CHANGES IN ANTIOXIDANT ENZYMES ACTIVITY OF DFD BEEF SUBJECTED TO HIGH PRESSURE PROCESSING DURING CHILLED STORAGE

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Abstract – Vacuum packed dark-firm-dry (DFD) beef loins were subjected to high pressure processing (HPP) at 200, 400 and 600 MPa, stored for 0, 3, 6 and 9 days at 4 ± 1°C and compared with control (0.1 MPa). HPP affected the development of lipid oxidation significantly ($p<0.001$). The activity of catalase (CAT) and glutathione peroxidase (GSH-Px) was recorded during storage. The GSH-Px activity was affected ($p<0.05$) by different pressure, however no clear effects were found on CAT activity. HPP promoted lipid oxidation significantly ($p<0.01$) at the first 3 days of storage, in which the activity of CAT and GSH-Px decreased ($p<0.05$) until the end of storage. Electronic nose discriminated the different aroma pattern from treated samples during chilled storage. In conclusion, HPP could affect the activity of antioxidant enzymes in DFD beef loin.

Key Words – Catalase, glutathione peroxidase, lipid oxidation.

I. INTRODUCTION

DFD beef is known having undesirable sensory attributes and high pH (>6.0) as a result of depleted muscle glycogen reserves prior to slaughter. The weak beef flavor, less acceptable colour and tenderness lowered the price. Moreover, it is spoiled by microorganism faster than the normal one [1]. As beef industry grows, the occurrence of DFD beef increases [2]. Chemical tenderization can be used to improve the quality. However, it may pose a problem, when recently the trend of additive-free products is increasing due to health issue.

HPP, a non-thermal food preservation technology, has been widely applied as a cold-pasteurization with minimal effect on nutritional content and to obtain extended stability of food freshness without using chemical additives. Many studies have been conducted to observe the particular effects of HPP on the quality parameters with impact on the meat quality such as color changes, lipid oxidation, and aroma volatile compounds [3, 4, 5]. The objective of present study was to determine the effects of HPP on antioxidant enzymes activity and aroma pattern of DFD beef.

II. MATERIALS AND METHODS

Sample and storage condition

The under grade *longissimus dorsi* muscles (*N* = 48) with dark-firm-dry (DFD) characteristics were purchased from local slaughterhouse, vacuum packed (350 g each) and distributed to HPP plant (Hyungkuk F&B, Korea) within an ice box. Vacuum packed samples were treated at 0.1 MPa (atmospheric pressure) as control, 200, 400 and 600 MPa for 3 min in a 350-L chamber (QFP 350L-600, Avure Technologies, US) using a pressurization medium of water at 14-17°C. Pressurization, holding and depressurization times were 56.3 s, 180 s and 12.2 s, respectively. Samples were then stored at 4 ± 1°C for 0, 3, 6 and 9 days.

pH & lipid oxidation

The pH value of the homogenized samples were recorded using a pH meter (Seven Easy pH, Mettler-Toledo GmbH, Switzerland) in triplicates. Thiobarbituric acid reactive substance (TBARS) were determined using a method as described by Sinhuber and Yu [6]. The results were calculated as mg malondialdehyde (MA) per kg meat.

Antioxidant enzymes activity

CAT activity was measured according to a modified version of a method described by Aebi [7]. The CAT activity was expressed as U/g sample. GSH-Px activity measurement was

performed according to DeVore & Greene [8] with slight modification. The GSH-Px activity was expressed as U/g sample.

Aroma pattern

A total of 2 g of sample was weighed into 10 ml-headspace vial and prepared in duplicate. The 2.5 mL-gas in the headspace of the samples was extracted by the automatic sampler syringe (HS 100, Alpha MOS, France) and detected using metal oxide sensors (MOS) array system (Alpha MOS, FOX 3000, France). Principal component analysis (PCA) was used for data processing using Alpha Soft package version 8.01 [9].

Statistical analysis

A 4 x 4 factorial design with three replicates was employed with pressure treatments and storage times as main effects using two-way analysis of variance (ANOVA) using R-version 3.1.2 with "Agricolae" library (The R-foundation for Statistical Computing, Austria). The statistical significance of the differences between means from different treatments was determined by Duncan's multiple range test ($p \leq 0.05$).

III. RESULTS AND DISCUSSION

High pressure processing has been known at promoting protein denaturation which causes a decrease of the acidic groups [10]. The elevation of pH occurs when actomyosin was denatured by pressure above 200 MPa [11]. However, present study showed that different pressure slightly affected the pH of DFD beef with no significant changes during chilled storage (Table 1). The stability of pH may figure out the freshness of the samples in vacuum packaging.

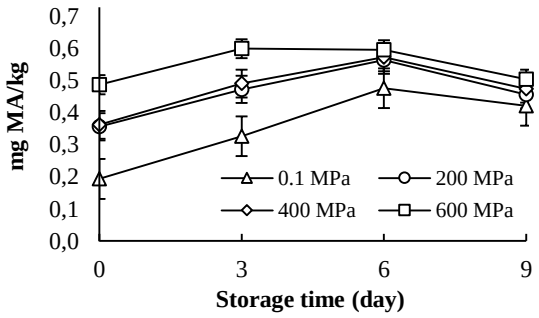
Table 1 Meat pH from different pressure treatment during chilled storage

Storage time (day)	0.1 MPa	200 MPa	400 MPa	600 MPa
0	6.17 ± 0.14	6.25 ± 0.24	6.32 ± 0.01	6.30 ± 0.07
3	6.13 ± 0.06	6.29 ± 0.05	6.36 ± 0.01	6.33 ± 0.12
6	6.18 ± 0.09	6.24 ± 0.17	6.29 ± 0.15	6.31 ± 0.03
9	6.12 ± 0.12	6.24 ± 0.10	6.32 ± 0.07	6.32 ± 0.16

Means within each row and column are not significantly different ($p>0.05$).

