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Myosin Denaturation in Pale, Soft, and Exudative (PSE) Porcine Muscle Tissue as Studied by Differential Scanning Calorimetry[a]

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Myofibrillar tissue from pale, soft, and exudative (PSE) pork was compared to tissue from normal pork by differential scanning calorimetry at pH 5.4. Thermograms of myofibrillar tissue from normal pork were characterised by three major peaks with temperature maxima at 58 and 66°C, associated with myosin denaturation, and at 78°C, associated with actin denaturation. In thermograms of PSE pork, the peak at 58°C was markedly reduced, and appeared as a shoulder. When the thermograms were divided into segments corresponding to the three major peaks, the area of the low temperature myosin segment was shown to be reduced by about 50% in PSE pork, as compared to normal pork. This indicates approximately 50% denaturation of the least thermostable parts of the myosin molecule. The more thermostable parts of the myosin molecule were largely unaffected, as was actin.

1. Introduction

Pale, soft and exudative (PSE) meat is undesirable for meat processing purposes. Both textural and binding properties in products made from PSE meat are inferior to the properties of products made from normal meat.[1] The PSE condition is believed to be caused by low pH and high temperature at the early post-mortem stage.[2]

Sarcoplasmic proteins of PSE meat have been shown to be partly denatured,[3,4] and it has been suggested that deposition of denatured sarcoplasmic proteins on to the myofilaments causes reduced extractability of myofibrillar proteins, without necessarily involving denaturation of these proteins.[5] However, sarcoplasmic and myofibrillar proteins denature in the same temperature range.[6] Studies on washed rabbit myofibrils heated to temperatures of between 35 and 42°C, have revealed pronounced myosin denaturation, particularly at low pH values (*ca.* 5.5).[7–9] The importance of myofibrillar protein denaturation in PSE muscle was demonstrated by Penny.[10] He found the degree of denaturation of myofibrillar proteins to be closely related to the decreased water binding capacity of the meat. This is in accordance with other studies which have shown that myosin and actomyosin are essential in developing the binding properties of meat,[11] and that denaturation of these proteins will result in a lower binding ability of the meat.[12]

Extraction of salt soluble proteins and measurement of ATP-ase activity of myofibrils have been used to study the denaturation of myofibrillar proteins in muscle.[7] Differential scanning calorimetry (DSC) offers a method for studying the denaturation of myofibrillar proteins in muscle tissue *in situ*.[6] The aim of the present study was to employ DSC for measuring the degree of denaturation of the myofibrillar proteins myosin and actin in PSE pork, as compared to normal pork.

2. Experimental

2.1. Materials

Experiments were performed with post-rigor samples of *M. longissimus dorsi* from market weight Norwegian Landrace pigs, following normal commercial slaughter and chilling procedures. The excised samples were frozen and stored at −20°C.

[a] A report on a preliminary study of this subject was delivered at the symposium 'Porcine stress and meat quality' held at Refsnes Gods, Jeløy, Norway, 17–19 November 1980.

Table 2. Apparent enthalpies of denaturation of myofibrillar tissue (ΔH_{app}=J g^{-1}±s.e. (mean)) for the main peak areas (A, B, C) of 5 normal and 5 PSE samples of porcine *M. longissimus dorsi*.

	Peak area		
Muscle	A	B	C
Normal	4.38±0.10	4.11±0.06	4.19±0.05
PSE	2.06±0.06	3.45±0.07	3.95±0.04
Differences	2.32	0.66	0.24
Differences (%)	53.0	16.1	5.7
Significance	***	***	***

***, $P<0.001$.

2.2. Methods

2.2.1. Sample preparation

Connective tissue and sarcoplasmic proteins were removed from the muscle tissue, and the pH value adjusted to 5.4±0.05 with 0.5N NaOH/HCl, as described by Stabursvik and Martens.[6]

2.2.2. Differential scanning calorimetry

Thermal denaturation of the proteins of myofibrillar tissue was studied in a Perkin-Elmer DSC-2, as described by Stabursvik and Martens.[6] A heating rate of 10°C min^{-1} and small (15 µl) sample pans (Perkin-Elmer part No. 219–0062) were used. After DSC analysis, the sample pans were punctured and the dry weight of the samples determined after drying at 105°C overnight.

Thermogram baselines between 49 and 83°C were drawn with the aid of a flexible ruler (Figure 1). The thermograms were divided into the segments A, B, and C, at 61 and 71°C, respectively, which were the mean temperatures of the curve minima observed. Peak areas of DSC thermograms were estimated by planimetry and used to calculate apparent enthalpy of denaturation (J g^{-1} dry matter). Each of the individual peak areas was divided by the total dry matter weight. Indium (heat of melting=28.42 J g^{-1}) was used as a standard.

