EFFECTS OF POWER ULTRASOUND ON OXIDATION OF BEEF PROTEINS DURING CURING PROCESSING

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Abstract – The aim of this study was to evaluate the effects of power ultrasound (PUS) treatment on the oxidation of beef proteins during the brining procedure. The results showed that carbonyl contents significantly increased after PUS treatment for 30 and 120 min (P<0.05), while total thiol groups significantly decreased after 120 min PUS treatment (P<0.05). SDS-PAGE showed protein aggregation through disulfide cross-linking by PUS treatment. Fourier transform infrared spectroscopy (FTIR) analysis found changes of protein secondary structure with the increase in β-sheets and decrease in α-helix structures due to protein oxidation. These results indicate that PUS could lead to oxidation and structure changes of beef proteins during curing processing.

Key Words –Carbonyl contents, Sulfydryl groups, Secondary structure

I. INTRODUCTION

Power ultrasound (PUS) processing has been gradually applied in food industry. The cavitation zone generated by high PUS in liquid medium could lead to extreme high temperatures and pressures to produce free radicals. These phenomena are responsible for the changes of characteristic, microstructure and molecular reactions of food [1].

Meat curing is known to be able to improve shelf-life, flavor, juiciness and tenderness of products by immersing meat in brine solution [2]. Recent studies have considered that 2-64 W cm^{-2} PUS assisting curing could reduce brining time without affecting meat quality [3-4]. However, there are possible quality impairments by PUS through the degradation of nutrition compounds and the production of off-flavors by lipid or protein oxidation during this processing [5]. However, no studies have investigated the effects of PUS treatment on protein oxidation during curing. In the present study, the effects of PUS on protein oxidation of beef with brining treatment were evaluated by carbonyl and sulfydryl contents. Fourier transform infrared spectroscopy was used to determine protein structure changes.

II. MATERIALS AND METHODS

The *longissimus dorsi* (LM) of beef were obtained from 5 carcasses (Limousin, pH ranging from 5.55 to 5.65) at 48 h post-mortem and vacuum packaged (slab shaped samples: 50 × 50 × 10mm). The basal components of brine for curing included sucrose, sodium tripolyphosphate, sodium pyrophosphate and sodium hexametaphosphate with the final concentration of 1.5%, 0.16%, 0.16% and 0.08% (w/v), respectively. NaCl concentration was set at 6% (w/v). The effects of ultrasonic intensity (2.39, 6.23, 11.32 and 20.96 Wcm^{-2}) and ultrasonic treatment time (30 and 120 min) on protein oxidation were studied.

The carbonyl content of beef protein was determined by the method of Zhang *et al*. [6] with slight modification. Total SH groups of the samples were determined according to the methods of Yongsawatdigul *et al*. [7] and Fu *et al.* [8]. The oxidation-induced aggregation of proteins were detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli [9]. FTIR spectra of samples were performed on a ThermoFisher Nicolet 6700 spectrometer using a DTGS (KBr beamsplitter) detector with Smart iTX accessory. Each spectrum from wave number 400 to 4,000 cm^{-1} was obtained with an average of 128 scans at a resolution of 4 cm^{-1}. The pretreatment

method for samples was following to the report of Sun *et al.* [10] with slightly modification.

Statistical analyses were performed using SAS 9.2 for Windows. For the analysis of protein oxidation and secondary structures changes, ultrasonic intensity and treating time were set as factors in a Mix-models analysis. A factor or their interaction was significant when *P* values were lower than 0.05. The Fisher's LSD (Least Significant Difference) and Bonferroni correction were used for multiple testing.

III. RESULTS AND DISCUSSION

As shown in Table 1, the carbonyl content could be significantly affected by PUS intensity (UI), treatment time (TT) and their interaction (UI×TT) (P<0.05). After 30 and 120 min PUS treatment, the carbonyl contents of beef proteins significantly increased compared to static brining. These results indicated that PUS could induce protein oxidation compared to static brining. Previous studies have demonstrated that reactive oxygen species (ROS) were responsible for the occurrence of protein oxidation [6]. Thus the free radicals produced by PUS might contribute to the increased protein carbonyl content in the current study.