Lipid oxidation is one of the main factors affecting meat quality. It is figured out by an increase of malondialdehyde content of the samples. As expected, pressurization at 200 MPa and above (Fig. 1) led a higher TBARS values than control ($p<0.001$). Interaction was found between different pressure and storage times ($p<0.01$). Both control and pressure-induced DFD beef showed an increase of malondialdehyde content during storage ($p<0.001$). Protein denaturation leads to the release of free-radicals catalyzing oxidation [12]. Moreover, increases in lipid oxidation have also been attributed to the release of ions from heme-iron complexes promoting auto-oxidation of lipids in pressurized meat [13]. However, those parameters were not observed in this study.

Figure 1. Changes in TBARS values of high pressure treated DFD beef during chilled storage

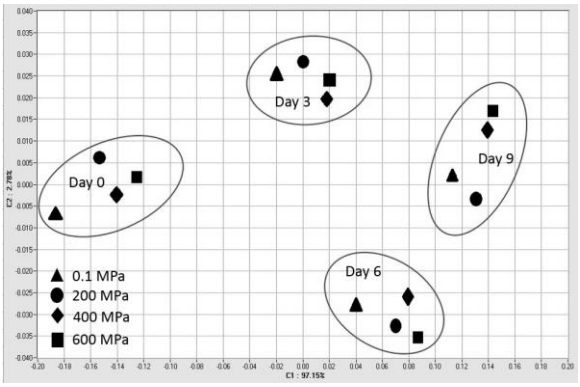


Aroma is an important sensory attributes affecting consumer preferences. As lipid oxidation occurred in DFD beef subjected to high pressure treatment, electronic nose discriminated the distinct aroma pattern from different pressure and storage times (Fig. 2).The first component (C1) explains 97.15% of the variability and the second (C2) 2.78% with positive discrimination index. These results showed that HPP alter the pattern of DFD beef aroma, which in agreement with Kang *et al.* [5], that found the similar results with further information regarding the changes in volatile compound in goat meat.

Muscle cells have their own defense system to inhibit lipid oxidation related to ageing with slow oxidative processes. The activity of self-defense enzymes against free radicals can figure out the mechanisms of oxidation in meat post-mortem [14]. In present study, the activity of CAT and GSH-Px was observed *in vitro*. Figure 1 shows the activity of CAT, in which no interaction was

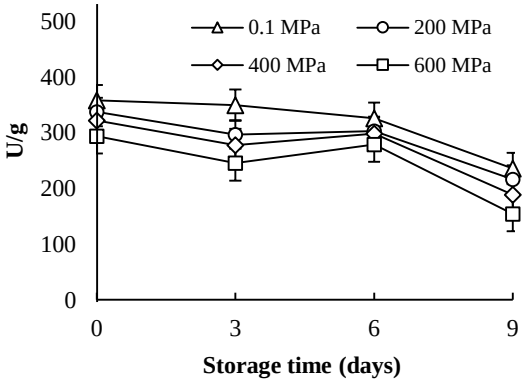
found between different pressure and storage times. The CAT activity was affected only by storage times and decreased until the end of storage significantly ($p<0.01$). The highest pressure-treated loins had slightly lower CAT activity than the others.

Figure 2. Principal component analysis of aroma pattern from DFD beef loin treated with different pressure and its changes during chilled storage as revealed by electronic nose



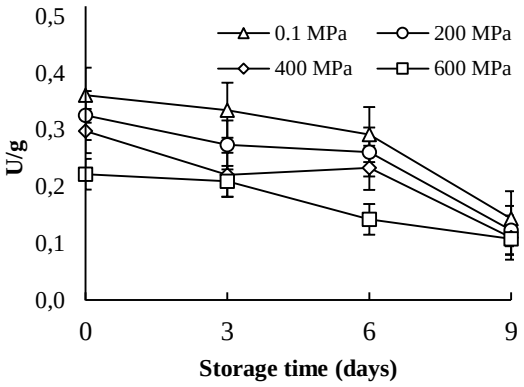
The activity of GSH-Px is shown in Figure 4. HPP decreased GSH-Px activity significantly ($p<0.05$), in which the highest pressure led to the lowest GSH-Px activity. No interaction was found from two factors but the activity of GSH-Px decreased very significantly until the end of storage ($p<0.001$).

Figure 3. CAT activity of different high pressure treated DFD beef during chilled storage compared with control



As HPP has potential for food preservation purposes through inactivating microorganisms growth and their enzymes activity [15], the activity of antioxidant enzymes such as CAT and GSH-Px in meat might be affected as well. The present study showed that pressurization until 600 MPa at temperature of 14-17°C did not fully inactivated those enzymes in DFD beef loins. Miyagawa *et al*. [16] distinguished four groups of enzymes inactivation; completely and irreversibly inactivated, completely and reversibly inactivated, incompletely and irreversibly inactivated, and incompletely and reversibly inactivated, based on loss and recovery activity under pressure treatment. The results suggest that CAT and GSH-Px are included in the group of incompletely and reversibly inactivated enzymes.

Figure 4. GSH-Px activity of different high pressure treated DFD beef during chilled storage compared with control



IV. CONCLUSION

High pressure processing influenced GSH-Px activity but had no effects on CAT activity in DFD beef. The activity of CAT and GSH-Px decreased during chilled storage. Electronic nose revealed that samples treated with different pressure had distinct aroma pattern. HPP up to 400 MPa might be applied for pasteurizing raw meat. We suggest further research to find the proper condition for keeping the oxidative stability of the meat.

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