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Influence of different freezing temperatures on some characteristics of fibrillar proteins of bovine longissimus dorsi muscle

LJILJANA PETROVIĆ, S. RAHELIĆ

Faculty of Technology, Institute of Meat, Milk, Oil and Fat and Fruit and Vegetable Technology, Novi Sad, Yugoslavia

Introduction

The effect of different freezing temperatures on both histological and ultrastructure of muscles is different (Cassens, 1971; Lawrie, 1966; Love, 1966; Tuchschneid-Emblich, 1959; Rahelić et al., nonreported data). On the basis of these changes, it can be expected that different freezing temperatures will variously influence the muscle proteins as well. One can find data in literature on the effect of storage of frozen muscles on the protein structure. The most numerous data on these changes have been obtained by investigation the structure of fibrillar proteins of fish muscles (Connell, 1962; Love, 1962; Matsumoto, 1980). Less have been obtained by investigation of frozen chicken muscles (Khan, 1966; Huber and Stadelman, 1970) and even less by that of bovine (Awad et al., 1968; Rahelić et al., 1974) and pork (Rahelić et al., 1974).

Review of Literature

Connell (1962) and Love (1962) reported the results on the investigation of fibrillar muscle proteins of frozen fish and showed that because of the influence of prolonged storage, the extractability of actomyosin i.d. the protein solubility was decreasing. In a concise monographic review of the effect of storage on the muscle proteins of fish, Matsumoto (1980) gives one more datum i.d. that during the storage of frozen fish muscle the viscosity of soluble actomyosin fractions is decreasing as well.

Khan et al. (1963) and Khan (1966) have reported the decrease of the extractability of chicken muscle, including the extractability of actomyosin fraction too. Awad et al. (1968) have established similar changes in bovine muscle. A similar result was reported by Huber and Stadelman (1970) with the datum that the proteins of myofibrills of muscles frozen at $-10^{\circ}C$ are more soluble than the ones frozen at $-196^{\circ}C$.

Khan et al. (1963) have reported that the amount of SH groups is decreasing during the storage of frozen chicken meat. Rahelić et al. (1974) have established that the amount of SH groups in the protein of pork muscle decreasses up to the 180th day of storage, and afterwards, during the following 180 days of storage, it is not changing, whereas in bovine muscle they have not established any considerable changes during the whole storage period. On the other hand, Hofmann and Hamm (1978) have reported that the amount of free SH groups is increasing in the frozen muscle.

As there are enough data on the changes of the fibrillar proteins influenced by the storage of frozen muscle, and very few ones on the influence of different freezing temperatures, it was decided that in this work the eventual influence of several different freezing temperatures on the fibrillar proteins of bovine long. dorsi muscle will be investigated.

Matherials and Methods

The long. dorsi muscle of domestic spotted cattle race, of the age of 10 to 18 months was used for the investigation. They were slaughtered in the usual way. Muscle pieces of the thoracal part were cut from the halves having been cooled for 24 hours. The weight of pieces was cca 600 - 700 g and they were frozen at the following temperatures: $-10^{\circ}C$ (in the refrigerator), -20° and $-30^{\circ}C$ (in the cooling house), $-78^{\circ}C$ (in dry ice) and $-196^{\circ}C$ (by dipping into liquid nitrogen). After the samples had reached the temperature of the medium they were frozen in, they were stored at that same temperature for 48 more hours, and after that, they were thawed in closed boxes in the refrigerator at $8^{\circ}C$ for approximately 24 hours. Muscles cooled for 24 hours were also investigated.

Preparation of myofibrills. Myofibrills were preparated from 25 g of minced sample by the

method of Perry and Grey as described by Penny (1976).

Extraction of myofibrills. The washed myofibrills were suspended in 5 mM Triss buffer pH = 8,2 (Solution I), and afterwards, the solution was dialysed against frequent changes of Tris buffer for 2 days at 0°C. The dialysate was centrifugated at 180,000 x g for 30 min (Extract I), and the precipitate was suspended in HS (Hasselbach-Schneider) solution (Solution II). The residual fibrillar proteins were extracted overnight at 4°C, and the extract was separated from the insoluble residues by centrifugation at 30,000 x g for 10 min (Extract II) (Penny, 1970).

Solubility. The protein content of Extract I is expressed as percents of the proteins in Solution I, and of Extract II as the percent of the proteins in Solution II. The sum of proteins of Extract I and Extract II, but converted in percents to the proteins in Solution I, i.d. to the total fibrillar proteins, expresses the solubility of total fibrillar proteins. The N content was determined by micro-Kjeldahl method, and by multiplying it with the factor 6,25, the content of proteins was obtained.

