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Comparative studies on the biochemical characteristics of natural actomyosin isolated from PSE and normal pork*

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Abstract: Biochemical changes of natural actomyosin from fresh pale, soft, exudative (PSE) and normal pork were studied, and the effects of different storage temperatures and different incubation temperature and times on sample superprecipitation, total sulfhydryl (-SH) content, and ATP (adenosine triphosphate) sensitivity were investigated. The results demonstrated that ATPase activity and thermal stability of PSE actomyosin were lower than those of normal pork; and that PSE actomyosin had higher -SH content than that of normal pork at all incubation temperatures and times tested.

Key words: PSE, Pork, Actomyosin, Biochemical characteristics

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INTRODUCTION

The meat industry is facing consumer demands for high, predictable, and consistent meat quality. One of the major problems is the change in exudation, colour, and firmness that characterize meat quality. High incidence of pale, soft and exudative (PSE) pork cause poor technological quality, drip loss, and shrinkage during storage as well as unattractive visual appearance for consumers. PSE pork also causes an economic impact on the industry because of product dehydration during processing.

It had been generally accepted that pH and temperature of pork, including rate of pH fall, final pH, and the chilling regime, are responsible for PSE (Greaser *et al*., 1969; Van Laak and Solomon, 1994). Myofibrillar protein from PSE muscle shows a decrease in functional properties such as solubility, gelling capacity, and water-holding capacity (Swatland, 1994; Offer, 1991; Goldspink and McLoughlin, 1964). Offer (1991) found that denaturation of muscle actomyosin happened when carcasses were exposed to pH value lower than 6.0 and temperature higher than 38 ºC. However, few studies have been reported on biochemical differences between normal and PSE pork.

This study was aimed at investigating the differences between normal and PSE muscle in superprecipitation, total -SH content, and ATP sensitivity.

MATERIAL AND METHODES

Extraction and purification of actomyosin

Actomyosin obtained without ATP was regarded as an analog of “Natural” actomyosin

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(AMN). AMN from PSE and normal pork were prepared by a modification of the methods used by Herring *et al.*(1969) and Karlsson *et al.*(1999). Twenty-five grams muscle was homogenized in chilled 100 ml 50 mmol/L phosphate buffer (pH 7.5) at 1500 rev/min for 3 min. After standing for 20 min, the extract was centrifuged at 2100×g for 10 min at 4 ºC. The precipitate was collected and the above procedure was repeated three times. 3 Volume of 0.45 mol/L KCl-phosphate (pH 7.5) were added to the precipitate. The mixture was homogenized for 30 sec; pH was adjusted to 7.5 with 0.5 mol/L Na_2HPO_4 if necessary and stored at 4 ºC for 20 h. Then the mixture was centrifuged at 5500×g for 20 min. The supernatants were filtered through a 80 mesh nylon screen and collected as crude actomyosin.

Ten volumes of chilled deionized water were added to the crude actomyosin. The mixture was stirred well, adjusted to pH 6.5−6.6, and centrifuged at 5500×g for 20 min at 4 ºC. Three mol/L KCl solution (pH 7.5) was added to the pellet until the mixture had 0.6 mol/L KCl; then the mixture was homogenized for 30 sec, and centrifuged at 5500×g for 15 min. The supernatant was collected and used as actomyosin. The actomyosin solution was adjusted to 2.65 mg/ml protein and kept at 0 ºC until used.

Protein determination

The protein content of actomyosin was determined by the method of Gornall *et al.*(1949) with bovine serum albumin (BSA) as the protein standard. The protein content was expressed as mg actomyosin per gram muscle.

Determination of actomyosin superprecipitation

Superprecipitation was measured at 660 nm according to the method described by Goldspink and McLoughlin (1964). The optical density changes were recorded using a spectrophotometer (Model 721) with cuvettes of 1 cm light-path. Solution A contained 0.30 ml actomysin solution (2.65 mg/ml protein), 0.15 ml of 0.5 mol/L Tri-maleat buffer (pH 7.0), and 2.50 ml of distilled water. Solution B contained 15 µl of 10 mmol/L ATP-$MgCl_2$ (pH 7.0). After Solution B was added into Solution A, the absorbency was recorded at one-minute intervals.

