RESEARCH REPORT

PORK QUALITY

Title: Post –Mortem Processes in RSE Pork **– NPB #98-167**

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Abstract

RSE (Red, Soft, Exudative) pork poses a major problem: it is difficult to identify, and it=s causes are unknown. At an estimated incidence of 30%, costs associated with extra weight loss (due to increased purge) of RSE pork are more than $ 40 million per yr. Thus, elimination of RSE would result in significant economic savings. In this study, the hypothesis that RSE is a mild form of PSE (Pale, Soft, Exudative), and the hypothesis that sarcoplasmic proteins denature faster (under the mild post-mortem conditions in RSE) than myofibrillar proteins were tested.

A total of 61 samples, 25 RFN (Red, Firm, Non-exudative), 22 RSE and 14 PSE samples, were analyzed. Measures of post-mortem metabolism, R-value and pH-decline, and protein solubility, a measure of protein denaturation, were assessed at various times post mortem. R- and pH-values indicate that, compared to RFN muscle, the post-mortem glycolysis in RSE muscle is accelerated but glycolysis is not as fast as in PSE muscle. Thus, RSE is a mild form of PSE. The increased rate of glycolysis in RSE muscle did not affect protein denaturation. Protein denaturation in RSE and RFN pork was similar. Protein solubility in PSE pork was significantly lower than in RFN and RSE pork.

The increased rate of glycolysis in RSE muscle may be a result of a more >white= fiber type. When selection pigs for faster growth and increased leanness a selection for the faster glycolyzing white fiber type occurs. As it is not known at what critical post- mortem time changes in muscle result in reduction of the water holding capacity, it is not clear if increased chilling rates could reduce or prevent the occurrence of RSE. As RSE pork has a higher glycolytic potential (indicating higher glycogen levels in live pig) than RFN pork, feed withdrawal might be used to reduce the incidence of RSE. However, results of the effect of feed withdrawal are highly variable and prolonged feed withdrawal is associated with reduced carcass weight.

Further research is necessary to determine the cause of the accelerated glycolysis in RSE muscle and to determine the process of water expulsion from RSE muscle.

Introduction

Pork has traditionally been grouped in one of three quality categories: PSE (Pale, Soft, Exudative), normal (also RFN=Red, Firm and Non-exudative) and DFD (Dark, Firm, Dry). A few years ago, a fourth category, RSE (Red, Soft, Exudative) pork, was recognized (Kauffman et al., 1992). RSE pork is red like RFN pork but exudative (low WHC) like PSE pork. RSE pork poses several problems for the industry. Industry surveys indicate that the incidence of RSE is 30% (Kauffman et al., 1992; Kauffman, 1996). Costs associated with extra weight loss, due to increased purge resulting from the low water holding capacity, are more than $ 40 million per year. Furthermore, RSE is difficult to identify, preventing effective sorting of quality.

The cause of RSE remains unknown. Warner et al. (1997) suggested that RSE is a mild form of PSE. PSE quality is the result of a rapid post-mortem glycolysis, resulting in extensive protein denaturation, pale color and low WHC (high drip losses). WHC or RSE is lower than WHC of RFN, but slightly higher than WHC of PSE pork. Also, RSE is slightly paler (but still red) than RFN pork. Thus, RSE quality might be the result of a slightly increased rate of glycolysis, resulting in denaturation of some proteins. Cheah et al. (1997) also suggested that RSE is result of an increased rate of post-mortem glycolysis. They suggested that RSE is associated with a genetic defect in membrane permeability causing an increased rate of post-mortem glycolysis. No data are available to support these hypotheses.

Offer and Knight (1988) concluded that a reduction in myofilament spacing, resulting from denaturation of myofibrillar proteins (particularly myosin), is the primary cause for expulsion of water (drip loss) from muscle. This conclusion is based on results obtained from PSE muscle in which the low WHC is strongly associated with myofibrillar protein denaturation. Myofibrillar protein denaturation of RSE meat is comparable to that of RFN meat. The only difference between normal and RSE meat is that denaturation of the sarcoplasmic proteins phosphorylase and creatine kinase is enhanced in RSE pork (Warner, 1994). Since sarcoplasmic proteins are not part of the myofibrillar structure which holds water in the muscle, they are unlikely to have any effect on WHC.

