THE EFFECT OF FREEZING AND HEATING ON CHANGES OF SOME SARCOPLASMATIC PROTEINS OF BOVINE *LONGISSIMUS DORSI*

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Introduction

Freezing is one of the best and the most used methods of extending the shelf-life of foods. Some consumers still now consider and believe frozen meat is of noticeablely worse quality than the chilled one. Žlender (1995) estimated that the rate and duration of freezing had no effect on physical, structural, chemical, hygienic and sensory quality parameters of meat.

Cooling meat to 0°C had little or no effect on the muscle structure, but the process of freezing animal tissues led to considerable denaturation both soluble proteins of the sarcoplasm and the contractile protein complex of actin-myosin + tropomyosin-troponin-actinin due to a disruption of cellular organeles like mitochondria, lysosomes, etc., which release the enzymes bound to these structures into the cell sap (Bendall, 1973; Hamm, 1979; Hamm et al., 1986). There are reports that freezing caused a remarkable release of cytochrome c oxidase from mitochondria (Barbagli in Serlupi-Crescenzi, 1981). The same happens to meat pigments.

The objective of this study was to investigate the changes of sarcoplazmatic proteins (soluble sarcoplasmatic proteins in general) and separately content of native and denatured myoglobin and specific activity of cytochrome c oxidase of bovine m.*longissimus dorsi* after freezing and heating.

Material and methods

24 to 48 hours *post mortem* three *longissimuss dorsi* muscles (LD) of normal quality (pH = 5.6) were cut off from the left and right halves of the young beef. On the right (fresh and unfrozen) and on the left halves (frozen to -30°C and thawed in 24 hours at +5°C) LD, divided into one fresh and three heated samples (T_i = 50°C, 58°C, 65°C), myoglobin quantification (Trout's Nit_{409} method (1991) modified by Špenkuch (1994)), determination of the percentage of denatured myoglobin, specific activity of cytochrome c oxidase (Appelmans et al., 1954) and electrophoretic characterisation of soluble sarcoplasmatic proteins on polyacrylamide gel using a modified method of Laemmli (1970) were carried out. Proteins for electrophoresis were extracted according to the method of Toldra et al. (1992).

Results and Discussion

Electrophoretic separation of myofibrillar proteins in polyacrylamide gels in the presence of sodium dodecyl sulphate (SDS) and mercaptoethanol is widely used for qualitative analysis of proteins (Claeys, 1995). With this method we wanted to evaluate the effect of freezing and thawing on the soluble sarcoplasmatic proteins of fresh and heated beef meat. The 15% gel concentration served to separate sarcoplasmatic proteins within a molecular weight range of 10 - 50 kDa (Figure 1). After comparison of proteins migration distance from the top of the separating gel to the center of the protein band on 15% SDS-PAGE gel we could resume that electrophoretic profiles of frozen and unfrozen samples of sarcoplasmatic proteins did not distinguish. Different intensity of separated proteins was due to the different protein concentrations in samples.

We calculated the relative mobility (R_f) of unknown proteins and estimated its molecular weight from a calibration curve constructed from the molecular weight of proteins in the Sigma standard (SDS-7 DALTON MARK VII-L) and their R_f values. We could predict that the most distant protein band was myoglobin with M_r of 17800.

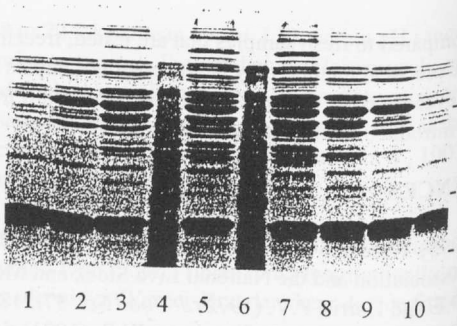

Figure 1 Electrophoretic profiles of sarcoplasmatic proteins of bovine LD separated on 15% SDS-PAGE gel

Legend: 1 = UF(65), 2 = UF(58), 3 = UF(50), 5 = UF(fresh), 6 = FT(fresh), 8 = FT(50), 9 = FT(58), 10 = FT(65), 4,7 = standard 7L; UF - unfrozen; FT - frozen/thawed; (T_i) - internal temperature (°C)

Table 1 Effect of freezing and heating on the content of native myoglobin in bovine LD samples (mg/g of meat)

thermal treatment	unfrozen $\bar{x}$	frozen $\bar{x}$	t-value
fresh [1]	$10.10^{4,3}$	$6.68^{4,3,2}$	2.96^{*}
50°C [2]	8.63^{4}	$5.81^{4,3}$	3.38^{*}
58°C [3]	6.91	4.76	3.94^{**}
65°C [4]	4.65	4.43	0.23
F-value	7.34^{**}	13.54^{***}	

Significance level of effect is described as ***, **, * for $p<0.001$, $p<0.01$, $p<0.05$

We noticed quite large differences, except for the group of samples heated to T_i = 65°C, in the content of myoglobin between frozen and unfrozen samples (Table 1 and Figure 2). In spite of careful freezing and thawing sarcolema and some cell structures were obviously demaged, and this could contribute to the loss of myoglobin in the drip from thawed and rather small frozen samples (9 x 9 x 5 cm) with relatively large surface.

