

DSC THERMAL SCANNING CALORIMETRIC STUDIES ON THERMAL PROPERTIES OF MUSCLE PROTEINS IN NORMAL AND PSE PORK

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ABSTRACT: This study was conducted to investigate the thermal properties of muscle proteins in PSE pork as compared to normal pork by differential scanning calorimetry. The fine ground excised Longissimus dorsi muscle was homogenized with 0.3M KCl to extract salt soluble proteins, and added, with 0.1mM CaCl₂, 5mM EDTA, as well as with 0.1mM CaCl₂ + 20mM ATP, respectively. Result was observed on thermogram of 0.3M KCl extracted sample with CaCl₂ was similar to the control, and there were two endothermic peaks appearing on the thermogram. T_{max} of the transition of the control and with CaCl₂ measured were ranged at 56.9°-57.9°C for the peak of myosin, and 78.2°-79.2°C for the peak of actin, but endothermic peak of myosin disappeared from thermogram as the control sample added with EDTA. In addition, T_{max} for the extracted sample with CaCl₂ and ATP was higher than that of the control. Normal whole muscle held for 24.5 hr postmortem showed three major peaks of transition which were same as PSE pork. T_{max} for myosin in PSE pork was lower than that of the normal pork, but T_{max} for actin in PSE pork was higher as compared to the normal pork. Both T_{max} changed with time postmortem. The enthalpy of denaturation for PSE pork was lower than the normal pork under the same conditions. This result was evidenced by the change of myosin endothermic peak in thermogram. An exothermic peak was observed on thermogram for normal muscle fibers and showed a progressive decrease in the size with postmortem time. However, the exothermic peak was only slightly evident in PSE pork as affected by pH and temperature. The enthalpy of denaturation for transition for samples of normal and PSE was also evaluated. In conclusion, the thermograms and ΔH were varied with muscle types, postmortem times.

INTRODUCTION: Pale, soft, and exudative(PSE) pork is obtained from PSS pig caused by stress. PSE meat is undesirable for meat processing and eating purposes. The functional properties in PSE meat are inferior to those of the normal meat. The importance of myofibrillar proteins for gelation, textural properties and binding of fat and water in processed meats is well documented (Samejima et al., 1981; Schmidt et al., 1981; Acton et al., 1983; Asghar et al., 1985). Penny(1969) found that the degree of denaturation of myofibrillar proteins to be closely related to the decreased water binding capacity of the PSE meat. DSC is used progressively to study thermogramic properties of muscle proteins, and it can offer a method for studying the denaturation of myofibrillar proteins in muscle tissue in situ. In order to establish a quality control system of raw meat DSC was conducted to study the properties of muscle proteins from PSE pork as compared to the normal pork.

MATERIALS AND METHODS:

Material source: Normal and PSS pigs(LYD crossbred) were selected from the local meat market and slaughtered following commercial procedures.

The samples were excised from the Longissimus dorsi (L. D.) between 5th and 11th ribs after 30 min slaughter, and placed in ice box, then transported back to lab immediately and stored at 4°C. PSE and normal muscles were defined according to the definition described by Briskey(1964). If the muscle dropped below 5.8 within one hour postmortem, it was defined as PSE muscle, or above 6.2 after 24 hours postmortem, the muscle was defined as DFD muscle, other samples were belong to normal muscles.

Surface fat and connective tissue of muscle tissue were carefully removed using a scalpel. At 0, 3, 5, 8.5 and 24.5 hr postmortem pH, temperature and calorimetry of the samples were measured. Additionally, the muscle samples were excised immediately from the L. D. muscle between 5th and 11th ribs were checked its pH and temperature.

Protein extraction: Myosin was extracted according to the procedures described by Quass and Briskey(1968). The muscle samples obtained from 0, 3, 5, 8.5 and 24.5 hr postmortem and placed in liquid nitrogen, and tempered in ice bath for 30 min, and then excised for extracting with 0.3M KCl solution under 2-4°C.

Sample preparation: Whole muscle fibers were removed from the chilled meat (4°C) and cut into 2 cm long for DSC analysis, or the whole muscle fibers were excised and stored in liquid nitrogen for 12 hr and then crumbled for another DSC analysis.