Determination of ATP sensitivity

ATP sensitivity of actomyosin was determined according to the method described by Hasnain *et al.* (1973) with a slight modification: Viscosity was measured at 25 ºC using a Cannon-Fenske viscometer. The viscosity of 10 ml actomyosin solution (2.65 mg/ml protein) was recorded as η_a, and the viscosity of actomyosin with addition of 1 mmol ATP-$MgCl_2$ solution (pH 7.0) was recorded as η_b. The ATP sensitivity was calculated by using the following formula:

$$\text{ATP Sensitivity}=(\log\eta_a-\log\eta_b)\times 100\ /\log\eta_b$$

Determination of total sulfhydryl (-SH) groups

Total -SH groups was determined by the method of Ramirez *et al.*(2000). Three ml 8 mol/L urea was added to 3 ml actomyosin solution (2.65 mg/ml protein) and a 3 ml aliquot was mixed with 20 µl DTNB (39.6 mg DTNB in 10 ml phosphate buffer, pH 7.0). After incubation for 20 min, absorbance at 412 nm was measured using a spectrophotometer. Sample blank was prepared by replacing DTNB with distilled water. Sulfhydryl groups were calculated from absorbance using the extinction coefficient of 13600 L/mol·cm. C_0=($A/E\times D$), where C_0 is molarity of -SH; A is optical density at 412 nm; D is extension rate; E is extinction coefficient 13600 L/mol·cm.

RESULTS AND DISCUSSIONS

Superprecipitation

It is generally known that actmyosin solution shows syneresis or super precipitation when actomyosin reacts with Ca^{+2}/Mg^{+2} and ATP. As a result the turbidity and absorbency increases due to the reaction of actin and myosin (Offer, 1991).

Absorbency reading is commonly used to monitor protein aggregates. Fig.1 shows that normal muscle exhibited higher rate in turbidity de-

velopment than that of PSE muscle. Normal muscle actomyosin consists of long filaments, in which approximately 1 mm long thin filaments of actin, tropomyosin, and troponin are conjugated with a great number of myosin all along the filament. Each myosin molecule is bound to the actin filament at its head portion with its tail portion sticking out. The results suggested that PSE muscle has less content of active myosin than normal, probably due to partial myosin denaturation.

Sulfhyldryl groups

SH groups determine the ATPase activity and structural stability of muscle proteins; therefore SH groups are usually used as an index for protein denaturation. Fig.2 shows the contents of SH group changing during storage at 0 ºC. PSE muscle had higher -SH contents than those of normal muscle. Changes in sulfhydryl content of actomyosin during heating are shown in Fig.3. Sulfhydryl content increased drastically after heat treatment at temperature of 30 ºC to 40 ºC, and decreased from 40 ºC to 55 ºC. A similar result was also observed when actomyosin solution was incubated at 35 ºC from 15 to 90 min (Fig.4). This was possible facilitated by the structural changes of AMN from normal/PSE muscle during heating which made the SH groups more susceptible to oxidation. The results suggested that disulfide bonds would play an essential role in actomyosin aggregation.

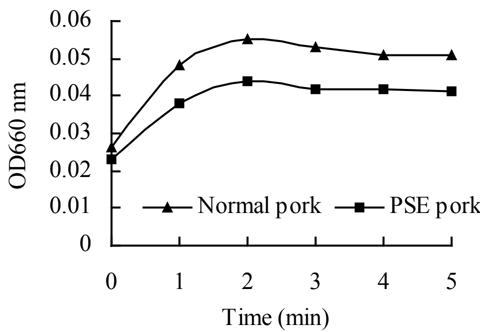

Fig.1 Estimation of actomyosin superprecipitation
The solution contained 0.30 ml actomysin solution (2.65 mg/ml protein), 0.15 ml of 0.5 mol/L Tri-maleat buffer (pH 7.0), 2.50 ml of distilled water and 15 μl of 10 mmol/L ATP-$MgCl_2$

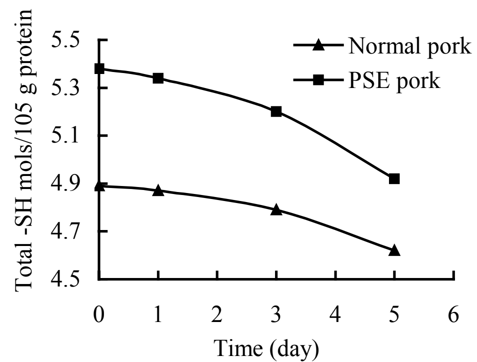

Fig.2 Effects of incubation time on total -SH when storing at 0 ºC
3 ml 8 mol/L urea was added to 3 ml actomyosin solution (2.65 mg/ml protein) and a 3 ml aliquot was mixed with 20 μl DTNB (phosphate buffer, pH 7.0)

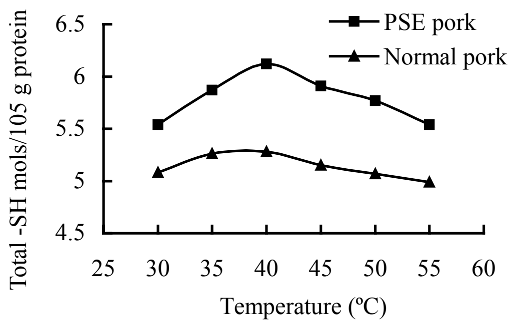

Fig.3 Effects of temperature on total -SH during incubation for 30 mins
3 ml 8mol/L urea was added to 3 ml actomyosin solution (2.65 mg/ml protein) and a 3 ml aliquot was mixed with 20 μl DTNB (phosphate buffer, pH 7.0)

