EFFECTS OF FREEZING AND TEMPERING ON COLLAGEN TYPES III AND IV IN PORK MUSCLE

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BACKGROUND

Muscles have a complex network of intramuscular connective tissue, in which the endomysium encloses the muscle fibre, and at a higher level, the perimysium surrounds bundles of muscle fibres. The property of the endomysium to act as a mechanical constraint on swelling (Bailey and Light, 1989), and the fact that it shrinks during heat treatment (Bailey and Light 1989; Light 1984) mean that it has an important effect on the functional properties of meat products. Knight and Elsey (1989) showed that stripped fibres, lacking the endomysial sheath, swell approximately five times more than intact fibres. The perimysium and the links between the perimysium and the endomysium are believed to be the key structures determining toughness in meat (Purslow, 1985; Light et al, 1985). Damage to the connective tissue is likely to affect both swelling and shrinkage during heat treatment, which in turn will affect both the water-holding capacity and texture of meat products. The diffusion of salt is facilitated by ruptures in the endomysium, which could lead to shorter times for processes such as presalting. Despite the importance of the connective tissue, little information is available about the role of intramuscular connective tissue in the processing of meat products.

OBJECTIVES

The aim of this study was to investigate effects of different freezing and tempering rates on the microstructure of intramuscular connective tissue. Knowledge about structural changes caused by ice crystals and its relation to functional properties and meat quality can enhance the use of frozen meat in processed meat products. The perimysium and endomysium were studied with light microscopy using immunological methods to specificly lable collagen types III and VI, constituents of the perimysium and endomysium .

METHODS

Pork foreleg was cut one day after slaughter and was packed and frozen the day after. It was packed in plastic bags, each bag containing 2 -3 kg. The meat was divided into two groups, one group was frozen at a "fast" rate and the other at a "slow" rate, table 1. Each of these groups was then tempered to -3.5°C at a "slow" and a "fast" rate, table 1., which gives a total of four groups. The meat was stored at -30°C for 12 weeks prior to tempering and preparation of samples. The chemical composition of the meat was 7.1% fat, 72.5% water, 20.3% protein, 0.36% hydroxyproline, and the pH was 5.4.

	Fast freezing	Slow freezing
Time to pass the interval 0°C to -5°C	1.5 hours	24 hours
Time to reach -18°C	3.5 hours	32 hours

	Fast tempering	Slow tempering
Time in temperatures over -5°C	1 hour	20 hours
Time to reach 3.5°C	5 hours	43 hours

Table 1. Time for slow and fast freezing and tempering of pork foreleg.

Preparation of samples for light microscopy

To detect the effects of freezing and tempering the samples were frozen in liquid nitrogen prior to cryo-section. A solution of Aniline blue and Orange G, staining the myofibrillar proteins yellow and the collagen - gelatine blue, was used to obtain an overview of the structure (Hermansson and Jordansson, unpublished; Ofstad et al, 1995). Polyclonal antibodies against collagen types III and IV were used as the primary antibodies, and Vectastain ABC Kit (pk 4005) was used for peroxidase as detection system in order to study the collagen more specifically (Egelandsdal et al, 1991).

RESULTS AND DISCUSSION

Studies of freezing and tempering showed that conditions during freezing determined the distribution of ice crystals in the structure. The distribution of ice crystals after freezing was seen to have an important effect on the structure during further frozen storage and tempering. Fast freezing gave small ice crystals evenly distributed in the structure, inside and between muscle fibres, whereas slow freezing gave large ice crystals mostly between the muscle fibres (results not shown here). Fast tempering gave relatively small changes in the structure compared to the structure after freezing. Slow tempering led to growth and transformation of the ice crystals. The growth of the small ice crystals caused more fractures in the intramuscular connective tissue than the growth of the large ice crystals.

Figure 1a shows collagen type III and figure 1b shows collagen type IV in a non-frozen control. The anti-collagen III antibody labels the perimysium and to lesser extent the endomysium, whereas the anti-collagen IV antibody only lables the endomysium.

Figure 2a shows collagen type III and figure 2b shows collagen type IV after fast freezing and fast tempering. The perimysium is to some extent affected by the freezing and tempering. It is more compact and thinner than in the control. Ice crystals have not caused any notable rupture of the perimysium. The endomysial tissue is relatively intact, although the shape is less uniform and muscle fibres are

less packed than in the control, figure 2b. The formation of ice crystals has caused gaps between the fibers, outside the sheaths of collagen type IV. Other staining techniques showed holes foremed by ice crystals even inside the fibres.

