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Effects of power ultrasound on oxidation and structure of beef proteins during curing processing

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ABSTRACT

The aim of this study was to evaluate the effects of power ultrasound intensity (PUS, 2.39, 6.23, 11.32 and 20.96 W cm^{-2}) and treatment time (30, 60, 90 and 120 min) on the oxidation and structure of beef proteins during the brining procedure with 6% NaCl concentration. The investigation was conducted with an ultrasonic generator with the frequency of 20 kHz and fresh beef at 48 h after slaughter. Analysis of TBARS (Thiobarbituric acid reactive substances) contents showed that PUS treatment significantly increased the extent of lipid oxidation compared to static brining ($P < 0.05$). As indicators of protein oxidation, the carbonyl contents were significantly affected by PUS ($P < 0.05$). SDS–PAGE analysis showed that PUS treatment increased protein aggregation through disulfide cross-linking, indicated by the decreasing content of total sulfhydryl groups which would contribute to protein oxidation. In addition, changes in protein structure after PUS treatment are suggested by the increases in free sulfhydryl residues and protein surface hydrophobicity. Fourier transformed infrared spectroscopy (FTIR) provided further information about the changes in protein secondary structures with increases in β-sheet and decreases in α-helix contents after PUS processing. These results indicate that PUS leads to changes in structures and oxidation of beef proteins caused by mechanical effects of cavitation and the resultant generation of free radicals.

1. Introduction

The use of power ultrasound (PUS) processing has progressively been taken up by some sectors of the food industry. Frequencies ranging from 16 to 100 kHz (power intensity in the range of 10–1000 W cm^{-2}) are commonly used for food processing, and at these power values, some physical and chemical properties of the food can be affected [1,2]. The cavitation zone generated by high PUS in a liquid medium can lead to extremely high temperatures and pressures, thereby free radicals produced. These phenomena are then responsible for the changes in characteristics, microstructures and molecular reactions of food [3–5]. Furthermore, previous studies have confirmed that the structure and the function of proteins can be modified by applying PUS. Li et al. [6] found that high-intensity PUS could be used to modify the functionality of PSE (Pale, soft and exudative)-like meat by changing the secondary structures of proteins, thereby improving the microstructure and rheological properties of gels. Hu et al. [7] reported that PUS treatment of soy protein isolates led to an increase in the surface hydrophobicity and solubility of proteins, again affecting its microstructure and rheological properties.

Meat curing is known to improve the shelf-life, flavor, juiciness and tenderness of products by immersing meat in a brine solution [8]. Recent studies using PUS have suggested that power settings between 2 and 64 W cm^{-2} can assist the curing process by reducing brining time without affecting meat quality. Thus, it is likely that PUS would be an emerging and promising technology for meat processing [9,10]. However, the use of PUS may result in quality impairments through the degradation of nutritional compounds, as well as the production of off-flavors caused by lipid and/or protein oxidation [11]. Many investigations have shown that highly reactive free radicals are generated from water molecules as a result of cavitation initiated by ultrasound and these degradation products can induce free radical chain reactions in foods [12–14]. Therefore similar reactions may be initiated by cavitation during PUS treatment causing hydrolysis or oxidation of lipid [15]. Wolff et al. [17] reported that the reactive oxygen species produced by lipid oxidation can modify or oxidize intracellular and membrane proteins in muscle. Xiao et al. [18] discovered that the free radicals

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generated from irradiation contributed to meat protein oxidation. Therefore, although there are benefits of PUS-assisted brining for accelerating mass transfer of ions and sucrose in meat, consideration should be given to the effects such a process may have on the promotion of oxidation reactions.

In the present study, the effects of PUS on lipid and protein oxidation of beef muscle with brining treatment were evaluated by the measurements of TBARS, carbonyl and sulfhydryl contents. Protein surface hydrophobicity and Fourier transformed infrared spectroscopy were used for determining structural changes in proteins. The aim was to provide insights into the relationship between protein oxidation and structural modifications during the PUS-assisted brining procedure.

2. Materials and methods

2.1. Sample and brining preparation

Five *longissimus dorsi* (LM) of beef was obtained from 5 carcasses (Limousin) at 48 h post-mortem and vacuum packed. The pH was recorded randomly at three locations along the muscle length using a pH-meter at 48 h postmortem (Hanna Co., Padova, Italy). The pH values of the 5 muscles ranged from 5.55 to 5.65. All visible fat and connective tissues were removed and slab shaped samples (50 × 50 × 10 mm), with myofibril direction parallel to the probe axis, were cored from the lean tissue with a sharp knife. Three sample locations were chosen randomly in each LM of the 5 animals. Following preparation of the samples, they were wrapped in plastic waterproof film and kept frozen storage at −20 °C until further treatment. Before the experiment, samples were slowly thawed at temperature of 2 °C for 24 h.