Sample extraction: The chilled meat of normal pig after 24 hr slaughter was minced and extracted with 0.3M KCl (1:3, w/v) for 20 min, and the filtrate was mixed with 5mM EDTA or 0.1mM CaCl₂(1:27, w/v) for 30 min and centrifuged by 3,000xg, 30min at 4°C, and

discarded supernatant, the EDTA or CaCl_2 washing was repeated two additional times. The final pellet was dissolved in 0.3M KCl solution and mixed with 5mM EDTA or 0.1mM CaCl_2 to a final concentration of 0.03M KCl, and centrifuged, the precipitate was used for DSC.

DSC was performed on a ULVac DSC-7000 (Sinku-Riko, Japan) equipped with thermal analyzer. Samples (15-20mg) were weighed in aluminum pans (No. 201-53090) and then sealed. The scanning temperature was 25°-99°C at a heating rate of 10°C/min, triplicate samples were analyzed in reference containing 12-13 mg distilled water was used. The instrument was temperature calibrated using indium. Each DSC analysis was repeated three times. After DSC analysis, the sample pans were punctured and the dry weight of the samples determined after drying at 105°C overnight. The enthalpy of denaturation of muscle proteins was also collected.

ATP d

etermination: ATP content was determined according to the method of Dieter and Jutta (1981).

RESULTS AND DISCUSSION:

The temperature of L. dorsi muscle obtained from the normal pork stored at 4°C for 24.5 hrs was ranged from 29.3°C to 4.2°C during 1.5 to 24.5 hrs postmortem, and ranged from 27.6 to 4.3 for PSE meat. The pH values were from 6.42 to 5.58, and 5.74 to 5.59 from 1.5 hrs to 24.5 hrs after slaughtering for the normal and PSE meats, respectively.

Hunter colorimetric value-L value (lightness) changed from 32.45 to 39.07, and 41.01 to 42.21 for the normal and PSE pork, respectively. As mentioned above, the changes of color, pH value could be used to confirm PSE and normal meats.

Fig. 1 showed a typical DSC thermogram of myosin in the normal muscle obtained from carcass immediately 30 min postmortem.

Fig. 2 showed the postmortem changes in DSC thermogram of the normal muscle fibers during storage at 4°C. A strong exothermic reaction appeared on the thermogram in the 48-58°C region of the normal muscle up till at least 8.5 hrs after slaughtering. This peak showed a progressive decrease in the size with postmortem time, and disappeared after 24.5 hrs postmortem. The changes were in accordance with the work of Martens and Vold (1976). They also found the reaction took place in the thermogram of calf muscle. The endothermic peaks of myosin from the normal meat appeared at 8.5 hrs after slaughtering, and its maximum transitional temperature was 58.2, and shifted to 56.9 after 24.5 hrs postmortem (Table 1). As shown in Fig. 2 and Table 1, the T_{max} of transitions of sarcoplasmic proteins were also gradually shifted to the lower temperature from 70.3 to 66.1°C for actin from 80.3 to 78.3°C after 24.5 hrs of postmortem storage. The reason for the endothermic peak of myosin was absent at 1.5 to 3.5 hrs after slaughtering might be caused by obscuring of the exothermic peaks appearing prior to denaturation of myosin in normal meat (Park and Lanier, 1988). This phenomenon also occurred on the thermogram of myosin in PSE meat (Table 3).

As shown in Fig. 3 and Table 2, the T_{max} of the transitions of the muscle proteins in PSE meat measured postmortem from 1.5 to 24.5 hrs after slaughtering. A large exothermic peak was very apparent near 55°C shortly after slaughtering, but disappeared after 3.5 hrs postmortem. This change had the same trend as changes in ATP level of the PSE meat (See Table 2 and 3). The meat being striking difference in exothermic peaks on the thermogram was detected between the normal and PSE meats. Table 2 showed a unstable change in T_{max} of transitions for myosin, sarcoplasmic proteins and actin in PSE meat. The T_{max} for sarcoplasmic proteins and actin in PSE meat were lower than those of the normal meat (Tables 2 and 1).

Table 4 showed that the changes in apparent enthalpies of denaturation of the muscle proteins in the normal and PSE meats. The ΔH of the normal meat could be measured after 8.5 hrs postmortem, the reason was mentioned above, the obscuring by exothermic energy or difference in energy between the exotherm and endotherm. However, the Δh for PSE meat was detected after 3.5 hrs postmortem. Very few information about the changes in thermal properties of the normal and PSE meats was available. The origin of the exothermal reaction in prerigor meat is at present not clear. It is possibly caused by some super-activation of one or more metabolic energies, possibly breaking down ATP or some other high-energy compounds (Martens and Vold, 1976).

