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MUSCLE PROTEIN CHANGES POSTMORTEM IN RELATION TO PORK QUALITY TRAITS

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ABSTRACT

Eight-four pork loins, representing four quality classes of pork [PSE (pale, soft, exudative), RSE (reddish-pink, soft, exudative), RFN (reddish-pink, firm, non-exudative) and DFD (dark, firm, dry)] were used to investigate postmortem changes in muscle proteins in relation to their varying quality traits. Protein solubility measurements (sarcoplasmic and myofibrillar) were lower ($P<0.05$) and myosin denaturation (quantified by myofibrillar ATPase activity) was higher ($P<0.05$) for PSE samples compared to samples from the other quality classes. RSE samples were similar ($P>0.05$) to RFN samples in protein solubility and myosin denaturation although RSE had lower ($P<0.05$) values then DFD samples for protein solubility measurements. RFN samples had lower ($P<0.05$) drip and thaw water loss then RSE samples and all water loss traits were lowest ($P<0.05$) for DFD samples. The concentration of the sarcoplasmic protein phosphorylase which was bound to the myofibril was the only measurement of protein denaturation that differentiated among PSE, RSE and RFN samples. Samples subjected to SDS-PAGE showed evidence that for PSE and RSE samples, the myofibrillar protein titin was less degraded and nebulin was more degraded in the first 24 hr postmortem, compared to RFN samples. In conclusion, although RSE samples have unacceptably high water loss, muscle protein denaturation was minimal and would not influence functionality for processed meats.

INTRODUCTION

It is well known that pork which is described as pale, soft and exudative (PSE), has high drip loss, a pale unstable color and exhibits denatured muscle proteins. The water-holding capacity (WHC) of pork has been reported to be influenced by a number of factors including ultimate pH (pH_u), protein denaturation, intra- and interfascicular spacing and sarcomere length (Offer and Knight, 1988). Degradation of structural proteins postmortem is potentially important in determining water-holding capacity (WHC) and the swelling of the myofilament lattice during extraction and solubilization of myofibrillar proteins (Offer and Trinnick, 1983). It is not clear if some or all of these factors are independent of each other and thus additive. The occurrence of pork which is acceptable in color but has excessive exudation has recently been described and given the name RSE (Kauffman *et al.*, 1992) but the biochemical and physio-chemical traits of pork of this quality have not been described, nor even recognized by many meat scientists. In attempting to understand the basic mechanisms of drip loss from pork, a fundamental question is what is the contribution, if any, that protein denaturation makes to drip loss and surface color? An experiment was designed to investigate muscle protein denaturation and degradation in pork samples in relation to pork quality traits.

MATERIALS AND METHODS

Eighty-four boneless pork loins from a commercial pork plant were selected at 24 hr postmortem, on 14 different occasions, to equally represent four quality conditions. The loins were transported in vacuum-sealed bags and measurements of physical, biochemical and water loss traits were conducted in the laboratory. Physical measurements of meat quality included color (L*- surface lightness), drip loss, pH_u and water loss during thawing and cooking. Measurements of pH_u , colour and drip were used to allocate samples to one of four quality classes; **Pale, Soft, Exudative** (PSE): $L^* > 50$, drip > 5%, **Reddish-pink, Soft, Exudative** (RSE): $L^* = 42\text{-}50$, drip > 5%, **Reddish-pink, Firm, Non-exudative** (RFN): $L^* = 42\text{-}50$, drip < 5% and $pH_u < 6.0$ and **Dark, Firm, Dry** (DFD): $L^* < 42$, drip < 5% and $pH_u \geq 6.0$. Biochemical measurements of protein denaturation included the solubility of the myofibrillar and sarcoplasmic proteins, myofibrillar ATPase activity and the binding of the sarcoplasmic protein phosphorylase to the myofibril. Extractability of myofibrillar proteins was determined by subjecting samples to SDS-PAGE electrophoresis after solubilization. The amount of phosphorylase adhering to the myofibril was confirmed using SDS-PAGE and densitometry. The degradation of myofibrillar proteins in the first 24 hr postmortem was determined using SDS-PAGE and Western Blot analysis. Data was anlayzed by analysis of variance and mean separation was achieved using the least squares means test.

RESULTS

Differences among quality classes in physical and biochemical traits are shown in Table 1. Physical measurements of pH_u, surface lightness and drip loss were used to allocate samples according to quality classes and thus, by design, the mean values of all traits (except L* between RSE and RFN) were significantly different ($P<0.05$) among quality classes. PSE samples had the lowest pH_u, lightest surface (high L*) and highest drip ($P<0.05$ for all). DFD samples were darker ($P<0.05$) and had lower drip ($P<0.05$) and higher pH_u ($P<0.05$) then the other samples. In contrast, PSE and RSE samples lost similar ($P>0.05$) amounts of fluid during thawing and cooking and DFD lost less water ($P<0.05$) then all other samples.

