EFFECT OF FREEZE-THAW PROCESS ON MYOGLOBIN OXIDATION OF PORK LOIN DURING COLD STORAGE

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Background and Objective

Although frozen storage is an important preservation method for muscle foods, quality deterioration cannot be avoid during freezing because of the formation of ice crystals, distorting the tissue structure, mechanical damage and denaturation of protein. Especially, the rapid meat color deterioration has been often observed in thawed meat compared to fresh meat at a commercial meat market. Myoglobin (Mb) oxidation is a major nonmicrobial factor responsible for the quality deterioration of fresh meat, and it is commonly assumed that lipid oxidation is closely related to Mb oxidation. Recently, we observed the formation of MetMb without lipid oxidation in thawed pork chop steak. During retail display, the rate of Mb oxidation is related to many factors, and physical and biochemical factors influence the displayable life of meat. Enzymes and other components are released during freezing and thawing. The release of mitochondrial enzymes such as β-hydroxyacyl CoA-dehydrogenase (HADH) into sarcoplasm have been reported (Gottesmann and Hamm, 1984; Chen et al., 1988; Toldra et al., 1991). Decker and Welch (1990) also reported that the rate of iron release from ferritin was influenced by temperature in muscle foods. In this research, we hypothesized that HADH activity could be partially responsible for the observed formation of MetMb without lipid oxidation in thawed pork loin during cold storage. The objective of this study was to determine whether HADH activity increased by freeze-thaw process could impact myoglobin oxidation without lipid oxidation in pork loin during cold storage.

Materials and methods

Commercial 10 pork loins were selected randomly at 24h postmortem, and the longissimus lumborum was used to make steaks (3 cm thickness). The steaks of each loin were packaged in a polyethylene bag, and samples were subjected to fresh group (Control), one cycle freeze-thaw group (Treatment 1) and two cycles freeze-thaw group (Treatment 2), respectively. All freeze-thaw samples were kept at -65℃ for 12 hours for freezing and stored at 4℃ for 12 hours for thawing. After thawing, samples were stored at 4℃ for 7 days to measure meat color (CIE L*a*b*), percentage of MetMb, thiobarbituric acid reactive substance (TBARS) value and HADH activity at 0, 3 and 7 days of storage.
Meat color (CIE L*a*b*) was measured by using a Minolta Chromameter (Minolta CR 301; Tokyo, Japan). Seven random readings were made from the surface of samples. Myoglobin was extracted from meat samples with phosphate buffer of pH 6.8. Samples were homogenized, centrifuged and filtered to obtain the absorbance of the resulting supernatant solution at 572, 565, 545, and 525 nm, respectively. Lipid oxidation was measured by TBARS value. Five grams of meat was weighed into a 50ml test tube and homogenized with 15 ml of deionized distilled. One ml of homogenate was transferred to a disposable test tube, and butylated hydroxyanisole (50, 10%) and thiobarbituric acid/trichloroacetic acid (TBA/TCA) (2 ml) were added, and then incubated in a boiling water bath for 15 min. The absorbance at 531 nm was used for TBARS value as milligrams of malondialdehyde per kilogram of meat. HADH activity was measured according to the method of Fernandez et al. (1999). Extract was obtained from meat sample by immersion in two volumes (4-6ml) of 0.1M phosphate buffer, pH 6.0, at room temperature. The HADH released in the extracts was assayed by mixing in a methacrylate disposable semi-micro spectrophotometer cell (10mm light path length, nominal working volume 1.5ml), 34 extract, 70 ethylenediaminetetraacetic acid (EDTA; 34.4ml) and 880 phosphate buffer (0.1M, pH 6.0). The mixture was kept at room temperature for 3 min and 20 NADH (1.5mM) and 20 acetoactyl-CoA (5.9mM) were added. The HADH activity was determined by measuring immediately the absorbance of the mixtures at 340nm.