Tumerman (1974) has found that myoglobin denatures only during prolonged heating at 60°C; after heating meat samples (one hour) with pH 5.5 at 60°C the content of denatured myoglobin was about 30%, at 70°C about 80% and at 75°C close to 100%. Our results (Figure 2) showed that the rate of denaturation was faster in unfrozen samples (relation between native myoglobin (Mb) and T_i: Mb= - $0.11 \cdot T_s + 12.7$) than in frozen one (Mb= - $0.05 \cdot T_s + 7.8$).

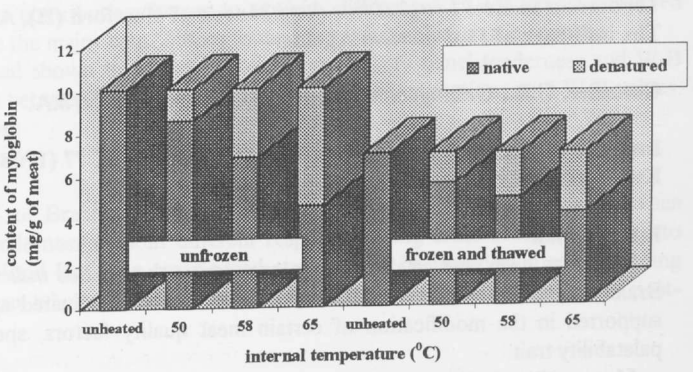

Figure 2 Content of native and denatured myoglobin determined by Nit_{409} method in fresh and heated samples

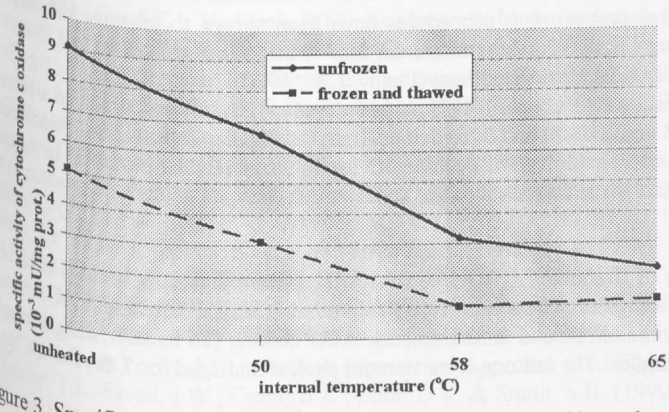

Figure 3 Specific activity of cytochrome c oxidase in fresh and heated samples before and after freezing

Table 2 Effect of freezing and heating on specific activity of cytochrome c oxidase (10^{-3} mU/mg of protein) in fresh and heated LD

thermal treatment	unfrozen $\bar{x}$	frozen $\bar{x}$	t-value
fresh [1]	$9.1^{3,4}$	$5.1^{3,4,2}$	1.29
50°C [2]	6.6	$3.1^{3,4}$	2.18
58°C [3]	3.2	1.0	1.71
65°C [4]	2.2	1.2	1.08
F-value	2.80	20.94^{***}	

Freezing decreased the enzyme activity but differences were not significant (Table 2). Generally, the specific activity of cytochrome c oxidase decreased with increased T_i. In fresh samples enzyme activity was significantly higher ($p \leq 0.05$) than in heated one.

Conclusions

1. Electrophoretic separation of sarcoplasmatic proteins on polyacrylamide gels in the presence of sodium dodecyl sulphate (SDS) and 2-mercaptoethanol is a suitable method for evaluating an effect of freezing and heating on the soluble sarcoplasmatic proteins of beef meat.
2. Correct freezing and thawing did not have any influence on the electrophoretic profile of sarcoplasmatic proteins and on the specific activity of cytochrome c oxidase.
3. As expected specific activity of cytochrome c oxidase in fresh meat was significantly higher ($p \leq 0.05$) than in the heated one.
4. The myoglobin level in fresh and heated to T_i = 58°C frozen meat was significantly lower ($p \leq 0.01$) than in unfrozen samples.

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