Table 1 shows the total sulfhydryl groups of beef proteins treated with or without PUS. The results indicate that the effects of UI, TT and their interaction (UI×TT) could significantly affect total thiol content (P<0.05) When PUS applied, there was no significant changes among different PUS

Table 1 Oxidations of meat proteins affected by different ultrasound intensity

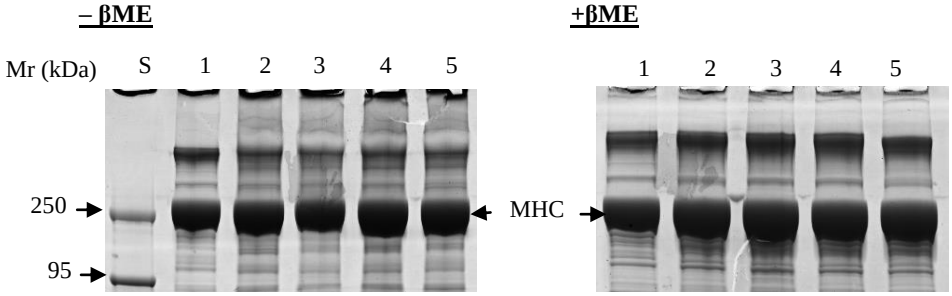
	Treat Time (min)	ultrasound intensity (W/cm^2)					SE	*P*-values		
		0	2.39	6.23	11.32	20.96		TT	UI	TT×UI
Carbonyl group (nmol/mg protein)	30	0.76^{cA}	1.09^{bB}	1.12^{abB}	1.27^{abB}	1.43^{aA}	0.06	<0.001	<0.001	<0.001
	120	0.75^{cA}	1.46^{bA}	1.79^{aA}	1.87^{aA}	1.95^{aA}				
Total Thiol Group (nmol/mg protein)	30	58.24^{aA}	57.53^{aA}	57.13^{aA}	57.22^{aA}	56.49^{aA}	1.02	<0.001	<0.001	<0.001
	120	59.75^{aA}	52.22^{bB}	51.66^{bB}	52.42^{bB}	51.45^{bB}				

[1] a~d different letters in the same raw indicate statistically significant differences at P<0.05.
[2] A~D different letters in the same column of treatment indicate statistically significant differences at P <0.05;
[3] TT means treat time, UI means ultrasound intensity

intensity at 30 min treating time (P>0.05). However, after 120 min sonication, the total sulfhydryl groups of static brining were significantly higher compared with four treatments with different PUS intensity (P<0.05). The results suggest the occurrence of protein oxidation and crosslinking of sulfhydryl could occur after long application duration of PUS treatment [11].

In order to evaluate how the PUS intensity affect protein-protein interactions of fresh beef during curing procedure, samples treated with static brining and PUS were subjected to SDS-PAGE analysis. The result showed the reduction of myosin heavy chain (MHC) and production of high molecular-weight polymers without adding β-mercaptoethanol (-β ME) after 120 min PUS treatment (Fig. 1, left). These displayed the formation of polymers when beef were exposed to different PUS treatment. However, the polymerization of proteins on the top of gels was almost completely recovered when samples were treated with +β ME (Fig. 1, right). Previous studies have shown that one of the common consequences of oxidation is protein aggregation through noncovalent or covalent interactions [12]. Our results indicated that the PUS treatment induced protein aggregation was mainly due to the formation of disulfide bond after longtime processing.