2.2.3. Statistical methods

A *t*-test was used for statistical evaluation of the meat quality characteristics (Table 1), and a mixed split-plot analysis of variance[16] for statistical evaluation of the apparent enthalpies of denaturation (Table 2). Eight to ten DSC scans for each sample were included in the calculation.

3. Results

Typical DSC thermograms of myofibrillar tissue from normal pork display three major peaks with temperature maxima (T_{max}) at 58, 66 and 78°C, respectively (Figure 1). Thermograms of PSE muscle reveal that the low temperature peak (T_{max} 58°C) was markedly reduced and appears as a shoulder (Figure 1), indicating that a partial denaturation of the myofibrillar proteins occurred in PSE muscle prior to the thermal denaturation in the calorimeter.

Calculations of apparent enthalpies of denaturation from the main DSC peak areas (A, B, and C) of myofibrillar tissue from normal and PSE muscle (Table 2) reveal a reduction of approximately 50% of segment A in PSE compared to normal muscle, whereas the apparent reductions of segments B and C are in the order of 15 and 5%, respectively. The differences between normal and PSE meat were found to be statistically significant at $P<0.001$ for all segments.

4. Discussion

In the present study collagen and sarcoplasmic proteins were removed during sample preparation, and consequently the thermograms are, for all practical purposes, related to the denaturation of

Table 1. Early (15 and 45 min) and 20 h post-mortem meat quality characteristics of 5 normal and 5 PSE samples of porcine *M. longissimus dorsi*

Muscle sample	Rigor value		Temperature (°C)		pH			Colour reflectance
	15 min	45 min	15 min	45 min	15 min	45 min	20 h	20 h
Normal	8.2±0.7[a]	9.6±1.3	40.3±0.7	37.7±0.6	6.80±0.07	6.65±0.07	5.52±0.03	70.7±0.5
PSE	14.8±0.8	15.6±0.6	42.6±0.2	39.4±0.2	5.47±0.08	5.32±0.02	5.41±0.05	56.2±0.7
Significance	***	**	**	*	***	***	n.s.	***

[a] mean±s.e. (mean).
n.s., not significant; *, $P<0.05$; **, $P<0.01$. ***, $P<0.001$.

Measurements of muscle pH (Orion Research model 201 digital pH-meter/Ingold LoT 406-M4 combined glass electrode), temperature (Digitherm model KM 2013 digital thermometer/needle probe PPC/D/st) and rigor state (Rigor meter model IVO, Holland)[13] of the carcass 15 and 45 min after exsanguination, and ultimate pH and colour reflectance (Göfo photoelectric instrument)[14] of the loin upon excision 20 h after slaughter, were used to select five definitely normal and five definitely PSE samples.[15] A high initial pH (≥6.5 at 15 min), combined with a normal colour reflectance (Göfo value 69–72) and an ultimate pH below 5.6, were the criteria for normal pork. A low initial pH (≤5.8 at 15 min) was the main PSE criterion, supplemented by high temperature (≥42°C at 15 min), an early developing *rigor mortis* (rigor values ≥12 at 15 min), and ultimate pH and colour reflectance values below 5.6 and 60, respectively.

As shown in Table 1, the differences between normal and PSE samples were statistically significant for all parameters with the exception of pH at 20 h.

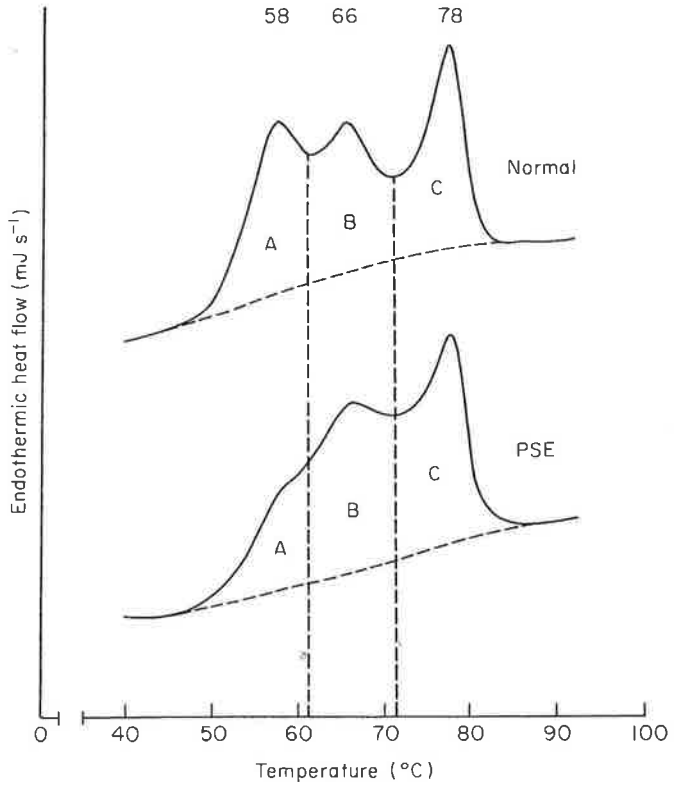

Figure 1. Thermal denaturation of myofibrillar tissue of porcine *M. longissimus dorsi*, at pH 5.4. Segmentation (A, B, and C) used for calculation of apparent enthalpies is indicated (see Table 2).