Recently, we determined that denaturation of sarcoplasmic proteins does result in a reduction of myofibrillar WHC (Wilson and van Laack, 1999). This finding shed some light on the mechanism of the low WHC in RSE meat. To find the cause of RSE we still need to determine why and when these sarcoplasmic proteins denature. If sarcoplasmic proteins denature under milder conditions than myofibrillar proteins then it is plausible that RSE is associated with a mildly accelerated post-mortem glycolysis.

Objectives

The objective of the present study was to test two hypotheses:

1. RSE is a mild version of PSE, i.e. the post-mortem glycolysis is intermediate of the glycolysis in PSE and RFN.
2. Sarcoplasmic proteins denature under milder conditions than myofibrillar proteins.

Procedures

Post-mortem processes that result in RSE quality were determined and compared to those that result in PSE and RFN quality. Furthermore, denaturation of the sarcoplasmic and myofibrillar proteins during the post-mortem pre-rigor process was evaluated. At time of slaughter it is not known what the quality of meat will be at 18 hr post-mortem (well into

rigor). Furthermore, incidence of PSE, RSE, RFN and DFD quality varies daily. Thus, it is not possible to select specific carcasses that will yield a certain quality. We evaluated post-mortem metabolism and protein denaturation in a large number of carcasses, and, after assessment of the quality of each carcass, assigned samples to the three quality categories.

Sample collection

The experiment was conducted at a commercial slaughter plant. The study consisted of three identical trials, each involving 50 randomly chosen carcasses. The left loin of each of these carcasses was used for analysis of post-mortem metabolism and protein denaturation during the first 18 hr post mortem. Rigor onset in RFN pork occurs between 3 and 5 hr post mortem (pm). In PSE pigs, rigor onset may occur as early as 15 min post mortem. Consequently, it was important to assess metabolic processes in the first few hrs after slaughter. At approx. 20, 40 and 90 min, and at 4 and 18 hr p, a 20 g sample was cut (with a coring device) from the longissimus lumborum (2 inches caudal and cranial to 10th costae). After, removal of external fat, the sample was put in a 50 mL tube, minced with surgical scissors, and frozen in liquid nitrogen. Subsequently, the samples were stored in dry ice for transport to the University of Tennessee, Knoxville.

At 18 hr pm, the right loin was excised, meat quality characteristics were assessed. Based upon color and WHC indicators (drip loss and filter paper wetness), loins were assigned to one of the 4 quality categories (PSE, RSE, RFN and DFD). DFD samples were excluded from further analysis because they were not needed to test the hypotheses.

After three trials, we had obtained 22 RSE samples. To keep the groups of RSE and RFN samples more or less equal in size (to prevent distorting the results) 25 RFN samples were included. In addition, 14 PSE samples were collected. These samples were subjected to the described tests.

Meat quality characteristics

Meat color (L*, a*, b*) and WHC (filter paper wetness and drip loss) were assessed to determine to which quality category each carcass belonged. Procedures were as described by Warner (1994).

Post-mortem metabolism

To test the hypothesis that RSE is a mild form of PSE, and that post-mortem glycolysis in RSE muscle is intermediate to that in RFN and PSE muscle, post-mortem metabolism was evaluated. R-value and pH are measures of post-mortem glycolysis. pH was measured using a portable pH meter (Orion, Model 250A with a Mettler-Toledo combination pH electrode). At 90 min pm, the carcasses were in a rapid chiller. At the low temperatures prevailing in this chiller, electronic equipment does not work. Consequently, pH could not be measured at that time.

R-value is a measure related to ATP (adenosine tri-phosphate) breakdown; a high R-value indicates a low level of ATP. R-value was assessed on the frozen muscle samples. These samples were pulverized mechanically with a hammer. The powdered sample was transferred to a plastic bag and stored at -70□C until analysis. R-value was assessed using the procedure described by Honikel and Fischer (1977).

Protein denaturation

To test the hypothesis that sarcoplasmic proteins denature under milder conditions than

myofibrillar proteins, protein solubility at various times post mortem was evaluated.

Two g of each pulverized sample was thawed in cold >rigor buffer= (75 mM KCl, K-phosphate, 2 MM EGTA, 2 mM $MgCl_2$, pH 7.2). The EGTA in this buffer sequestered Ca^{+2} preventing further ATP breakdown and lactate formation. In addition, the K-phosphate buffer prevented any pH changes. Two indicators of protein denaturation, protein solubility and the presence of phosphorylase in the myofibrillar fraction were measured.