Fig. 4 indicated the T_{max} of the transitions of salt soluble proteins extracted with 0.3 M KCl added with 0.1mM CaCl_2 (Fig. 4a), and added with 5mM EDTA solution (Fig. 4b). The sarcoplasmic fraction was removed from myosin extraction, therefore its transitional peak did not appear on the thermogram. There were two transitional peaks being observed on thermogram in Fig. 4a, but only one peak appearing on the thermogram in Fig. 4b. This could be noted that Ca^{2+} ion did not affect myosin thermal denaturation, but EDTA solution did. The reason for this effect has not understood yet, it needs to do more work. This result is not in agreement with the reaction of whole muscle sample (Park and Lanier, 1988). The destabilization by EDTA is mainly due to the Ca^{2+} ion, as Von Hippel and Schieich (1969) reported that Ca^{2+} was particularly effective destabilization of the native conformation of the soluble proteins. Park and Lanier (1988) stored the intact muscle in 100mM CaCl_2 solution or 10mM EGTA solution, the myosin T_{max} of transitions appeared on thermogram, but it did not appear on this experiment. In conclusion, the thermal transition properties and apparent enthalpy varied with muscle types, treatment, temperature and postmortem time as well as sensitivity of instrument.

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Table 1. The change of transition temperature (Tmax°C) of normal muscle fiber during storage at 4°C for 24.5hr.

Time(hr) post-mortem	Transition temperature(Tmax°C)			
	1.5	3.5	8.5	24.5
Ta1	*	*	58.2±1.6	56.9±1.1
Ta2	70.3±0.7	68.9±0.8	66.9±0.9	66.1±0.6
Ta3	80.3±0.2	80.2±0.2	78.5±0.4	78.3±0.7

Values are the mean±standard error
 Ta1, Ta2 and Ta3 represents the first (myosin), second (actin) and third (protein) Tmax°C that muscle is denaturated by heating.
 * is unable to detect

Table 2. The change of transition temperature(Tmax°C)PSE muscle fiber during storage at 4°C for 24.5hr.

Time(hr) post-mortem	Transition temperature(Tmax°C)			
	1.5	3.5	8.5	24.5
Tm1	59.0±0.9	57.8±1.8	57.9±2.4	58.9±1.8
Tm2	66.5±0.4	67.4±1.5	67.3±1.6	66.6±2.5
Tm3	79.1±0.7	78.9±0.8	78.4±1.0	78.5±0.4

Values are the mean±standard error

Tm1, Tm2 and Tm3 represents the first, second and third Tmax°C that muscle is denaturated by heating.

Table 4. The postmortem change of transition heat (ΔH) of normal and PSE muscle fiber during storage at 4°C

Time(hr) post-mortem	transition heat (ΔH)			
	1.5	3.5	8.5	24.5
Normal	*	*	*	6.262±1.838
PSE	*	3.871±1.380	3.136±0.706	4.354±1.507
DFD	6.653±2.513	6.196±1.358	6.652±1.134	5.279±0.998

Values are the mean±standard error

The unit of ΔH is kcal/per mg dry matter

* is unable to detect

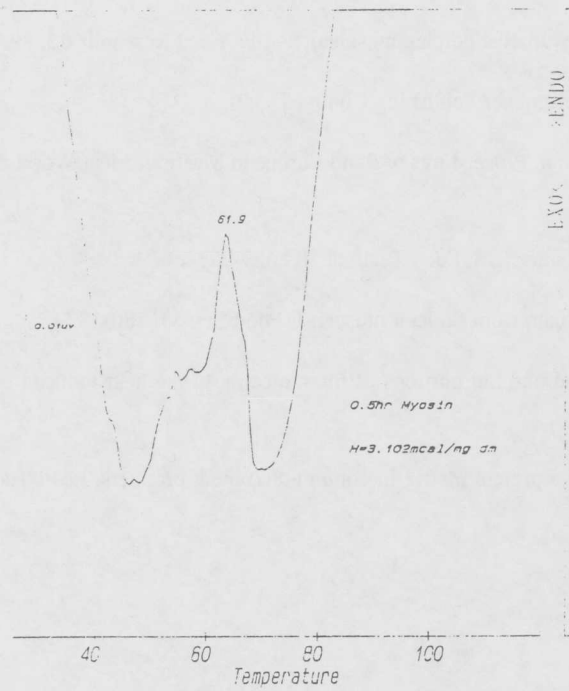


Fig 1. A typical DSC thermogram of myosin extracted from the normal porcine muscle.