Figure 3a shows collagen type III and figure 3b shows collagen type IV after fast freezing and slow tempering. Fast freezing followed by slow tempering caused severe rupture of the perimysium. Fragments of the perimysium are shown by the arrows, figure 3a. The ruptures in the perimysium by ice crystals could have an influence on the tenderness of frozen meat. Labelling of collagen type IV, figure 3b, shows pronounced fractioning of the endomysium. The collagen sheath surrounding the muscle fibre is ruptured, see arrows figure 3b, which will affect swelling in the presence of salt, as well as the endomysial shrinkage during heat treatment, which affects the water-holding capacity of meat products.

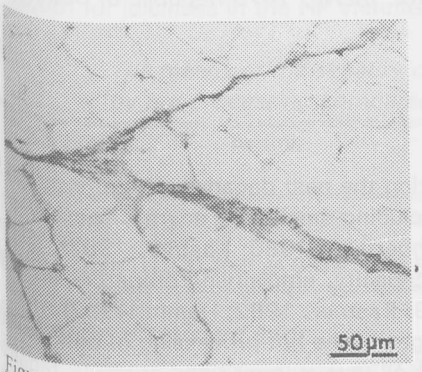

Figure 1a. Type III collagen; non-frozen control.

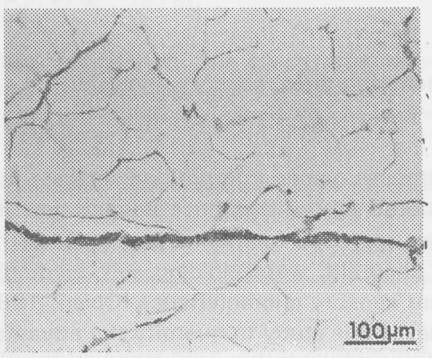

Figure 2a. Type III collagen; fast freezing - fast tempering.

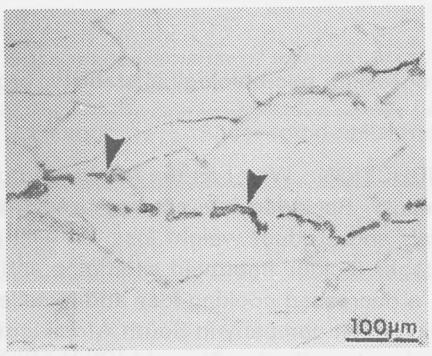

Figure 3a. Type III collagen; fast freezing - slow tempering.

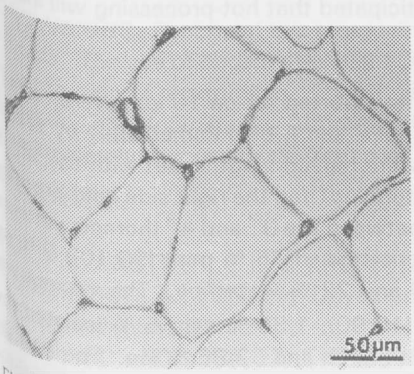

Figure 1b. Type IV collagen; non-frozen control.

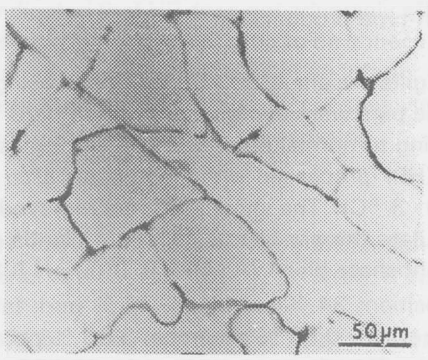

Figure 2b. Type IV collagen; fast freezing - fast tempering.

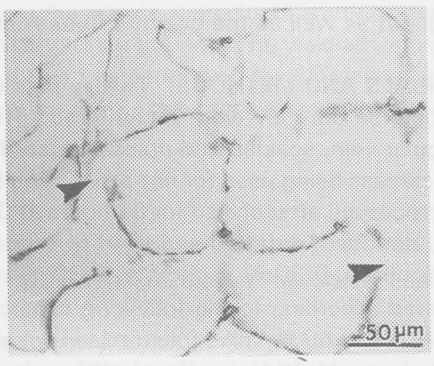

Figure 3b. Type IV collagen; fast freezing - slow tempering.

CONCLUSIONS

The results illustrate that immunological staining techniques supply accurate information on how ice crystals affect types III and IV collagen. The conditions during freezing and tempering determine the distribution of ice crystals, wich affects the connective tissue. The combination of fast freezing, with ice crystals evenly distributed inside and between the muscle fibres, and slow tempering, with transformation and growth of the ice crystals through the tissues led to pronounced damage to both the endomysium and the perimysium. As the endomysium and the perimysium highly influence the swelling of musclefibers in the presence of salt, shrinkage during heat treatment and the texture in meat, these results are likely to have a great impact on properties like water-holding and texture in meat products.

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