

Sensory properties of DFD beef and normal beef as related to thermal denaturation of meat proteins

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INTRODUCTION

Texture changes during heating of meat are related to the intramolecular denaturation processes of the two structural components of meat, myofibrillar proteins and intramuscular collagen. Protein denaturation is followed by intermolecular aggregation processes which determine the nature of the texture changes.

Differential scanning calorimetry (DSC) offers a method for studying denaturation of muscle proteins *in situ* (Stabursvik and Martens). In DSC only the denaturation process can be followed. However, since the denaturation process initiates the aggregation process, the denaturation process can be used to monitor the aggregation process.

Texture changes during heating of beef with a normal ultimate pH have been extensively studied. It is usually found that overall tenderness decreases when meat is heated to ca. 50°C (first toughening phase), increases when heated to ca. 60°C, and decreases again when heated to ca. 70°C (second toughening phase) (Davey and Gilbert, Machlik and Draudt). The decrease in tenderness at ca. 50°C, as measured by shear-force, is associated with loss of myosin solubility, presumed to indicate denaturation of myosin (Davey and Gilbert). The increase in tenderness at ca. 60°C has been related to denaturation of collagen, corresponding to the collagen shrinkage reaction (Machlik and Draudt). Giles found that the sarcomere showed a 20 per cent contraction at ca. 70°C, causing shrinkage of the meat along the fibres at this temperature. He also reported that the I-band (actin) became disrupted at ca. 70°C producing gaps in the sarcomere structure. Later Davey and Gilbert related the toughening phase at ca. 70°C to the shrinkage along the fibres at the same temperature.

Recently Martens *et al.* studied textural changes in normal beef, as measured by sensory methods, and compared these changes to the thermal denaturation of myofibrillar proteins and collagen, as studied by differential scanning calorimetry (DSC). They found that myosin, collagen and actin in normal beef denature in the temperature ranges 40-60°C, 55-63°C and 65-73°C, respectively, and that the second toughening phase at ca. 70°C could be associated with denaturation of actin (as observed by DSC).

DFD meat (Dark, Firm, and Dry) has a higher ultimate pH than normal meat. DFD meat binds more water and is more tender when cooked, compared to normal meat (Bouton *et al.*, 1971).

pH has little influence on collagen denaturation between 5.4 and 7.0 (Stabursvik and Martens), and the properties of collagen fibres (Finch and Ledward) or denatured collagen systems (Veis) show little or no pH dependence in the actual range.

The effect of pH on the myofibrillar structure is believed to be responsible for texture changes. Bouton *et al.* (1972) recently attributed the difference in tenderness caused by pH, as cooking temperature is increased above 60°C, to heat denaturation of myosin, based on the knowledge that myosin was denatured below 60°C. It has also been pointed out by Currie and Wolfe that in meat with a high pH, the water content is high, and that water content must be considered an important factor in determining meat tenderness. Ledward stressed that the pH dependence of cooked-meat toughness is primarily related to the effect of pH on the mode of aggregation of the myofibrillar proteins and, as such, is related to the interactions between these proteins and the solvent, i.e. their ability to hold water. pH influences both denaturation and aggregation by changing the electrostatic forces within a protein and between proteins (Lapanje).

The aim of the present investigation was to study the texture changes of DFD beef during heating, compared to those of normal beef, and to relate these changes to the thermal denaturation of the myofibrillar proteins myosin and actin. Denaturation of meat proteins was studied by DSC. Texture properties at different end temperatures were evaluated by analytical sensory methods.

