues (Ashie et al., 2002). Studies have also shown that papain treatment increases meat tenderness. Wang and Maynard (1955) found that immersion of frozen-dried pork chops in a solution of papain led to a significant increase in muscle tenderness. Gerelt et al. (2000), Istrati (2008) and, Whitehurst and Van Oort (2009) also reported the fragmentation of myofibrils and improvement of meat tenderness after enzymatic tenderization with papain. However, one problem commonly associated with the enzymatic system is finding an effective way of increasing enzyme diffusion in meat. A number of methods have been developed to introduce proteolytic enzymes into meat cuttings. These include both dry methods, such as powder application, and humid ones, such as immersion of meat or rehydration of freeze-dried samples in an enzyme solution and injection of enzymes in the muscle (Gerelt et al., 2000; Istrati, 2008). This is why, dry and humid methods are time consuming and by injection of an enzyme to meat, needle damage is observable on tissue (Jayasooriya, Torley, D'arcy, & Bhandari, 2007). The industry is, hence, seeking to find novel, non-destructive methods for accelerating meat tenderization or enzyme penetration.

Ultrasound radiation is a cost-effective, non-invasive technique that has been mainly used for improving meat properties (Awad, Moharram, Shaltout, Asker, & Youssef, 2012; Turantaş, Kılıç, & Kılıç, 2015). In this technique, the compression and depression induced by ultrasonic waves produce microbubbles in the structure whose implosion leads to a phenomenon known as cavitation that propagates shock waves of high energy throughout the tissue, thereby causing damages (Got et al., 1999; Awad et al., 2012). Ultrasound applied to meat for tenderization increases the cathepsin and calcium releases as a result of physical disruption of the muscular tissues (Got et al., 1999; Turantaş et al., 2015). Dickens, Lyon, and Wilson (1991) investigated the effect of low frequency ultrasound radiation (40 kHz, 15 min) on broiler breast muscle and cooked meat to find that it improved the tenderness and textural properties of meat samples. Dolatowski and Twarda (2004) and,

☆ Improvement of meat tenderness.

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Jayasooriya et al. (2007) also reported enhanced tenderness in beef *Semitendinosus* and *Longissimus lumborum et thoracis* muscles exposed to ultrasonic radiation.

Ultrasound in combination with other methods is reported to be effective for improving the general quality such as margination, tenderness and modifying the functional properties in meat and poultry products (Turantaş et al., 2015). Xiong, Zhang, Zhang, and Wu (2012) reported that contribution of mechanical disruption by ultrasound (24 kHz for 4 min) and endogenous proteolytic enzymes can be a suitable method for improving tenderness and decreasing the cooking loss of hens. They suggested that combined treatment, such as ultrasonic with enzyme treatment could have a synergistic effect on meat properties. Numerous publications are based on the effect of high-intensity ultrasound on meat properties. However, few have focused on the effect of ultrasound combined with enzyme treatments, and neither have reported the influence of simultaneous application of ultrasonic radiation and exogenous enzyme on properties of meat. So, the present study was conducted to evaluate the effects of ultrasonic alone and in combinations with papain on the tenderness and certain other physicochemical properties of *Longissimus lumborum (LL)* muscles in an attempt to develop a novel method for improving beef tenderness.

2. Materials and methods

2.1. Materials

Papain (EC 3.4.22.2), K-casein of bovine milk (PubChem CID: 73995022), L-Cysteine hydrochloride (PubChem CID: 60960), L-Tyrosine (PubChem CID: 6057), and Tris-HCl buffer (PubChem CID: 95373) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All the chemicals and reagents used were of analytical grade.

2.2. Meat preparation

Longissimus lumborum muscles, approximately 2–3 kg in weight, were purchased from a selected local slaughterhouse. The muscles were removed from five Holstein bulls with approximate age of 2.5 years and weight of 500 ± 50 kg immediately after slaughter. All animal had the same feeding regime. They were offered a mixed diet consisting of hay, barley and concentrate feed. These animals were slaughtered humanely according to the Islamic method of slaughtering without electrical stimulation. The muscles were transferred to the laboratory for pH measurement. The pH was measured after 12 h storage of meat at 16 °C by a Consort C831 pH-meter (Consort N.V., Turnhout, Belgium) and only three muscles with ultimate pH levels in the range of 5.5–5.8 were selected for further treatment to ensure the absence of Dark, Firm and Dry (DFD) meat in the samples. Surface fat, silver skin and external connective tissues of the whole *Longissimus lumborum* muscles were carefully removed with a sharp knife. Each muscle was cut into pieces of 3 × 3 × 3 cm in size. The meat cuttings from each muscle were finally sealed in individual plastic bags, labeled, and kept frozen at −18 °C until use.