Protein solubility

After overnight incubation in the rigor buffer, the samples were homogenized with a Polytron homogenizer and centrifuged. The supernatant contains the sarcoplasmic proteins. Protein concentration in the supernatant was the measure for sarcoplasmic protein solubility. A low solubility indicates denaturation.

The myofibrillar pellet was washed four times, i.e. resuspended in rigor buffer and centrifuged. After the last wash, myofibrils were resuspended in 0.0625 M Tris buffer and protein concentration assessed with the Biuret procedure (Gornall et al., 1949). This suspension was prepared for measurement of phosphorylase in the myofibrillar fraction (see below), and for assessment of myofibrillar protein solubility.

Based upon the protein concentration of the suspension, the volume needed to obtain 30 mg protein was calculated. This amount was transferred to a 50 mL tube and centrifuged. The supernatant was discarded and the pellet was resuspended in 7.5 mL 0.5 M K-phosphate/0.55 M KI pH=7.4 buffer. This buffer solubilizes myofibrillar proteins. Denatured proteins will not be solubilized. After overnight incubation at 0-2□C, a sample was taken to determine protein concentration in the suspension (this should be 4 mg/mL, but because of various steps such as centrifugation etc. this concentration might be somewhat different). Then, the suspension was centrifuged and the supernatant filtered over a Whatman No 1 filter. Protein concentration of the supernatant is a measure of myofibrillar protein denaturation. To correct for possible variation in total protein concentration, the ratio of protein concentration in supernatant vs total protein concentration of the suspension was calculated. Lower ratios indicate increased denaturation.

Phosphorylase denaturation

Phosphorylase is a sarcoplasmic protein that becomes insoluble (=ends up in the myofibrillar fraction) upon denaturation. Higher levels of phosphorylase indicate denaturation of sarcoplasmic proteins (Warner, 1994). Presence of phosphorylase in the myofibrillar fraction is determined via electrophoresis.

After assessment of the protein concentration of the myofibrillar suspension, protein concentration of 1 mL of this suspension was adjusted to 4 mg/mL with 0.0625 M Tris. This diluted sample was then mixed with equal volumes of 2x sample buffer (12.5% 0.5 M Tris, pH 6.8, 4% SDS, 20% glycerol, 0.02% bromophenol blue solution, 10% mercaptoethanol), heated for 10 min at 90□C and stored at -20□C. These samples were analyzed by electrophoresis on a 12.5% polyacrylamide gel. With this method proteins are separated based upon molecular weight. A standard sample with proteins of known molecular weight was run simultaneously. Thus, the various proteins on the gel could be identified. After staining, the gels were scanned with a laser scanner to determine relative concentration (staining intensity) of the various proteins. The ratio of the proteins phosphorylase vs actin was calculated. To assure that differences in staining intensity were

not due to differences in amount of samples loaded, or variation in staining procedure, actin was used as a reference. Actin is a myofibrillar protein that does not denature or change under post-mortem conditions in muscle.

Statistical analysis

To determine significance of differences between quality categories, data were subjected to ANOVA using the GLM procedures, with quality by replication as the error term (SAS, 1996).

Results and Discussion

Quality groups

Meat quality characteristics of the three categories are presented in Table 1. By definition, the color L*-values of PSE meat will be higher than of RFN and RSE pork. Both RFN and RSE are red. In the present study, L*-value of RSE pork was significantly higher than of RFN pork. Other researchers have observed similar differences in L*-value of RSE and RFN pork (Kauffman et al., 1993; Warner, 1994; Joo et al., 1995; van Laack and Kauffman, 1999).

Filter paper wetness (FPW) and drip loss, measures of WHC, were significantly higher in RSE pork than in RFN pork. This was expected because by definition, WHC of RFN is higher than of RSE. In this study, WHC of RSE pork was higher (lower FPW and drip loss) than WHC of PSE pork. This indicates that RSE pork was exudative but its WHC was not as low as that of PSE pork.