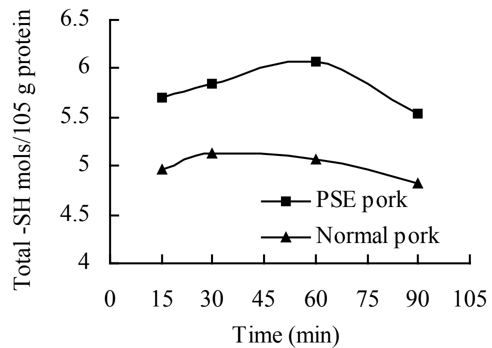

Fig.4 Effects of incubation time on total -SH when storing at 35 ºC
3 ml 8 mol/L urea was added to 3 ml actomyosin solution (2.65 mg/ml protein) and a 3 ml aliquot was mixed with 20 μl DTNB (phosphate buffer, pH 7.0)

ATP Sensitivity

The dissociation of actomyosin by ATP into actin and myosin was most easily demonstrated by the dramatic reduction in viscosity of actomyosin suspensions after the addition of ATP. Fig.5 shows that ATP sensitivity of PSE muscle decreased faster than that of normal muscle during storage at 0 °C. After 5 day's storage, ATP sensitivity of PSE muscle was only 70% that of normal muscle. Fig.6 and Fig.7 show changes of ATP sensitivity of actomyosin during heating and incubation. Both normal and PSE muscle exhibited decreases of ATP sensitivity.

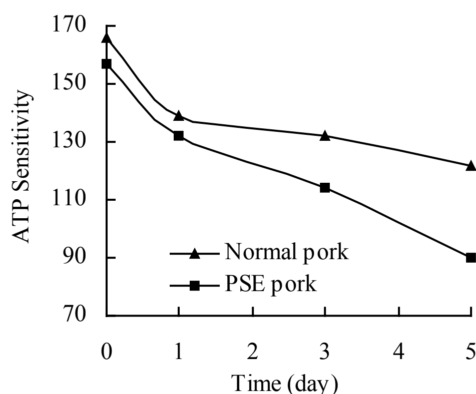

Fig.5 Effects of incubation time on ATP sensitivity when storing at 0 °C
Viscosity of 10 ml actomyosin solution (2.65 mg/ml protein) was measured at 25 °C using a Cannon-Fenske viscometer

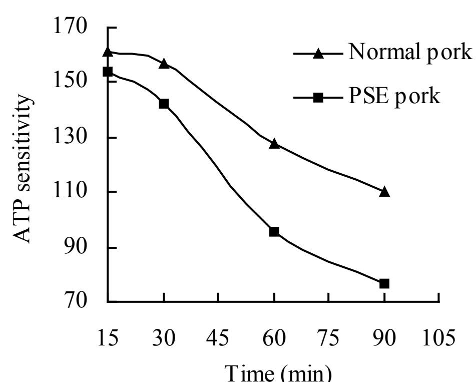

Fig.6 Effects of incubation time on ATP sensitivity when storing at 35 °C
Viscosity of 10 ml actomyosin solution (2.65 mg/ml protein) was measured at 25 °C using a Cannon-Fenske viscometer

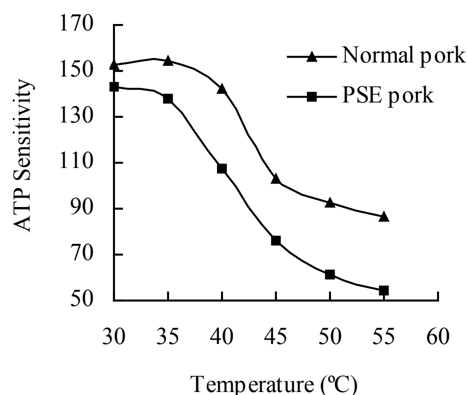

Fig.7 Effects of temperature on ATP sensitivity during incubation for 30 mins
Viscosity of 10 ml actomyosin solution (2.65 mg/ml protein) was measured at 25 °C using a Cannon-Fenske viscometer

However, PSE muscle had faster decrease rate than normal muscle under both test conditions. Deng *et al.*(2002) and Parsons and Patterson (1986) found that myosin was more sensitive to heat than actin. Therefore, our results suggest that PSE actomyosin underwent more denaturation than normal actomyosin.

CONCLUSIONS

PSE muscle had lower actomyosin superprecipitation than normal muscle.

PSE muscle had higher -SH value than normal muscle.

There is good correlation between SH group, ATP sensitivity, and superprecipitation, particularly between SH group and ATP sensitivity. Therefore, changes of SH group and ATP sensitivity could be used to distinguish normal pork from PSE pork.

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