The basal components of brine for curing included sucrose, sodium tripolyphosphate, sodium pyrophosphate and sodium hexametaphosphate with final concentrations of 1.5%, 0.16%, 0.16% and 0.08% (w/v), respectively. Sodium chloride (NaCl) concentration was set at 6% (w/v). The temperature of brining solution was maintained at 10 °C before each treatment. The weight ratio of meat to brine was set to 1:20 for all treatments.

Table 1
Calorimetric study on VCX 750 ultrasonic probe by applying different power to 50 g water for 3 min.

Power setting (W)	Diameter of emitting surface (mm)	Actual power output (W)	Actual ultrasonic intensity[1] (W cm^{-2})
150	13.00	3.18	2.39
200		8.27	6.23
250		15.03	11.32
300		27.82	20.96

[1] Ultrasonic intensity is equal to the power output measured by calorimetry divided by the area of the emitting surface (1.327 cm^2).

2.2. PUS treatment

The effects of ultrasonic intensity (2.39, 6.23, 11.32 and 20.96 W cm^{-2}) and ultrasonic treatment time (30, 60, 90 and 120 min) on protein oxidation and structural changes were studied. The PUS intensities measured by calorimetry are shown in Table 1. Each level of ultrasonic intensity was performed for 30, 60, 90 and 120 min at 6% NaCl concentration. For ultrasonic treatment, each sample was immersed in a 500 mL beaker and the entire ultrasonic probe was inserted into the brine 20 mm from the beef surface. The cooling reservoir around the beaker maintained the temperature at 10 ± 0.5 °C (Fig. 1). The non-sonicated samples having the same brining time as mentioned above were used for control. After treatment, the samples were taken out of brine and rinsed in distilled water for 20 s to remove adhered surface salt. The samples were then wiped dry using filter paper and wrapped in plastic waterproof bag and frozen (−20 °C) until required for analysis. Each experiment was replicated independently three times.

2.3. Lipid oxidation

Lipid oxidation was determined by measuring 2-thiobarbituric acid reactive substances (TBARS) according to the method of Zhang et al. [19] with slight modification. One gram of PUS-treated meat was homogenized in 5 mL 7.5% TCA solution (containing 0.1% EDTA) in an ice bath. Samples were centrifuged at 12,000×*g* for 5 min and 2 mL of 0.02 M TBA were added to 2 mL supernatant. Then the mixed liquid was subjected to a water bath (100 °C) for 40 min. The samples were cooled and the absorbance of the samples was measured at a wavelength 538 nm and the amount of TBARS was calculated from a standard curve prepared from a series of diluted malonaldehyde (MDA) solutions. The results were expressed as mg MDA per kg muscle.

2.4. Protein oxidation measurement

2.4.1. Carbonyl group content

The carbonyl content of beef protein was assessed with 2,4-dinitrophenylhydrazine (DNPH) using the method of Zhang et al. [19] with slight modification. One gram of each of the treated beef samples about 2 mm on the surface was minced and homogenized for 15 s in 10 mL pyrophosphate buffer (pH 7.4) consisting of 2 mM $Na_2P_4O_7$, 10 mM Tris–maleate, 100 mM KCl, 2 mM $MgCl_2$ and 2 mM EGTA. One mL of 20% trichloroacetic acid (TCA) was added

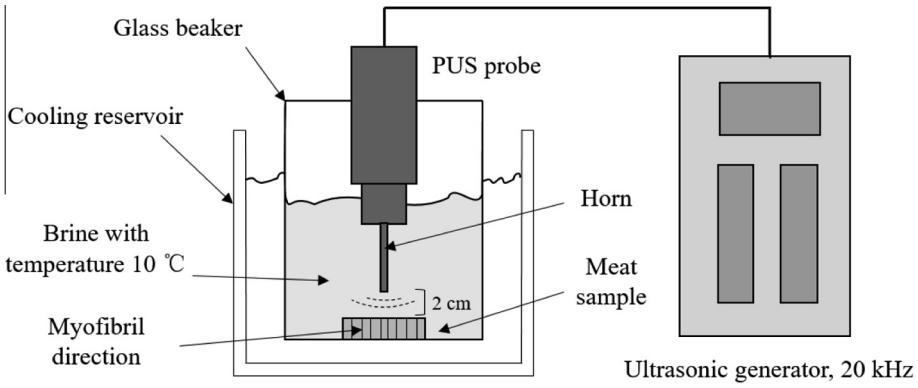

Fig. 1. Schematic diagram of experimental set-up for PUS assisted brining.