myosin and actin.[6] The peak maximum values found for porcine *M. longissimus dorsi* at pH 5.4 (Figure 1) are practically identical to those found for muscle from bovine *M. semimembranosus*.[6] As shown in work by, among others, Martens and Vold[17] and Wright *et al.*,[18] the high temperature peak (T_{max} 78°C) is evidently due to actin denaturation, while the two peaks with T_{max} values 58 and 66°C, respectively, are ascribed to denaturation of myosin. Stabursvik and Martens[6] interpreted the peak with T_{max} 66°C at pH 5.4 as representing denaturation of heavy meromyosin (HMM) or its subfragment 2 (HMM S-2), while the peak with T_{max} value 58°C at pH 5.4 was interpreted as representing denaturation of light meromyosin (LMM) plus a possible contribution from subfragment 1 from heavy meromyosin (HMM S-1).

Dividing the thermograms into three segments, A, B, and C (Figure 1) revealed differences in the extent of denaturation of the various parts of the actomyosin complex.

Segment A in Figure 1 corresponds roughly to the less thermostable myosin peak (T_{max} 58°C), while segment B accordingly corresponds to the more thermostable myosin peak (T_{max} 66°C). Segment A of the PSE thermograms is about half the size of the A segment of the normal pork thermograms, whereas the B segment is reduced by only 15%. It should be noted, however, that the myosin transitions associated with segment A will tail to a smaller or larger extent into segment B. Thus the myosin transitions associated with segment B may be largely the same in PSE as in normal pork. Correspondingly, segment A also comprises parts of the myosin transitions associated with segment B, implying that the real enthalpy decrease of the transitions associated with segment A may actually be larger than indicated here.

Segment C (T_{max} 78°C) corresponds to the actin peak. A reduction of approximately 5% in apparent enthalpy was observed here. Some degree of tailing may also be responsible for this rather limited reduction in apparent enthalpy, although the possibility of denaturation occurring on a small scale cannot be ruled out, as is also the case for segment B.

In accordance with these findings it is proposed that the PSE condition is primarily characterised by extensive denaturation of that part of the myosin molecule represented by segment A, whereas the part of the myosin molecule represented by segment B is left nearly intact. An alternative explanation, involving total denaturation of approximately 50% of the myosin molecules, while leaving the remaining myosin molecules intact, would imply reduction of segments A and B to the same extent.

The assumption that subfragment 1 contributes to the myosin peak at 58°C is supported by studies on ATP-ase activity in PSE muscle: Myofibrils from PSE muscle have less than 50% of the ATP-ase activity of myofibrils from normal muscle.[19] The ATP-ase activity of myosin is well established to be associated with subfragment 1. There is good agreement, therefore, between studies on ATP-ase activity and the interpretation that the peak at 58°C in PSE thermograms is reduced due to denaturation of subfragment 1. Loss of ATP-ase activity indicates denaturation of the S-1 fragment, but does not give any additional information about the molecular state of other parts of the actomyosin complex.

Penny[7] compared the influence of pH and temperature on the protein extractability and ATP-ase activity of washed myofibrils, and showed that ATP-ase deactivation and loss of extractability seem to be directly related. The extractability of salt soluble myofibrillar proteins (actomyosin) from PSE muscle has been found to be 50% or less, compared to that of normal muscle.[20] Extraction studies of actomyosin from PSE and normal muscle in a Hasselbach Schneider solution, which is known to split the actomyosin complex into myosin and actin, showed a myosin : actin ratio of 5 : 1 in extracts from PSE muscle compared to 10 : 1 in extracts from normal muscle, when subjected to electrophoresis.[21]

Thus, both ATP-ase activity and extractability of myofibrillar proteins closely agree with the approximately 50% reduction of segment A observed in the PSE thermograms, which indicates that extractability and ATP-ase activity are determined by denaturatuon of the less thermostable parts of myosin.

Differential scanning calorimetry is probably not suitable as a routine method of analysis for meat processing purposes. The advantage of the method lies in its ability to characterise the molecular state of actomyosin in myofibrillar tissue in greater detail than is possible by use of extractability or ATP-ase activity determinations.

For practical purposes the solubility of a protein is very important,[22] and may be the only protein property with a major effect in meat processing.[23] Therefore, solubility determinations may represent the method of choice for practical rough estimations of protein denaturation in meat.

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