EFFECT OF HIGH POST-MORTEM TEMPERATURE ON ANTIOXIDANT ENZYME ACTIVITIES AND MEAT QUALITY IN PORK

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Abstract – The objective of this study was to investigate the influence of high post-mortem (pm) temperature on the activity of antioxidant enzymes and meat quality in pork M. *Longissimus dorsi* (LD). Pork LD was taken from the left and right side of 15 pig carcasses at 30 min pm. The LD of one carcass side was kept at 40°C in an hot air oven for 4 hours and then moved to a chiller at 4°C until 24 hours pm (delayed chilling), whereas the other side was immediately placed in the same chiller (normal chilling). Delayed chilling resulted in PSE characteristics, heat shortening and lower protein solubility compared to normal chilling (P<0.001). The activities of catalase, superoxide dismutase and glutathione peroxidase at 24 hours after cutting were lower in the delayed chilling compared to the normal chilling treatment (P<0.05). Colour *L values were higher during 8 days of display (P<0.001) and *a** values were significantly lower from day 4 of display on (P<0.05) in the delayed compared to the normal chilling treatment. TBARS values were lower after 8 days of display in the delayed chilling treatment (P<0.01). It was concluded that induction of PSE characteristics by high pm temperature reduced the activity of antioxidant enzymes and colour stability but not lipid stability.**

Key Words – Catalase, Glutathione peroxidase, *longissimus dorsi*, Pork, Superoxide dismutase, Post-mortem temperature, PSE

- INTRODUCTION

The combination of high temperature and fast pH decline in muscle early post-mortem (pm) may lead to more protein denaturation compared to when a normal pattern of rigor mortis is present. This results in the occurrence of PSE meat, i.e. pale, soft and exudative meat. The causes and characteristics of PSE meat have been abundantly studied but the concomitant changes in oxidative stability upon further storage or display have been less well documented.

Meat oxidative deterioration leads to a loss of nutritional value and reduced sensory quality. Several mechanisms are present in muscle to protect against oxidation including the endogenous antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). In a previous study in beef, we found that the activities of CAT and GSH-Px were lower, whereas lipid oxidation and formation of metmyoglobin were higher in the deeper laying part of the M. *Biceps femoris* compared to the superficial part, which was associated with an increased degree of heat shortening and protein denaturation [1]. Protein denaturation resulting from severe heat shortening may affect the activity of these endogenous enzymes. Hence, it may be argued that heat shortening conditions result in a reduced activity of these enzymes and thereby negatively affect the oxidative stability of meat.

Installing different chilling regimes allows to induce different meat qualities, e.g. hot boning followed by rapid chilling may result in improved meat quality, whereas delayed chilling is expected to worsen meat quality. Therefore, the objective of this study was to investigate the influence of two chilling treatments, i.e. high temperature early pm (40°C for 4 hours and then 4°C until 24 hours) versus a normal chilling regime (4°C for 24 hours), on antioxidant enzyme activities and meat quality in pork M. *Longissimus dorsi* (LD) with emphasis on the meat oxidative stability.

- MATERIALS AND METHODS

Animals
A total of 15 pigs originating from commercial farms in Belgium were slaughtered according to common practices on 2 days in the same slaughterhouse.

Sampling
A piece of 30 cm long of the LD was cut from the left and right side of the carcasses within 30 min pm. The LD muscle of one carcass side was kept in an hot air oven at 40°C for 4 hours and then moved to the chilling room at 4°C until 24 hours pm (delayed chilling). The other carcass side was immediately placed in the chilling room at 4°C for 24 hours (normal chilling). The samples for analysis of enzyme activity were taken at 0, 4 and 24 hours after cutting the LD (30 min pm) and put in liquid nitrogen, then stored at -80°C until analysis. For the simulated retail display, meat samples were wrapped in oxygen permeable foil and displayed at 4°C under constant fluorescent light (approximately 1200 lux) for 8 days.