EXPERIMENTAL

Material and sample preparation

Experiments were performed using post rigor bovine *M. semitendinosus* from 1½ year old bulls. The muscles were excised following normal slaughterhouse procedure. Sixteen muscles with pH 5.4-5.7 were selected as normal muscles, and sixteen muscles with pH 6.4-6.8 were selected as DFD muscles. The muscles had not been electrically stimulated, and no drugs had been administered to obtain high pH values. The muscles were frozen post rigor, and cut in 1.0 cm. thick slices along the fibres. The slices were vacuum packed and stored at -20°C for 2-6 weeks. The meat was thawed at 4°C, heated in plastic bags at 1.2°C/min to 9 different end temperatures (20°C (raw), 48°C, 54°C, 60°C, 65°C, 69°C, 73°C, 79°C, and 85°C, respectively), was held at these end temperatures for 10 min, and then rapidly cooled to room temperature (20°C). Cooking loss (ml/100 g raw muscle) and shrinkage along the fibres were measured.

Sensory evaluation

The sensory evaluation of the meat was performed in blue light by a trained laboratory panel of 12 persons. Each judge received, as one sample, two strips of each slice, cut parallel to the muscle fibers (approx. 1 x 1 x 1.5 cm). Six samples were served in each session together with two reference samples. Each sample was judged in two replicates. The order of preparation and serving to the judges was randomized.

The following characteristics were evaluated on 9 point scales:

Firmness, i.e. the force necessary to compress the meat strip about 30 per cent with the molar teeth perpendicularly to the fibre direction (1 = very low, 9 = very high).

Bite-off force, i.e. the force necessary to bite off the meat strip (after compression) by the front teeth, also perpendicularly to the fibre direction (1 = very low, 9 = very high).

Total chewing work, i.e. the work necessary for chewing the strip ready for swallowing (1 = very little, 9 = very much).

Juiciness, i.e. juiciness perceived during chewing (1 = very dry, 9 = very juicy).

Meat flavour, i.e. intensity of meat flavour perceived by mouth (1 = none, 9 = very strong).

Total preference, i.e. total impression of texture and flavour-by-mouth (1 = very bad, 9 = very good).

The uncertainty measure given for each sensory variable is \bar{s} , the standard error of the mean of the replicates, averaged (in absolute value) over the different treatments.

Differential scanning calorimetry

Thermal denaturations were studied in a Perkin-Elmer DSC-2, and preparation of myofibrillar muscle samples was performed according to Stabursvik and Martens.

RESULTS AND DISCUSSION

Thermal denaturation of myofibrillar proteins

In DSC the heat denaturation of a protein is detected as an endothermal peak as a function of increasing sample temperature - a DSC thermogram. Fig. 1A shows a thermogram of myofibrillar tissue from unheated normal beef (20°C). The least thermostable DSC peak (58°C) is interpreted as representing denaturation of LMM (light meromyosin) and HMM-S1 (Subfragment 1: globular head of heavy meromyosin) of the myosin molecule, while the middle peak (65°C) is interpreted as representing the HMM-S2 (subfragment 2: helical fragment of heavy meromyosin) of the myosin molecule (Goodno *et al.*, Stabursvik and Martens). The most thermostable peak (79°C) represents actin denaturation (Wright *et al.*, Martens and Vold).

pH is found to influence the thermograms of myosin between pH values 5.4-7.0, in contrast to thermograms of actin which are very little influenced by pH in this range. When pH is increased, the least thermostable myosin peak becomes slightly stabilised, and the more thermostable peak is destabilised. At pH values above 6.0, as found in DFD muscle, the two peaks merge into one. pH is thus the main cause of the difference between thermograms obtained for normal and DFD beef (Stabursvik and Martens). Fig. 1B shows a thermo-

gram of myofibrillar tissue of DFD beef (20°C) where only one single myosin peak (63°C) can be observed. However, Figs. 1A and 1B show that myosin and actin in DFD beef denature in the same temperature ranges as these proteins do in normal beef.

Tenderness and juiciness

Fig. 2 (A, B, C) show total chewing work, firmness and bite-off force as functions of protein denaturation in normal and DFD beef. The changes in overall tenderness (total chewing work) of normal beef during heating, as measured by sensory methods, were in accordance with the changes described above: The overall tenderness (Fig. 2A) decreased at ca. 50°C, which is the denaturation range of the HMM S-1 and LMM parts of myosin. The observed increase in tenderness at ca. 60°C is usually attributed to denaturation of collagen. The possible influence of the denaturation of HMM S-2 of the myosin molecule on texture at this temperature is unknown and should be investigated. The tenderness decreased in the actin denaturation range at ca. 70°C.