Post-mortem metabolism

R- and pH-values for each quality category are presented in Table 2. At 40 min post mortem, the pH of PSE loins was < 6.00. A pH of 5.90-6.00 at 40 min post mortem is often used to select carcasses that will yield PSE (Bendall and Swatland, 1988). At around pH 5.9, rigor onset occurs. Before slaughter, pH of muscle is around 7.2, the physiological pH. Thus, in PSE carcasses the pH declines from 7.2 to 6.0 in less than 40 min. Such a rapid pH decline will result in protein denaturation which then results in loss of WHC and pale color (Offer and Knight, 1988; Offer, 1990). Indeed sarcoplasmic protein solubility, of PSE samples was lower than protein solubility of RFN samples (a lower solubility indicates more denaturation; Table 2). The pH decline in RSE carcasses was slower than in PSE carcasses, but faster than in carcasses that yielded RFN pork, suggesting that RSE is intermediate of PSE and RFN. In earlier research by Kauffman et al. (1993), pH at 45 min post mortem was also slightly (but not significantly) lower in RSE carcasses than in RFN carcasses.

In addition to pH, R-value was assessed. The R-value is a measure of ATP breakdown; higher R-values indicate lower levels of ATP. At rigor onset the R-value is $\geq$ 1.00. R-value correlated with pH values (Table 2); muscle with a low pH had a higher R-value than muscle with a high pH. At 40 min pm, R-value of PSE carcasses was 0.97, i.e. the muscle was close to rigor onset. Rigor onset (R-value >1.00) between 40 and 90 min pm resulted in RSE, and rigor onset between 90 min and 4 hr pm yielded >normal=, i.e. RFN, quality meat.

As observed by others (Warner, 1994; van Laack and Kauffman, 1999), the pHu of RSE pork was lower than pHu of RFN meat, but higher than pHu of PSE pork. These differences were not significant.

Protein denaturation

The procedure to measure myofibrillar protein solubility used was different from the >traditional= method. The modified procedure was more difficult to standardize and less sensitive compared to the traditional procedure; small differences in solubility were not detectable (results not shown).

Sarcoplasmic protein solubility (denaturation) of RSE and RFN pork was similar (Table 2), and did not change during first 18 hr post mortem. Possibly, sarcoplasmic solubility measurements are not sensitive enough to assess mild denaturation. Sarcoplasmic proteins are globular proteins which become insoluble only after severe denaturation. Warner (1994) did not find a difference between sarcoplasmic solubility of RSE and RFN either. She found that the increased denaturation of the sarcoplasmic protein phosphorylase in RSE vs RFN could be detected via electrophoretic analysis of the myofibrillar fraction; denaturation of phosphorylase will result in presence of phosphorylase in the myofibrillar fraction.

In an effort to determine under what conditions the sarcoplasmic proteins denature, the presence of phosphorylase in the myofibrillar fraction was monitored over time (Table 3). As can be seen, the results varied considerable and there was no detectable difference in the amount of denatured phosphorylase in RSE vs RFN, or PSE vs RFN. It has been shown repeatedly (Fischer et al., 1979; Greaser et al., unpublished results; Warner, 1994) that phosphorylase denaturation is significantly higher in PSE pork than in RFN pork. The lack of a difference between phosphorylase in RFN vs PSE in the present study suggests that the method used may not have been sensitive or accurate enough.

The results concerning post-mortem glycolysis in RSE vs RFN and RSE vs PSE pork strongly suggest that RSE is associated with a moderately fast post-mortem glycolysis. The reason for this increased rate of glycolysis in RSE carcasses still needs to be determined. A possible hypothesis is that RSE quality results from moderate stress before slaughter. However, if pre-slaughter stress were the cause of RSE, one would expect a strong seasonal variation in the incidence of RSE, i.e. more RSE in the summer when pigs are more easily stressed than in the winter. Such a seasonal variation has not been established.

A more likely cause for the faster glycolysis in RSE vs RFN muscle would be a difference in fiber type between RFN and RSE muscle. Muscle of a >whiter= type has a higher ATP-ase activity (breaks down ATP faster; Solomon et al., 1998) and thus will have a faster glycolysis. Leaner, faster growing animals, tend to have a whiter muscle. In addition to the higher ATP-ase activity and the lower fat content, the whiter muscle has a higher glycolytic potential than red muscle (Solomon et al., 1998). The results by van Laack and Kauffman (1999) and the observation that pHu of RSE pork is lower than pHu of RFN pork (Kauffman et al., 1993; Warner, 1994; van Laack and Kauffman, 1999) indicate that glycolytic potential of RSE pork is higher than of RFN pork, and thus suggest a difference in fiber type of RSE vs RFN pork. Huff-Lonergan et al. (1999), reported that meat from pigs selected for leanness had a lower pHu and developed RSE characteristics. Combined these results suggest that the occurrence of RSE pork is a consequence of the selection for leanness possibly related to a change in muscle fiber type.