Figure 1. SDS-PAGE profiles of total muscle protein from fresh beef loin muscle treated with (3, 4 and 5) or without (1) ultrasound for 120min. S: molecular weight standards; 1: static brining 120min; 2, 3, 4 and 5 represent ultrasound intensity 2.39 W/cm^2, 6.23 W/cm^2, 11.32 W/cm^2 and 20.96 W/cm^2 treated 120min, respectively. Electrophoresis was run under non-reducing (−βME) and reducing (+βME) conditions. MHC: myosin heavy chain



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In order to obtain further information about protein structural changes induced by oxidation, the FTIR spectra of static brining and PUS treatment samples between wavelengh number 1700 and 1600 cm^{-1} were analyzed. Fig. 2 shows the typical deconvoluted FTIR spectra of freeze drying myofibrillar protein of beef with or without PUS treatment in present study. Five major peaks related to the certain protein secondary structure could be observed in the amide I region.

As shown in Table 2, PUS treatment and treating time had significant effects on the secondary structures and relative contents of both α-helix and β-sheet (P<0.05). For the static brining, treating time had no significant influence on the secondary structures of beef proteins (P>0.05). When PUS was applied, the α-helix contents were decreaed while β-sheet contents were increased with PUS intensity or processing time. These findings indicated that PUS could result in the unfolding of α-helical region followed by the formation of β-sheet, and

Figure 2. Typical deconvoluted FTIR spectra of the freeze drying beef protein

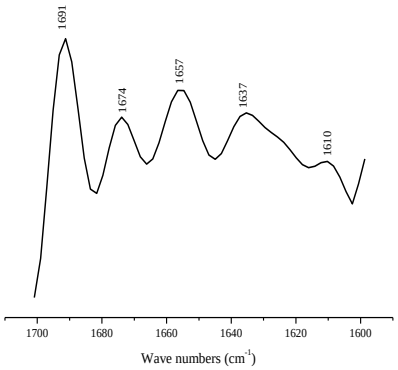

Table 2 Determined frequencies of the amide I component bands and their distribution percentage as part of the secondary structural content of the myofibrillar proteins

Structure distribution (%)	Treat Time (min)	ultrasound intensity (W/cm^2)					SE	*P*-values		
		0	2.39	6.23	11.32	20.96		TT	UI	TT×UI
α-helix (1657 cm^{-1})	30	19.32^{aA}	19.85^{aA}	19.16^{aA}	18.17^{aA}	17.34^{aA}	0.01	<0.001	<0.001	0.405
	120	16.88^{aA}	15.07^{abB}	14.31^{abB}	14.06^{abB}	13.51^{bB}				
β-sheet (1610, 1637 cm^{-1})	30	18.32^{bA}	18.38^{bB}	16.53^{bB}	24.43^{aA}	24.64^{aA}	0.03	0.002	0.029	0.031
	120	16.04^{bA}	25.52^{aA}	24.39^{aA}	24.66^{aA}	24.88^{aA}				

[1] a~d different letters in the same raw indicate statistically significant differences at P<0.05.
[2] A~D different letters in the same column of treatment indicate statistically significant differences at P <0.05;
[3] TT means treat time, UI means ultrasound intensity

high PUS intensity could induce significant secondary structure changes after 120 min treatment. The results of present study were similar to the finding of Jiang *et al*. [13] who discovered that PUS treatment was responsible for decreased α-helix and increased β-sheet compared with native black-bean protein isolate. In addition, previous studies have confirmed that the increase of β-sheet component could contribute to protein aggregates in meat system [14]. Moreover, oxidation could also induce partial unfolding of proteins and expose the buried sites within proteins. Sun et al. [15] showed increased β-sheets and decreased α-helix in porcine muscle after treatment with oxidizing agent. In this study, protein oxidation occured after brining assisted with PUS treatment due to the production of free radicals by cavitations (Table 1) which could also contribute to the protein secondary structure changes.

IV. CONCLUSION

This study demonstrated that beef with PUS treatment during brining could result in protein oxidation. The main consequences of protein oxidation were increased carbonyls contents and decreased total sulfydryl groups which led to protein aggregation with covalent cross-linking. FTIR spectroscopy analysis showed the secondary structural changes of beef proteins with increase in β-sheets and decrease in α-helix. Therefore, we conclude that PUS could induce protein oxidation during curing processing due to the mechanical and radical effects of cavitations.

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