The present study demonstrates, however, that tenderness (total chewing work) of DFD beef during heating differs significantly from that of normal beef. DFD beef was found to be slightly tougher than normal beef before heating, but became more tender than normal beef at ca. 60°C. Above 65°C the difference in tenderness became much more pronounced, since DFD beef in contrast to normal beef showed no further decrease in tenderness.

When expressed in terms of its two contributing characteristics, firmness (Fig. 2B) and bite-off

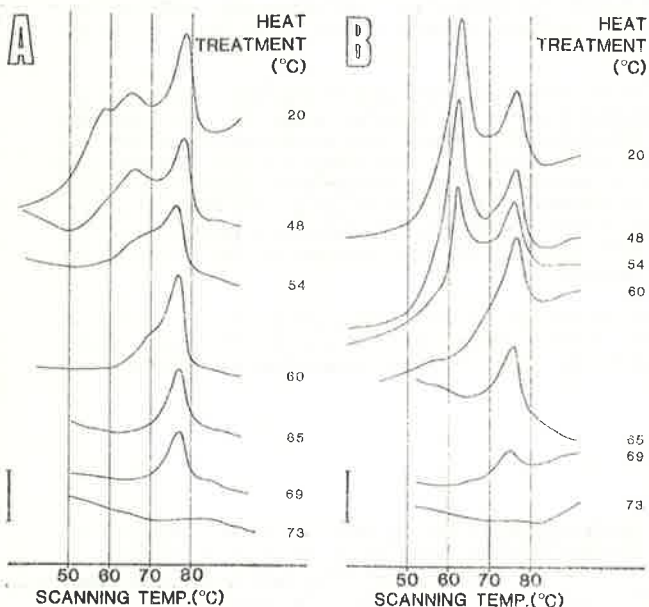


Fig. 1. Thermograms of myofibrillar tissue from normal (A) and DFD (B) bovine *M. semitendinosus* recorded after subjecting the samples to heat treatment at various temperatures. Scanning rate 10°C min⁻¹. The bar represents a change in the value of dQ/dt of 0.05 mcal s⁻¹.

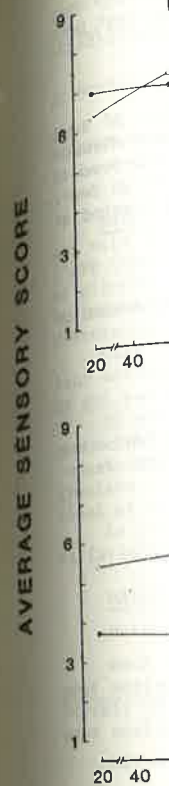


Fig. 2. Mean temperature error

force (Fig. 2C) be due to:

