CALPAIN ACTIVITY AND PROTEIN DEGRADATION OF RED, FIRM AND NON-EXUDATIVE AND RED SOFT AND EXUDATIVE PORK LOINS

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I. INTRODUCTION

Lesser-known and understood, red, soft, and exudative (RSE) lean pork has the color of red, firm and non-exudative (RFN) pork, but possesses pale, soft, and exudative meat texture and water holding capacity. This condition was first described in the literature by Warris and Brown [1]. Warner et al. [2] reported RSE meat possessed divergent 24-hour postmortem myofibrillar protein denaturation patterns compared to RFN meat; however, denaturation patterns during extended aging time remains unknown. The objective of this study was to determine differences in myofibrillar protein degradation patterns of pork categorized as RFN or RSE during 14 days of aging.

II. MATERIALS AND METHODS

Twenty-four hours postmortem, pork loins were visually classified as RFN (n = 25) and RSE (n = 25). Subjective and objective color, subjective firmness, and pH were collected and the ventral portion of the *longissimus dorsi* muscle was gathered and shipped to the Muscle Biology/Meat Science Laboratory at the University of Georgia. Loins were fabricated into nine 1.27-cm chops and three-chop groups were randomly assigned to a day of postmortem aging (DPA; day 2, 7, or 14). At each DPA, samples were finely minced and sub-samples were collected for casein zymography and Western-Blotting.

The methods of Phelps et al. [3] were followed for casein zymography and myofibrillar protein degradation. Extracted calpains were separated on 12.5% SDS-casein PAGE gels, incubated in a calcium-rich buffer, visualized with Coomassie brilliant blue stain, and band size (amount degraded by the enzymes) was quantified by densitometry. Total band density was normalized to a pooled sample consisting of samples from the current study. Protein was separated via SDS-PAGE, transferred to nitrocellulose membranes, and intact and degraded desmin (DES) and troponin-T (TnT) were visualized using Western Blotting methodology and quantified using densitometry. All bands were normalized to the intact DES band from a 7-day aged beef sample.

Data were analysed as a completely randomized design with a 2 × 3 factorial arrangement with loin as the experimental unit. Fixed effects included meat category (CAT), DPA, and their interaction. Data were analysed using the Mixed procedure of SAS 9.4. Statistical significance was determined at $P < 0.05$ and tendencies at $0.05 < P < 0.10$.

III. RESULTS AND DISCUSSION

There were no CAT effects for all 24-hour loin color and pH measures ($P > 0.31$), indicating loins did not differ in subjective and objective color when selected for analyses (Table 1). Red, soft, and exudative loins had lower ($P < 0.01$) firmness scores (softer) than RFN loins.

Table 1 Twenty-four-hour pH and color measurements of loins categorized as RFN or RSE

Item	Red, firm, and non-exudative	Red, soft, and exudative	SEM	*P* Value
Day 0 pH	5.71	5.74	0.045	0.64
Firmness[1]	4.4	1.1	0.14	<0.01

Day 0 JCS color[2]	3.0	2.9	0.15	0.49
Day 0 NPB marbling[3]	2.1	1.9	0.14	0.31
L*	52.1	52.6	0.76	0.66
a*	15.3	15.5	0.31	0.66

[1]Firmness scale: 1 = very soft; 5 = very firm. [2]Japanese(JCS) color scale: 1 = pale pinkish grey to white, 6 = dark purplish red. [3]National Pork Board (NPB) marbling scale: 1 = 1% intramuscular fat content, 10 = 10% intramuscular fat content.

There were no CAT × DPA interaction or DPA main effect for calpain activity ($P > 0.22$). Over the three postmortem aging periods, RSE meat had more ($P = 0.03$) calpain activity compared to RFN meat (Table 2). There were no CAT × DPA interactions for intact and both degraded forms of DES and TnT ($P > 0.20$;). There were no CAT main effects for intact or the 38 kDa degraded form of DES ($P > 0.53$); however, RSE meat had more ($P = 0.04$) 45 kDa degraded DES than RFN meat over the three aging periods. While there were no DPA main effects for the two degraded forms of DES ($P > 0.19$), there was a DPA effect ($P < 0.01$) for intact DES. Day 2 meat had more intact DES than the other two aging periods ($P < 0.01$), that did not differ ($P = 0.14$) from each other.

There were no CAT main effects for intact and 36 kDa degraded TnT ($P > 0.22$); however, RSE meat tended to have more ($P = 0.06$) 34 kDa degraded TnT and more ($P = 0.03$) 30 kDa degraded TnT. There were DPA main effects for all forms of TnT ($P < 0.01$). All three postmortem aging periods for all forms of TnT differed from each other, with intact and 36 kDa degraded TnT decreasing over the three aging periods, while 34 and 30 kDa degraded TnT increased ($P < 0.04$).

Table 2 Category main effect means of calpain activity and desmin and troponin-T degradation products from loins categorized as red, firm, and non-exudative (RFN) or red, soft, and exudative (RSE)

				P Value[1]		
Item[2]	RFN	RSE	SEM	CAT	DPA	CAT × DPA
Calpain activity	89.1	100.0	6.3	0.03	0.23	0.22
Desmin						
Intact	3.1	3.0	0.56	0.79	<0.01	0.55
45 kDa degraded	0.4	0.6	0.14	0.04	0.19	0.78
38 kDa degraded	0.4	0.5	0.12	0.53	0.41	0.20
Troponin-T						
Intact	6.4	6.3	0.61	0.79	<0.01	0.87
36 kDa degraded	4.3	4.8	0.58	0.22	<0.01	0.33
34 kDa degraded	2.0	2.4	0.27	0.06	<0.01	0.73
30 kDa degraded	2.1	2.5	0.26	0.03	<0.01	0.50

[1]CAT; loin visual category. DPA; days of postmortem aging. [2]Percent normalized value. Calpain bands normalized to a pooled sample consisting of samples from the current study. Desmin and troponin-T bands normalized to the intact desmin band of a 7-day aged beef longissimus lumborum sample.

IV. CONCLUSION

Data indicated RSE loins had greater calpain activity and desmin and troponin-T degradation than RFN loins, which may contribute to the soft texture and exudative nature of these loins.

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