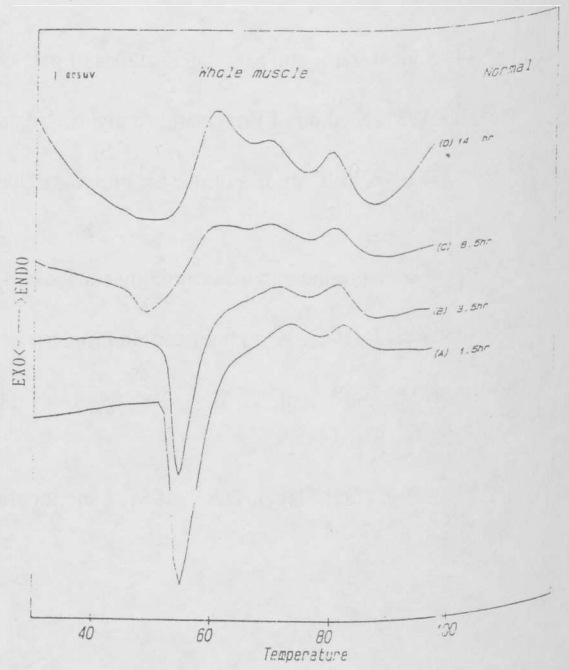


Fig 2. The postmortem change in DSC thermogram of normal muscle fiber during storage at 4°C.

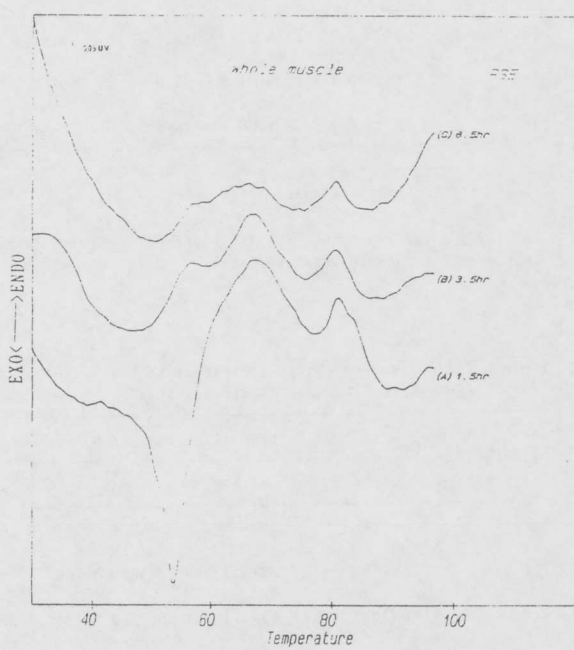


Fig 3. The postmortem change in DSC thermogram of PSE muscle fiber during storage at 4°C.

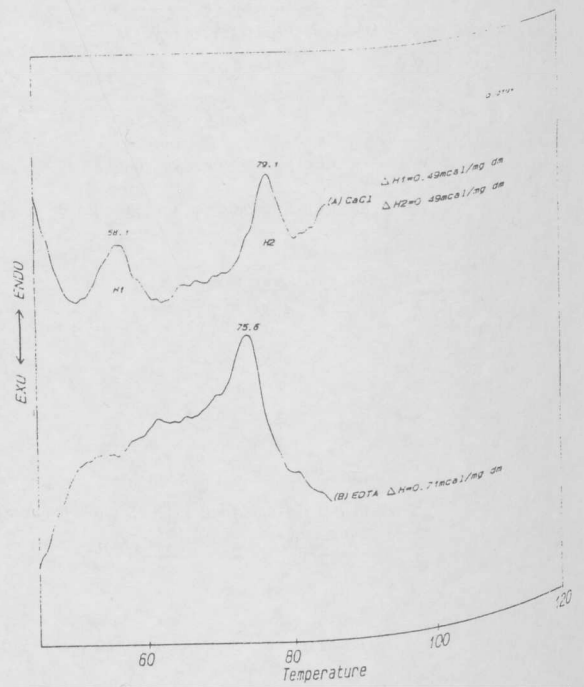


Fig 4. DSC thermogram of salt-soluble proteins (A) extracted by 0.3M KCl and 0.1M CaCl₂ (B) extracted by 0.3M KCl and 5mM EDTA.