Results and discussion

Sampes of control showed significantly ($p<0.05$) higher CIE a* value compared to those of treatments at 7 days of cold storage. Moreover the a* value of Treatment 1 was higher than that of Treatment 2. On the

contrary, MetMb percentages of Treatments were significantly ($p<0.05$) higher than those of control at 3 and 7 days of cold storage. Treatment 2 showed significantly ($p<0.05$) higher MetMb percentage compared to Treatment 1 at 3 days of storage. There were no significant ($p>0.05$) differences in TBARS values between control and treatments during cold storage. However there were significant ($p<0.05$) differences in HADH activity between control and treatments at 3 days of cold storage. Samples of treatments showed higher HADH activity compared to those of control. There was no significant ($p>0.05$) difference in HADH activity between Treatment 1 and Treatment 2.

These results suggested that the freeze-thaw process could accelerate meat color deterioration, i.e. decreased redness and increased MetMb percentage in pork loin during cold storage. However the freeze-thaw process did not affect on lipid oxidation of pork. This implied that autoxidation of Mb in freeze-thaw pork loin was influenced by somehow except lipid oxidation products. It could be possible that the freeze-thaw process leads to a local concentration effect of metal ions that might accelerate Mb oxidation. The rate of iron release from ferritin could be influenced by temperature in muscle foods (Decker and Welch, 1990). It could be also possible that the thawing process likely makes for a lower solubility of oxygen in the tissue that would lead to decreased OxyMb. The damage to cellular and sub-cellular compartments form physical disruption by ice crystals during freezing and thawing could release enzymes into sarcoplasm, and the enzymes could potentially be involved in MetMb formation in thawed meat finally. In this research, we confirmed the increasing of HADH activity by freeze-thaw process. HADH is involved in generation of NADH that has MetMb reductase activity in mitochondira intermembrane space. Because NAD^+ (Nicotinamide adenine dinucleotide) could not generate NADH (Reduced Nicotinamide adenine dinucleotide) effectively with presence of HADH, MetMb reductase activity might be reduced by increased HADH activity in postmortem muscles. Therefore, results suggested that the rapid Mb oxidation without lipid oxidation could be affected by partially the increased HADH activity during freezing and thawing of pork loin.

Conclusions

Freeze-thaw process of pork loin accelerated color deterioration during cold storage. The oxidation of Mb was not related to lipid oxidation. It was suggested that released HADH into sarcoplasmic from mitochondria could be involved in the autoxidation of Mb in freeze-thaw pork loin.

References

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Table 1. Changes in CIE a*, MetMb %, TBARS and HADH activity by freeze-thaw cycles of pork loin during 7days of cold storage.

Measurements	Treatments [1)]	Storage period (days)		
		0	3	7
CIE a*	C	7.26±1.15	7.11±0.79	6.24 ± 0.37^{b}
	T1	7.29±0.66	7.20±1.09	5.80 ± 0.64^{ab}
	T2	6.83±1.15	6.58±0.94	5.15 ± 0.44^{b}
MetMb (%)	C	8.03 ± 3.72^{Aab}	8.30 ± 2.31^{Ac}	14.00 ± 4.01^{Bb}
	T1	4.29 ± 0.35^{Bb}	13.35 ± 1.79^{Ab}	15.35 ± 3.83^{Ab}
	T2	8.68 ± 2.58^{Ca}	17.37 ± 0.95^{Ba}	20.98 ± 1.10^{Aa}
TBARS (MA mg/g)	C	0.04 ± 0.01^{B}	0.14 ± 0.01^{A}	0.16 ± 0.01^{A}
	T1	0.04 ± 0.02^{B}	0.12 ± 0.01^{A}	0.14 ± 0.01^{A}
	T2	0.04 ± 0.01^{B}	0.12 ± 0.01^{A}	0.14 ± 0.01^{A}
HADH activity (Unit /)	C	3.26 ± 0.25^{C}	5.55 ± 0.17^{Bb}	9.65 ± 0.24^{A}
	T1	3.66 ± 0.91^{B}	9.00 ± 0.10^{Aa}	9.60 ± 0.47^{A}
	T2	3.30 ± 0.23^{B}	9.02 ± 0.17^{Aa}	9.59 ± 0.45^{A}

[A,B] Means± SD with different superscript in the same row are significantly different($p<0.05$)

[a,b] Means± SD with different superscript in the same column are significantly different($p<0.05$)

[1)] C: Control, T1: Treatment 1(one cycle freeze- thaw), T2: Treatment 2(two cycles freeze-thaw)