MYOSIN DEGRADATION AND IMPAIRED GELATION OBSERVED IN PSE PORK

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Background

The ability of muscle proteins to form a cohesive thermally-induced gel matrix, either in isolated protein model systems or in processed meats is determined by a number of factors, including; protein structure and concentrations, ionic strength, pH, heating rate and muscle fiber type. In addition, postmortem conditions (pH and temperature decline and glycolysis rate) can have an impact. Pork with pale, soft, and exudative (PSE) characteristics is a significant meat quality problem. Rapid pH decline prior to reduction in carcass temperature is associated with the PSE condition (Offer, 1991). Recently, we observed that increased degradation of the heavy chain subunit of myosin was associated with rapid postmortem glycolysis in turkey breast meat (Rathgeber et al., 1999a). Breast meat from rapid glycolyzing birds also had PSE-like characteristics, including poor water binding and reduced processed product cohesion (Rathgeber et al., 1999b). These characteristics result in major economic implications for turkey processors.

Objectives

To monitor myosin heavy chain degradation in pork *longissimus* exhibiting accelerated postmortem metabolism and determine if low meat quality is associated with increased proteolytic activity.

Materials and Methods

Carcass selection: On two separate days, the pH of the *longissimus* (LD) muscle of pork carcasses on a commercial processing line was measured at ~ 45 min postmortem using a stab probe pH electrode. Carcasses with a pH < 5.80 were classified as rapid declining (RD) and carcasses with a pH > 6.00 were classified as moderate declining (MD). Samples from a total of 10 RD and 9 MD carcasses were retained for further evaluation. At 2 and 24 h postmortem, a 15 to 20 g LD sample was frozen in liquid nitrogen for evaluation of proteins by SDS-PAGE and Western blotting techniques. At 24 h postmortem the remaining LD was removed for product quality measurements.

Measurements on fresh pork: The drip loss of 1-cm thick LD slices was determined over a 30 h period at 4°C. Slices were also allowed to bloom for 60 min before Hunter colour (*L*a*b**) values were measured. Sarcoplasmic and myofibrillar proteins were extracted after homogenization in low (LIS; 0.05 M potassium phosphate; pH 7.5) and high (HIS; 0.05 M potassium phosphate 0.5 M KCl; pH 7.5) ionic strength buffers, respectively. Homogenized samples were centrifuged at 17,000 x *g* and protein concentration of the supernatant measured using the Biuret protein assay. Results were expressed as mg/g meat.

SDS-PAGE and Western blotting: Myofibrils were prepared from all 2 and 24 h postmortem samples as described by Rathgeber et al. (1999a). Protease inhibitors were added to all buffers used in the wash procedure to limit degradation during sample preparation. SDS-PAGE was performed as outlined by Laemmli (1970) at 35 milliamps constant current. The proteins were electrotransferred to nitrocellulose at 40 volts for 2.5 h. Nitrocellulose membranes were probed with monoclonal antibodies developed against the myosin heavy chain subunit of myosin (clones F27 and F59, gifts from Dr. F. E. Stockdale, Stanford University, Palo Alto, CA, USA). Several polyclonal antibodies (Sigma) were also used. Cross reactivity was detected by using secondary antibodies coupled to alkaline phosphatase or horseradish peroxidase.

Meat product preparation: A low-fat, high added-moisture bologna was prepared from the remaining meat from each loin at ~48 h postmortem. The meat batter was adjusted to 11% meat protein by altering the ratio of meat:water. The batter included 1.9% sodium choride, 2.6% dextrose, 0.4% sodium tripolyphosphate, 0.02% sodium nitrite and 0.07% sodium erythorbate. The mixture was chopped in a Hobart bowl chopper and passed through an emulsion mill. The batter was stuffed into moisture-proof casings and stage cooked in a water bath to 74°C and cooled.

Cooked product measurements: Purge was calculated as percentage of weight lost from vacuum packaged bologna slices following upright storage (2 h and 4 weeks) at 4°C. Expressible moisture (EM) was determined by measuring the mass lost from 15x8 mm cores of bologna following centrifugation at 750 x *g* for 10 min. Texture Profile Analysis (TPA) was determined using a Food Technology Corporation TMS-TP Texture Press (Rockville, MD, USA) by compressing 3.2x2.5 cm thick bologna cores to 75% compression. For torsional gelometry, cores were machined into capstan-shaped samples with a center diameter of 10 mm. Samples were twisted to fracture with a torsion gelometer (Gel Consultants, Inc., Rancho Cucamongo, CA, USA). Shear stress and strain values at failure were calculated on the basis of torque and angular displacement.

Statistical analyses: Samples were classified into one of three groups: (i) MD, moderate pH decline (n=9); (ii) RD. rapid pH decline, samples exhibited little myosin degradation (n=4); and (iii) RD-Pos, rapid pH decline, samples positive for myosin degradation at 2 hours postmortem (n=6) and analyses of variance were performed.