Measurements and analyses
The temperature and pH were measured at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 24 hours after cutting the LD (30 min pm). The duration of 'heat shortening' was calculated based on the definition of pH <6.0 and temperature >35°C [2]. The Pork Quality Meter (PQM) was used to measure the conductivity at 24 hours. Meat colour was measured with a Hunterlab Miniscan colour meter (D65 light source, 10° standard observer, 45°/0° geometry, 1-inch light surface, white standard) at 1, 2, 3, 4, 5, 6, 7 and 8 days of display. Lipid oxidation was assessed at day 8 of display by the thiobarbituric acid reactive substances (TBARS) method based on Tarladgis et al. [3]. Sarcoplasmic and myofibrillar protein solubility was measured on 24 hours samples according to Claeys et al. [4]. Determination of drip loss, thawing loss, and cooking loss was carried out as described by Uytterhaegen et al. [5].

Antioxidant enzyme activity assays
The SOD activity assay was performed as described by Marklund et al. [6] by measuring the inhibition of pyrogallol autoxidation. The activity of GSH-Px was determined by measuring the oxidation of NADPH according to Hernandez et al. [7]. The CAT activity was determined according to the method of Aebi [8].

Statistical analysis
Enzyme activities were analyzed using a model with the fixed effects of chilling treatment, sampling time, slaughter day, and their interaction terms. Meat quality variables were analyzed with a model including the fixed effects of chilling treatment, slaughter day and their interaction. For the colour a* values during display this model was extended with the repeated measures effect of day of display. The General Linear Model (GLM) procedure of SAS® Enterprise Guide®, version 4.3 (SAS Institute Inc., Cary, NC, USA) was used. Post hoc tests were performed at a significance level of $P<0.05$ using Tukey.

- RESULTS AND DISCUSSION

In Fig. 1 the pH versus temperature fall in pork LD is presented. The duration of heat shortening was estimated at 3.1 ± 0.67 hours in the delayed chilling treatment, whereas no heat shortening was observed in the normal chilling treatment.

Figure 1. Mean values (n = 15) for pH and temperature in pork LD. Error bars represent standard deviations. The marks from left to right correspond to the measurements at 0, 1, 2, 3, 4, 5, 6, 7, 8 and

24 hours after cutting the LD (30 min pm).

The mean values for meat quality traits according to the chilling treatments are shown in Table 1. PQM values were higher in the delayed chilling compared to the normal chilling treatment whereas protein solubility was lower (P<0.001). Meat in the delayed chilling treatment was much paler with a difference of 7 units in the L* value at day 1 of display (P<0.001), whereas there was no difference for the initial a* value. On the other hand, ultimate pH, drip loss, cooking loss and thawing loss were not significantly different between the two chilling treatments. The lower protein solubility and higher PQM values indicate that the high pm temperature increased protein denaturation and cell membrane disruption respectively, which was however accompanied by only a numerical increase in drip loss.

Table 1. Effect of delayed versus normal chilling on meat quality traits in pork LD (mean ± SD, n=15)

	Chilling regime		P-value
	Delayed	Normal	
PQM 24 h pm (µS)	16.07±1.30	6.63±2.88	<.001
pH 24 h pm	5.57±0.07	5.53±0.06	0.116
Drip loss (%)	5.68±1.04	5.13±1.29	0.188
Thawing loss (%)	8.88±1.45	8.83±2.13	0.932
Cooking loss (%)	25.10±1.01	25.48±1.94	0.493
Sarcoplasmic protein solubility[1]	62.28±4.07	74.11±2.43	<.001
Myofibrillar protein solubility[1]	11.90±0.89	14.93±0.53	<.001
*L** d 1	60.40±3.91	53.46±2.26	<.001
*a** d 1	9.11±1.18	9.11±1.11	0.943
TBARS d 8[2]	0.16±0.06	0.24±0.06	0.002