2.3. Meat treatment

The samples thus prepared were divided into two groups: those to be treated solely with ultrasonic radiation and those to be treated simultaneously with both the papain enzyme (0.1 g/100 ml) and ultrasonic radiation.

In the ultrasound treatment, meat pieces were loaded into glass beakers to be immersed in 100 ml of deionized water. Ultrasonic radiation was then applied using a probe (Adeeco, Iran) at an operating frequency of 20 kHz with maximum nominal power of 400 W and a constant amplitude of 100%. The samples were sonicated at powers of 100 and 300 W for 10, 20, and 30 min (pulse durations, including 7 s on-time and 2 s off-time). During the sonication process, the 12 mm diameter cylindrical titanium (Ti-6Al-4V) ultrasound probe was retained 1 cm above the meat surface and temperature was maintained at 11–17 °C by application of water bath. During the process, samples were being rotated manually in set pause times in order to subject all the meat faces equally to the ultrasonic waves. The control sample from this group was immersed only in deionized water in the absence of ultrasonic radiation.

In the combined papain and ultrasound treatment, meat pieces were loaded into glass beakers filled with 100 ml of the papain solution (0.1% w/v) while simultaneously exposed to ultrasound waves as described above. The enzyme control samples were solely immersed into the papain solution in the absence of any ultrasound radiation.

Since papain reaches its optimal activity at temperatures in the range of 65–80 °C, the enzyme treated samples were incubated at 65 °C for 30 min. The ultrasound-radiated samples were also incubated at 65 °C for 30 min in a temperature-controlled incubation chamber (Model HCP 246, Memmert, Germany) for realistic comparisons under identical conditions. For each treatment, 5 pieces of meat from each muscle were randomly selected. Hence, each experiment was repeated totally 15 times for each treatment.

2.4. Papain activity

To determine the effect of ultrasonic radiation on the enzymatic activity of papain, a papain solution (0.1% w/v) was prepared and exposed to ultrasound radiation at 100 and 300 W for 10, 20, and 30 min. Enzyme activity was determined as described in Bruno et al. (2010) with some minor modifications. Briefly, 0.1 ml of the papain solution was mixed in a 0.1 M Tris–HCl buffer (pH 8.5) containing 12 mM of 5% cysteine. The reaction was started by incubating the mixture at 42 °C for 60 min and stopped by adding 1.8 ml of 10% (w/v) trichloroacetic acid (TCA). It blanks were prepared by adding TCA to the mixture before adding the substrate solution. Each test tube was then centrifuged for 10 min at 1800*g* (Z36 HK, Hermle, Germany) and the absorbance of the supernatant was recorded at 280 nm. Absorbance values were compared against a tyrosine standard curve and enzyme activity was expressed as the equivalent amount in micromoles of tyrosine per minute released from casein.

2.5. Total proteolytic activity

The total proteolytic activity of the enzymes extracted from *Longissimus lumborum* was evaluated after the treatment. Enzymes were extracted according to the method described in Natalia, Hashim, Ali, and Chong (2004) with some modifications. Briefly, each sample was homogenized at 16000 rpm for 2 min with two volumes (w/v) of cold distilled water. Then, 5 g of the homogenate were placed into a 50 ml test tube and centrifuged at 10,000*g* for 15 min. After enzyme extraction, the activity was determined according to the method described in Bruno et al. (2010).

2.6. Filtering residues

Filtering residues were determined according to the freeze-drying method of Chang, Xu, Zhou, Li, and Huang (2012) with some modifications. Briefly, meat samples were homogenized at 16000 rpm for 30 s (IKA, ULTRA-TURRAX, Germany) with four volumes (w/v) of cold isolating phosphate buffer (20 mM, pH 7.5). The homogenate was placed into a 50 ml test tube and centrifuged for 15 min at 2000*g* before the supernatant was decanted. The pellet was treated again according to the blending and centrifuging procedures described above. Then, the pellet was transferred to a test tube where it was homogenized in four volumes (w/v) of a 0.5 M NaCl solution for 1 min. The homogenate was filtered through a layer of the cheese cloth filter and washed twice in a solution of 0.5 M NaCl (Li et al., 2014). Finally, the material retained