Viscosity of extracts of myofibrills was determined at 21°C, in an Ostwald viscosimeter of 2,5 ml capacity (Briskey and Fukazawa, 1971). The reduced viscosity was calculated as

$$\eta_{red} = \frac{\text{specific}}{C}$$

(C - concentration of proteins mg/ml in the extracts investigated).

The content of SH groups was determined by spectroscopic method (Kalab, 1970), the colour was developed with DTNB, and the absorption of the solution was measured at 412 nm. The content of SH groups is expressed as umoles SH/mg of proteins/ml.

Results

The analysis of the results presented in Table 1 shows a solubility decrease of myofibrillar proteins of muscles frozen at -20°C, comparing to the one of the fresh muscles. The solubility of myofibrillar proteins of muscles frozen at -10° and -30°C is approximately the same as of fresh muscles, whereas that of muscles frozen at -78°C is somewhat higher, and that of muscles frozen at -196°C considerably higher.

A similar finding was established in Extract II and exactly the same relationship was established when calculating the solubility of total fibrillar proteins of the muscles frozen at different temperatures.

Solubility of myofibrillar proteins of bovine long. dorsi muscle in 5 mM Tris buffer, pH = 8,2 (Extract I) in HS solution (Extract II) (in comparison to Solution I and Solution II) and total solubility before freezing and frozen at different temperatures

Table 1.

Freezing temperature (°C)	% of extracted proteins in Extract I	% of extracted proteins in Extract II Solution II	% of extracted proteins in Extract II Solution I	Solubility of total myofibrillar proteins (%)
fresh muscle	51,12	64,49	31,52	82,64
-10	55,66	63,78	28,28	83,84
-20	45,04	59,01	32,43	77,47
-30	54,91	65,08	29,34	84,25
-78	58,38	75,96	31,61	89,99
-196	75,82	71,54	17,30	93,12

As the results presented in Table 2 show, the viscosity of Extract I of muscles frozen at -30°C is the lowest and that of muscles frozen at -10°C somewhat higher. The viscosity of Extract I of fresh muscles and of the ones frozen at -20° and -78°C is even higher, while of muscles frozen at -196°C is the highest. The viscosity of Extract II of frozen muscles is somewhat higher than the one of extracts of the unfrozen muscles, and it is rather uniform, except that the viscosity of the Extract II of muscles frozen at -30°C is pronouncedly lower.

By the investigation of content of SH groups (Table 3) in Extract I it was established that it was the highest of the muscles frozen at -10°C, somewhat lower in the extracts of muscles frozen at -20° and -30°C, and the lowest in the extracts of muscles frozen at -78° and

Viscosity of extracts of myofibrillar proteins obtained with 5 mM Tris buffer, pH = 8,2 (Extract I) and HS solution (Extract II) from bovine long. dorsi muscle before freezing and frozen at different temperatures

Table 2

Freezing temperature (°C)	Extract I η_{red}	Extract II η_{red}
fresh muscle	0,220	0,107
-10	0,194	0,110
-20	0,219	0,117
-30	0,139	0,062
-78	0,246	0,121
-196	0,315	0,116

Content of SH groups in the extracts of myofibrillar proteins obtained with 5 mM Tris buffer, pH = 8,2 (Extract I) and HS solution (Extract II) of bovine long. dorsi muscle before freezing and frozen at different temperatures (umoles SH x 10^{-2}/ mg of proteins/ ml)

Table 3

Freezing temperature (°C)	Extract I	Extract II
fresh muscle	1,29	1,66
-10	1,65	2,10
-20	1,33	2,27
-30	1,45	2,14
-78	1,25	2,05
-196	1,27	2,49

-196°C. The content of the protein SH groups in Extract I of these two muscles is similar to the one of the unfrozen muscle. The content of SH groups is higher in Extract II of frozen muscles than in the ones of unfrozen muscles. In the extracts of frozen muscles the content of SH groups is fairly similar, but it is the highest in the extracts of muscles frozen at -196°C.