Results and Discussion

Early postmortem myosin degradation and gelation properties of pork *longissimus* with a rapid postmortem pH decline were compared to LD samples with moderate pH decline. An 82-kDa fragment of myosin heavy chain was detected by Western blotting for all samples. Additional fragments of 50, 53, 72, or 93 kDa were observed for 6 of 10 RD samples collected at 2 h postmortem, while none of the 2 h MD samples showed these additional fragments. For 24 h samples, 8 of 10 RD and 1 of 9 MD exhibited these additional myosin fragments.

The loins positive for additional myosin fragments at 2 h postmortem exhibited characteristics typical of PSE pork. The Hunter color values (L*, a*, b*) and drip loss were higher ($P < 0.05$) and myofibrillar protein extractability was lower compared to both MD loin samples and the RD samples negative for extra myosin fragments at 2 h (Table 1). Previously, Rathgeber et al. (1999a) observed that turkeys with a rapid postmortem pH decline exhibited a reduction in the extractability of sarcoplasmic and myofibrillar proteins and increased degradation of both nebulin and myosin heavy chain. The effects observed were more pronounced when birds were delayed from chilling for 30 min. In the present study, all carcasses had been chilled relatively rapidly (the average loin internal temperatures at 2 and 5 h were 18-21 and 6-8°C,

respectively). Reduced protein extractability in PSE pork has been attributed to denaturation of both sarcoplasmic and myofibrillar proteins, particularly myosin (Stabursvik et al., 1984; Offer, 1991). This work further suggests that degradation may also play a role. Myosin is generally not degraded by calpains that are generally accepted as making the greatest contribution to protein degradation postmortem. It is likely in these RD samples that cathepsins may be involved.

Gelation properties were evaluated by preparing a bologna-style product. All treatments were of similar pH due to the addition of phosphate (Table 2). Bologna prepared from the control MD loins and the RD loins with no additional fragments at 2 h were similar in texture and water holding capacity. Bologna from RD loins with more extensive myosin degradation had impaired gelation properties (lower TPA hardness, TPA springiness and strain at fracture) compared to the other RD loins and the MD samples. The fresh meat from these RD-Pos loins also exhibited lower protein extractability. However, it is unclear if a causal relationship exists. It has been previously shown that myofibrillar protein extractability is positively correlated to gel cohesiveness and water holding of turkey meat gels (Rathgeber et al., 1999a). In the present study, the correlation of myofibrillar protein extractability to strain was also significant (r=0.66).

Rathgeber et al. (1999a) had roughly estimated that in RD turkey *pectoralis,* degradation of myosin heavy chain was as high as 15-20%. It appears that the amount of degradation in pork is lower, but this requires further study and may depend on the severity of PSE. Further research is underway to determine the effect of myosin degradation on gelation.

Conclusions

Much of the research on PSE and muscle characteristics has focused on the reduction in protein solubility or extractability. This research and that of Rathgeber et al. (1999a) show that the PSE condition is more complex then just a reduction in extractability of proteins. The observed increase in myosin degradation in a number of the RD samples suggests that the low pH and high temperature conditions that creates PSE impacts protein degradation either by increasing catheptic activity in the meat and/or by altering structure to increase the sensitivity of muscle proteins to degradation. That myosin degradation could be detected as early as 2 h postmortem (our earliest sampling time) is also significant as there would be little opportunity to influence the pH / temperature decline in pig carcasses to help alleviate this problem.

References

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Acknowledgements

This research was support by strategic research funding by Saskatchewan Agriculture and Food. The technical assistance of H. Silcox, D. Pobereznek, G. Henriksen, J. Lerouge and E. Kerc is gratefully acknowledged. Thanks are also expressed to Mitchell's Gourmet Foods, Saskatoon, SK for supplying the pork loin samples.

Table 1. Properties of fresh pork as influenced by rate of pH decline and presence of myosin fragments.

Group[1]	pH, 2 h	pH, 24 h	Hunter L*	Hunter a*	Hunter b*	Drip, %	LIS, mg/g	HIS, mg/g
MD	5.96a	5.57a	48.75a	15.98a	11.04a	3.41a	96.34a	64.37a
RD	5.60b	5.49ab	48.05a	16.88ab	11.20a	4.01a	95.55a	65.55a
RD-Pos	5.45b	5.35b	52.94b	17.76b	13.39b	7.18b	88.03b	21.30b

[1]MD=Moderate pH decline; RD=Rapid pH decline with little degradation; RD-Pos=Rapid pH decline with degradation.
[ab]Means within a column with the same letters are not significantly different ($P < 0.05$).

Table 2. Properties of low-fat, high moisture bologna as influenced by rate of pH decline and presence of myosin fragments.

Group[1]	pH, 2 h	Protein, %	Hardness, N	Stress, kPa	Strain	EM, %	Purge, % 2 h	Purge, % 4 wk
MD	6.28a	11.98a	116.3a	33.91a	1.54a	18.22a	3.73a	7.99a
RD	6.34a	11.73a	112.9a	31.93a	1.48a	20.22a	5.07b	8.49a
RD-Pos	6.22a	11.89a	74.1b	25.92a	1.15b	24.98b	5.86b	9.31a

[1]MD=Moderate pH decline; RD=Rapid pH decline with little degradation; RD-Pos=Rapid pH decline with degradation.
[ab]Means within a column with the same letters are not significantly different ($P < 0.05$).