- 1) A smaller range
- 2) No increase to normal

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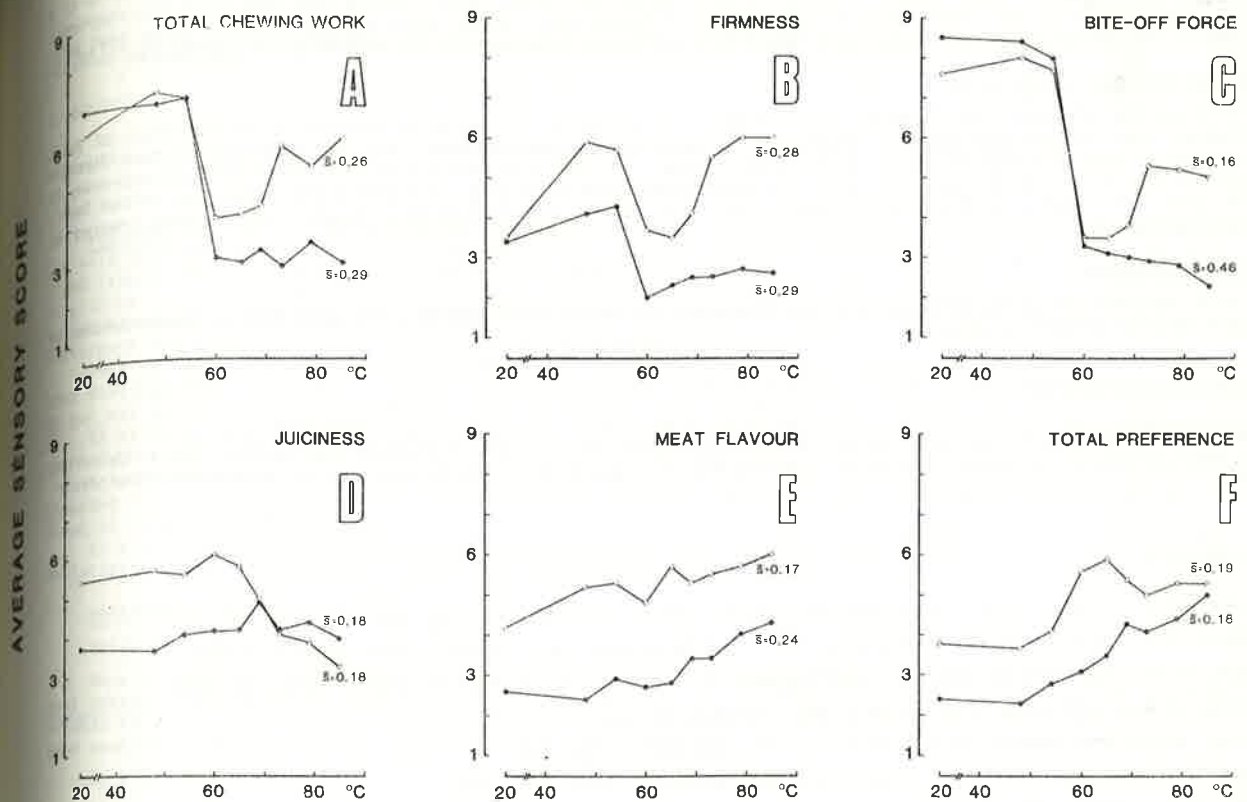


Fig. 2. Mean of sensory scores for bovine *M. semitendinosus* as a function of the temperature of heat treatment. o—o Normal beef. ●—● DFD beef. s = average standard error of the mean.

force (Fig. 2C), the improved tenderness of DFD beef during heating, as compared to normal beef, seems to be due to:

- 1) A smaller initial increase in firmness (a less pronounced hardening effect) in the myosin denaturation range
- 2) No increase in firmness and bite-off force of DFD meat in the actin denaturation range, in strong contrast to normal meat.

This demonstrates the importance of the contribution of the pH-sensitive myofibrillar proteins to meat texture above ca. 60°C. The two characteristics, firmness and bite-off force, are both strongly pH-dependent in the actin denaturation range.

The juiciness of DFD beef (Fig. 2D) was low and largely unaffected by the temperature of heat treatment. In contrast, the relatively high initial juiciness of normal beef was much reduced above 65°C, presumably due to actin denaturation. Thus, above 73°C normal beef was found to be less juicy than DFD beef.

Juiciness is probably not directly influenced by pH, i.e. it is not water bound by electrostatic forces which is the most effective in imparting juiciness to meat (Hamm). However, juiciness is indirectly affected by pH, by the way in which pH influences the microstructure (pore size) of meat (Clark *et al.*). The microstructure is to a great extent dependent upon aggregation patterns.

The difference in juiciness between the two types of beef above 73°C, may to a great extent be explained as being caused by a considerably higher loss of water from normal beef than from DFD beef. At 73°C normal beef had lost ca. 33 per cent as drip and cooking loss while DFD beef had lost ca. 12 per cent as cooking loss.