The question remains if and how RSE pork can be prevented. It is evident that post-mortem glycolysis is faster in RSE pork than in RFN pork, and this may be the actual cause for the low WHC of RSE pork. Since it is not known when the protein denaturation

responsible for the low WHC of RSE pork occurs, it remains unclear if a slow down of glycolysis, for instance by faster chilling, would result in an improvement of pork quality.

The ultimate pH of RSE pork is somewhat lower than of RFN pork. Possibly an increase in pHu prevents development of RSE quality. An increase in pHu may be achieved by a reduction in glycolytic potential through extended fasting before slaughter. All animals in the present study were fasted for 12 hrs. Longer fasting is disadvantageous because it will result in weight loss. Furthermore, as reported by Ellis and McKeith (1999), the effect of fasting on pHu is highly variable.

Conclusions

The results of this study indicate that RSE is a mild form of PSE. Post-mortem rate of glycolysis in RSE muscle is intermediate to that in RFN and PSE muscle. Slowing of post-mortem glycolysis may be an effective means to prevent RSE quality.

The hypothesis that sarcoplasmic proteins denature under milder conditions than myofibrillar proteins could not be tested. More sensitive methods to measure sarcoplasmic protein denaturation in pre-rigor muscle are needed.

Future research

Intermediate rate of post-mortem glycolysis is the cause of RSE. Factors (other than those already known such as stress and temperature) that influence rate of glycolysis need to be determined. Cheah et al. (1997) suggested that RSE occurrence is related to a genetic defect. One of the factors that influences rate of glycolysis is membrane permeability, determined by membrane composition (Stanley, 1991). In recent studies, we (van Laack and Spencer, 1997) assessed that there is a genetic difference in the membrane composition of muscle from 7 halothane-negative pig lines. These differences may influence rate of glycolysis. As membrane composition is influenced by nutrition as well, there may be possibilities to influence WHC of pork by nutrition. Fiber type also influences rate of glycolysis. The possible relationship between fiber type and incidence of RSE needs to be studied.

Although myosin denaturation seems the primary cause of low WHC of meat, it is not yet clear how water is expelled from the muscle. Purslow (personal communication) suggested that interfilamental linkages are involved in the process of water transfer from interfilamental spaces to extracellular spaces. In RSE pork these linkages were >stronger= than in RFN pork (Mielche and Purslow, 1999), causing more water expulsion in RSE than in RFN pork. We are in the process of evaluating the integrity of desmin in RSE, PSE, and RFN pork at various times post mortem. Results of this analysis will be reported as soon as they are available.

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Table 1: Meat quality characteristics of RFN, RSE, and PSE pork longissimus muscle.

	RFN (n=25)	RSE (n=22)	PSE (n=14)
L*-value	50.7a*	52.8b	58.3c
Filter paper wetness (mg)	33a	91b	136c
Drip loss (%)	4.0a	6.5b	8.5c

*Within rows, means lacking a common superscript are significantly different ($p<0.05$).

Table 2: Post-mortem processes in RFN, RSE, and PSE pork longissimus muscle.

		RFN (n=25)	RSE (n=22)	PSE (n=14)
pH	20 min pm	6.61a	6.30b	6.15b
	40 min pm	6.48a	6.16b	5.95c
	4 hr pm	6.04a	5.68b	5.53c
	18 hr pm	5.63a	5.55ab	5.49b
R-value	20 min pm	0.86	0.90	0.91
	40 min pm	0.85a	0.91b	0.97c
	90 min pm	0.96a	1.09b	1.18c
	4 hr pm	1.09a	1.22b	1.27b
	18 hr pm	1.28a	1.35b	1.34b
Sarcoplasmic protein solubility (mg/g muscle)		77a	75a	69b

*Within rows, means lacking a common superscript are significantly different ($p<0.05$).

Table 3: Phoshorylase:actin ratio in myofibrils from RFN, RSE and PSE pork longissimus muscle at various times post mortem.

Time post mortem	RFN (n=25)	RSE (N=22)	PSE (n=14)
20 min	0.10	0.08	0.14
40 min	0.15	0.20	0.13
90 min	0.08	0.11	0.18
4 hr	0.11	0.10	0.11
18hr	0.11	0.11	0.12