Protein denaturation - PSE samples exhibited lower ($P<0.05$) protein solubility for both protein fractions (sarcoplasmic and myofibrillar) compared to RSE, RFN and DFD although the relative differences were smaller among classes for sarcoplasmic protein solubility. RSE and RFN samples had similar protein solubilities for both extraction methods and RFN and DFD samples were also similar ($P>0.05$ for all). RSE samples had lower ($P<0.05$) protein solubility for all three methods when compared to DFD samples. The calcium-activated myofibrillar ATPase activity was lower ($P<0.05$) for PSE samples but the activity was similar ($P>0.05$) among RSE, RFN and DFD samples. PSE samples had the highest ($P<0.05$) amount of phosphorylase bound to the myofibril compared to the other three quality classes. RSE samples had more ($P<0.05$) bound phosphorylase then RFN and DFD samples. DFD and RFN samples had a similar ($P>0.05$) and low amount of bound phosphorylase.

Table 1: Meat quality measurements (least squares means ± standard errors) on loin samples derived from four quality classes

Quality Class	N	pH_u	Lightness (L*)	Drip Loss (%)	Thaw Loss[1] (%)	Cook Loss[2] (%)	ATPase Activity (umol Pi/mg/min)	Protein Solubility Sarcoplasmic (mg/g)	Protein Solubility Myofibrillar (mg/g)	Bound Phosph. (ng)
PSE	26	5.30±0.04^{a}	55.5±0.6^{a}	9.6±0.3^{a}	10.9±0.6^{a}	29.8±0.9^{a}	0.064±0.018^{a}	50.4 ±2.0^{a}	54.2±7.0^{a}	42.7±3.6^{a}
RSE	19	5.44±0.04^{b}	47.3±0.7^{b}	7.2±0.4^{b}	10.3±0.7^{a}	27.4±1.0^{ab}	0.287±0.021^{b}	65.8±2.3^{b}	97.6±8.6^{b}	25.8±4.1^{b}
RFN	19	5.59±0.04^{c}	45.5±0.7^{b}	3.4±0.4^{c}	8.2±0.7^{b}	25.3±1.0^{b}	0.273±0.021^{b}	70.5±2.3^{bc}	119.0±7.8^{bc}	10.8±4.6^{c}
DFD	20	6.29±0.04^{d}	38.3±0.7^{c}	1.3±0.4^{d}	3.1±0.6^{c}	16.1±0.9^{c}	0.270±0.021^{b}	75.7±2.3^{c}	123.8±8.1^{c}	6.4±4.3^{c}

[1] Thaw loss = difference between pre-thawed weight and pre-cooked weight expressed as a percent of pre-thawed weight.
[2] Cook loss = difference between pre- and post-cooked weight expressed as a percent of pre-cooked weight.
[abcd] Within columns, means with different superscripts are significantly different (P<0.05).

Protein degradation- PSE and RSE samples showed evidence of breakdown of nebulin (results not presented), as indicated by a fainter nebulin band and more protein bands immediately below the nebulin band, in both the gel and the blot. In contrast, RFN and DFD samples showed no evidence of nebulin breakdown as the nebulin band appears as a singlet, with strong staining intensity. In addition, RFN and DFD samples showed evidence of titin breakdown whereas PSE samples showed no evidence of titin breakdown and RSE samples show little evidence. This was indicated by the presence of a singlet band for titin for PSE and RSE samples and a doublet for RFN and DFD samples.

DISCUSSION

RSE is a quality class which has only recently been described (Kauffman *et al.*, 1992) although the existence of pork which is acceptable in color but has high drip loss has been previously reported (Warriss and Brown, 1987). Our study shows that compared to RFN samples, RSE samples had similar protein solubility and myosin denaturation characteristics but exhibited increased binding of the sarcoplasmic protein phosphorylase bound to the myofibril. For RSE and PSE samples, the high molecular weight protein titin was less degraded in RSE samples, and nebulin was more degraded, compared to RFN samples. Reduced degradation of titin in PSE samples has previously been reported by Boles *et al.* (1992) but differences in nebulin breakdown between quality classes of pork have not been reported. Monin and Laborde (1985) suggested that precipitation of sarcoplasmic proteins may cause the increased drip in PSE pork. Our results show that for RSE samples, precipitation of phosphorylase onto the myofibril is associated with samples which have an unacceptably high drip loss, but have no observable effect on surface lightness. The mole ratio of phosphorylase to myosin is calculated to be 1:30 for RSE samples. It is not clear whether precipitation of phosphorylase onto the myofibril is a causative factor in the increased drip loss in RSE samples or simply an indicator of pre-rigor conditions causing some denaturation of the most sensitive sarcoplasmic proteins. In summary, RSE samples were intermediate in drip and pH_u between RFN and PSE and similar in color to RFN. RSE samples were not different in myosin denaturation, extractability of myofibrillar proteins or extractability of sarcoplasmic proteins from RFN samples. RSE had a higher proportion of the sarcoplasmic protein phosphorylase remaining with the myofibril fraction after extensive homogenizing, washing and centrifugation then RFN samples. This implies that pre-rigor conditions in RSE muscle caused precipitation of the sarcoplasmic proteins which are most sensitive to pH/temperature conditions existing immediately postmortem. However, the pre-rigor conditions in RSE samples did not cause extensive denaturation of myofibrillar or sarcoplasmic proteins. Thus as RSE pork exhibits normal protein solubility, it would be an acceptable product in terms of its protein functionality in processed meats, where extraction of proteins is important. But RSE pork has unacceptably high losses during storage and cooking. The only practical measurement that differentiated RSE from RFN was pH_u but the differences were too small to be used reliably under commercial conditions.

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