Both normal and DFD beef lost ca. 10 per cent of the total weight in the actin denaturation range (65-73°C). This is consistent with normal beef and DFD beef showing the same shrinkage in this range. However, denaturation of myosin (40-60°C) resulted in ca. 10 per cent loss of water from normal beef, in contrast to DFD beef where no cooking loss was observed. This indicates a fundamental difference in denaturation and aggregation between normal and DFD myosin. When comparing Figs. 1A and 1B, the most obvious feature is the difference in myosin denaturation and aggregation between the two types of beef. In normal meat, myosin must be assumed to denature and subsequently aggregate in two more or less separate steps, while in DFD meat the myosin molecules presumably denature and aggregate more simultaneously, resulting in a different structure with a higher water binding capacity. Nevertheless, at this stage DFD beef was still perceived as less juicy than normal beef. Myosin denaturation and aggregation thus influences tenderness and apparent juiciness only to a limited degree in the myosin denaturation range, possibly due to intact collagen at these temperatures.

The most conspicuous result of myofibrillar denaturation is seen in the actin denaturation range. In spite of a probably very similar denaturation process in both cases, as judged from cooking loss and actin thermograms, actin aggregation in DFD beef caused no decrease in tenderness, while actin aggregation in normal beef produced a very tough meat, probably as a result of a different structure of the myosin-actin aggregates.

Flavour and total preference

The intensity of meat flavour (Fig. 2E), which is a property not directly related to denaturation of meat proteins, was judged considerably lower in DFD beef than in normal beef, but the intensity of meat flavour increased at higher temperatures in both types of beef. At 85°C the meat flavour of DFD beef had improved to such an extent that DFD beef had about the same total preference (Fig. 2F) among the judges as normal beef. The latter still had a stronger meat flavour, but was dryer and considerably tougher after being treated at this temperature.

Recommendations

A final temperature of 60-65°C for the heat treatment of normal beef and 85°C for DFD beef is recommended to obtain optimal sensory quality.

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REFERENCES

- Bouton, P.E., Harris, P.V., and Shorthose, W.R. (1971). *J. Food Sci.*, **36**, 435.
Bouton, P.E., Harris, P.V., Macfarlane, J.J., and Shorthose, W.R. (1982). *Meat Science*, **6**, 27.
Clark, A.H., Saunderson, D.H.P., and Suggett, A. (1981). *Int. J. Peptide Protein Res.*, **17**, 353.
Currie, R.W., and Wolfe, F.H. (1980). *Meat Science*, **4**, 123.
Davey, C.L., and Gilbert, K.V. (1974). *J. Sci. Fd Agric.*, **25**, 931.
Finch, A., and Ledward, D.A. (1972). *Biochim. Biophys. Acta*, **278**, 433.
Giles, B.G. (1969). 15th Eur. Meet. of Meat Res. Work., Helsinki, 289.
Goodno, C.C., Harris, T.A., and Swenson, C.A. (1976). *Biochemistry*, **15**, 5157.
Hamm, R. (1972). *Kolloidchemie des Fleisches*. P. Parey, Berlin/Hamburg.
Lapanje, S. (1978). *Physicochemical Aspects of Protein Denaturation*. Wiley and Sons, N.Y.
Ledward, D.A. (1979). *Effects of heating on foodstuffs*. Applied Science Pub. Ltd., London, 157.
Machlik, S.M., and Draudt, H.N. (1963). *J. Food Sci.*, **28**, 711.
Martens, H., Stabursvik, E., and Martens, M. (1982). *J. Text. Stud.* (in press).
Martens, H., and Vold, E. (1976). 22nd Eur. Meet. Meat Res. Work., Malmö, J9.
Stabursvik, E., and Martens, H. (1980). *J. Sci. Fd Agric.*, **31**, 1034.
Veis, A. (1964). *Macromolecular Chemistry of Gelatin*. Academic Press, N.Y.
Wright, D.J., Leach, I.B., and Wilding, P. (1977). *J. Sci. Fd Agric.*, **28**, 557.