[1]mg/g meat; [2] µg malondialdehyde/g meat

Table 2 indicates that the enzyme activities were all influenced by chilling treatment (P<0.02), sampling time (P<0.001; except for GSH-Px P>0.05) and chilling treatment × time (P<0.02). For CAT and SOD, the activity measured at 24 hours after cutting in the delayed chilling treatment was significantly lower compared to the activity at earlier time points in this treatment and compared to the activities at all sampling times in the normal chilling treatment. For GSH-Px, the activity at 24 hours after cutting in the delayed chilling treatment was significantly lower than the activity at 4 and 24 hours after cutting in the normal chilling treatment. This illustrates that the higher temperature early pm and the heat shortening conditions in the delayed chilling treatment reduced the activity of CAT, SOD and GSH-Px. This is in accordance with our previous study in beef [2], in which we found lower CAT and GSH-Px activities in the deeper laying part of the M. *Biceps femoris* compared to the superficial part, associated with a slower temperature fall and increased protein denaturation in the inner muscle part.

Fig. 2 illustrates the effect of chilling treatment on the decrease in a* value during 8 days of display as a measure of colour stability. Day of display and chilling treatment × day of display were significant (P<0.001). From day 4 of display on, the a* values were lower in the delayed chilling compared to the normal chilling treatment (P<0.05), illustrating the faster pigment oxidation and lower colour stability in the delayed chilling treatment. Surprisingly, this was not associated with higher lipid oxidation. On the contrary, TBARS values after 8 days of display were lower in the delayed chilling treatment (P<0.002). In a previous study in beef [2], we had indications that higher lipid oxidation was associated with reduced activity of antioxidant enzymes. In the present study, this did not appear to be the case, but on the other hand reduced colour stability and antioxidant enzymes activity were apparently correlated when comparing the chilling treatments.

Table 2. Effect of delayed versus normal chilling on activities (Unit[1]) of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in pig LD at 0, 4 and 24 hours after cutting the LD (30 min pm) (mean ± SD, n = 15)

Chilling	Delayed			Normal			P-values		
Time	0	4	24	0	4	24	Chill	Time	C×T
CAT	2355±208[a]	2347±241[a]	1865±315[b]	2336±210[a]	2295±263[a]	2289±209[a]	0.022	<.001	<.001
SOD	21.7±5.4[b]	23.8±6.1[ab]	13.3±10.7[c]	20.5±2.7[b]	25.9±3.1[a]	23.6±3.4[ab]	<.001	<.001	<.001
GSH-Px	0.57±0.26[ab]	0.53±0.20[ab]	0.38±0.14[b]	0.52±0.18[ab]	0.60±0.20[a]	0.65±0.20[a]	0.018	0.611	0.016

[a, b, c] Within a row, mean values with different superscripts differ significantly at $P<0.05$. [1]One unit of SOD was defined as the amount of extract needed to inhibit the rate of oxidation by the control (no SOD) by 50%. One unit of GSH-Px activity was defined as the amount of extract needed to oxidise 1 µmol of NADPH per min at 25°C. One unit of CAT was defined as the amount of extract needed to decompose 1 µmol of H_2O_2 per min at room temperature.

Figure 2. Effect of delayed versus normal chilling on the colour *a** value in pork LD during display. From day 4 on the difference between normal and delayed chilling is significant.

More research on the contribution of the antioxidant enzymes to protection against colour oxidation is warranted. Metmyoglobin reducing activity was not assessed in the present study. It would therefore be interesting to evaluate the effect of delayed chilling on this parameter in relation to colour stability.

- CONCLUSION

Delayed chilling of pork LD induced protein denaturation and PSE characteristics, and reduced the activity of the antioxidant enzymes CAT, SOD, and GSH-Px at 24 hours pm. This was accompanied by a lower colour stability during subsequent cooled display, but lipid stability was not negatively affected.

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