Discussion

Reviewing the results of the determination of protein solubility in Extract I (5 mM Tris buffer, pH = 8,2) (Table 1), it can be noticed that the obtained amount is higher than the ones reported by other authors (Perry and Corsi, 1958; Penny, 1970; Penny, 1976). This finding is probably partially the consequence of the fact that the proteins were determined by micro-Kjeldahl method and the established nitrogen content was multiplied by the factor 6,25 (some authors, - Connell, 1960, - have used a lower factor). Besides, the presence of myosin was established in that Extract I by electrophoretograms. The obtained results are only partially in agreement with the data given in literature that the exposing of muscles to freezing temperatures causes a decrease of fibrillar protein solubility (Connell, 1962; Love, 1962; Khan, 1966; Awad et al., 1968). Namely, by these investigations it was established that the amount of extracted proteins in Extract I and Extract II was decreased only in samples of muscles frozen at -20°C. The content of extracted proteins in Extract I of muscles frozen at temperatures -10° and -30°C doesn't differ significantly from the one in extracts of the unfrozen sample. However, in e tracts of muscles frozen at -78° and -196°C it was found even more i.d. considerably more dissolved proteins than in the one of unfrozen muscle. The findings that the content of extracted proteins was decreased in extracts of muscles frozen at -20°C, and was considerably increased in extracts of muscles frozen at -78° and -196°C, are in agreement with the ones of histological investigations. Namely, Gawwad and Rahelić have found that the changes of histological (1979) and ultrastructure (nonreported data) of long. dorsi muscle frozen at -22°C are more expressed than of muscles frozen at -10° and -30°C. The authors explain these expressed changes by the fact that a great part of water is still crystallizing between the fibers in muscles frozen at -22°C, but a considerable part is crystallizing also in the fibers and predominantly in the zone of I band. The appearance of inter- and intrafibrillar ice crystals causes greater changes in the structure of sarcomere and that is effecting the state of proteins as well. On the other hand, it can be seen on the electronmicrographs of the muscles frozen at -78°C that the ice crystals appear to a greater extent in the sarcomere, but in the muscles frozen at -196°C to still greater extent. As a consequence, the myofillaments of the muscles frozen at -78°C are considerably more damaged by the formed ice crystals, and the structure of the ones frozen at -196°C is completely changed and the myofillaments are being frequently torn. These findings lead to an explanation that there are more proteins in the extracts of muscles frozen at -78° and -196°C, because by freezing the structure of myofillaments has been damaged, and so their protein extractability was increased.

The results obtained by the establishing of viscosity (η_{red}) of Extract I (Table 2) can't be explained with the results obtained by the investigation of the solubility of fibrillar proteins in 5 mM Tris buffer pH = 8,2 (Table 1). Namely, the decreased viscosity of the extracts of muscles frozen at -10° and -30°C could be explained by protein denaturation of muscles when frozen at relatively high temperatures, though that denaturation didn't effect the extractability. One of the possible explanations is the assumption that a part of actin in these extracts got denaturated by the separation of subunits in the molecule of G-actin, so the friction during flowing was being decreased because the globular molecules got decreased. The increased viscosity of Extract I of muscles frozen at -78° and -196°C can be explained with the results obtained by the establishing of solubility (Table 1). As the amount of the extracted proteins in these extracts is considerably higher than the sum of the amounts of actine, tropomyosin, troponin and alfa actinin, that proves that myosin is present in that extract, as it has been previously explained. As a consequence of the presence of a relatively higher amount of fibrillar proteins of myosin in the extract of globular actin, the viscosity has been increased because of the presence of molecules of different shape.

Though the viscosity of Extract II of frozen muscles is somewhat higher (except of muscles frozen at -30°C) compared to the one Extract II of fresh muscles, it couldn't be said that the freezing temperature influences the viscosity of soluble fractions in HS solution, in the first place since in that extract there are mainly myosin and its subunits present, being of fibrillar structure and, as such, while flowing they are "arranging".

When analysing the content of SH groups of proteins in Extract I of unfrozen and frozen muscles (Table 3) it is evident that their content was increased in the extracts of muscles frozen at -10°, -20° and -30°C, the increase being the smallest in those frozen at -20°C. This finding is in agreement with the established viscosity (η_{red}) of the extracts of these muscles (Table 2) because their lower viscosity i.d. the higher fluidity of extracts may be explained by the higher content of free SH groups by which the cross-linkage of the proteins is weaker. Contrary to the finding obtained by the investigations of Extract I, the content of SH groups in Extract II of muscles frozen at all the temperatures was increased. This finding is in agreement with that of Hofmann and Hamm (1978) that the content of free SH groups is increasing when the meat is being frozen. It should be also brought into connection with the increased sensitivity of myosin to denaturation (Connell, 1962; Matsumoto, 1980). It is possible that the established increase of the content of SH groups of Extract II is expressed in this way because their content in the myosin is considerably higher (65%) than in other myofibrillar proteins (Hofmann and Hamm, 1978). As myosin is sensitive to denaturation the consequence of denaturation is always the release of a certain number of SH groups.

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