IMPACT OF CHILLING RATE AND HALOTHANE GENOTYPE ON THE FREQUENCY OF PSE-LIKE ZONES AND THE PROCESSING YIELDS OF HAMS

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Abstract – The aim of this work was to further investigate the effects of chilling rate on the frequency of PSE-like zones on pork hams, following on from a previous study [1]. An 8°c difference in chilling rate (4 hours post-mortem) was applied to the opposite carcass sides, from halothane genotyped pigs. In order to increase the frequency of PSE-like zones, pigs were slaughtered after a limited fasting time and without resting. Biochemical analysis (glycogen potential, protein solubility) were performed on *Semimembranosus* samples to explore local specificities of PSE-like zones. As expected, the overall meat quality was low but the frequency of PSE-like zones was noticeably high (44%). Chilling rate did not have a significant effect on meat quality and the frequency of PSE-like zones, except for *Semimembranosus* pH4 and L*; it however had a reduced influence on processing yields. Under these altered slaughter conditions, halothane genotype (Nn vs NN) had a massive effect on the frequency of PSE-like zones (61% vs 15%, respectively).

Key Words – PSE-like zones, chilling rate, glycogenolysis.

I. INTRODUCTION

Among the most influencing factors on the frequency of PSE-like zone defects that have been investigated (transport and resting time before slaughter [2], halothane genotype [3]), carcass chilling rate seems to have a major effect [1]. The aim of this study was to follow investigations into the effect of chilling rate on the frequency of PSE-like zones, using a less biased protocol (chilling differences applied on the same carcass) and including the halothane genotype mutation effect. To make the experiment design statistically stronger, comparisons were studied on a deliberately increased PSE-like zone frequency population, applying degraded slaughter conditions. In order to improve biochemical knowledge about the defect, a particular focus was applied to the level of protein degradation and the regulation of glycolytic metabolism.

II. MATERIALS AND METHODS

A balanced mix of 266 pigs from Large White x Piétrain sire and Piétrain sire were slaughtered in four groups (one breeding farm/sire per day), straight from transportation without resting and after a limited fasting period (18h), in order to increase the frequency of PSE-like zones. After classification, the right and left sides of the carcasses were split. The left sides went directly into the equalization chill room (5°), whereas the right sides followed classical tunnel chilling (1-2°, 195 min. with fast air velocity) before the chill room. The pH and the core temperature were measured on the *Semimembranosus* muscle using a SYDEL pH-meter equipped with a Xerolyt© electrode LoT type (Mettler Toledo) after 30 min. (pH1 and Temp1, right side only), 4 hours (pH4 and Temp4, both sides) and 24 hours post mortem (pH24 and Temp24, both sides). Halothane genotyping (Certagen Gmbh, Rheinbach, Germany) was performed on ear samples using a DNA test [4]. Directly after deboning (24h pm), meat color was determined with a CR-300 Minolta colorimeter on the internal side of the *Semimembranosus* muscle without a blooming period, and PSE-like zone defect quotation was performed according to the IFIP scale [5]. Ninety six hams were randomly selected in pairs (left and right sides from a single carcass) and individual "jambon cuit supérieur" cooked ham processing was performed as described by Vautier et al. [6]. Cooking yield and slicing defect rates (holes, slice cohesion defects, "paste-like" defects) were recorded. Paste-like defect quantification was similar to the destructuration quantification used by Hugenschmidt et al. [7] but recorded binary.

For each ham selected, two 10 g. *Semimembranosus* muscle samples (internal and external sides) were collected, then frozen in liquid nitrogen until analysis. Glycogen, glucose, glucose-6-phosphate and lactate content were measured with enzymatic procedure [8]. Glycolytic potential (GP) was calculated according to Monin and Sellier [9]. Sarcoplasmic protein degradation was estimated with protein solubility determination using a 0.025 M potassium phosphate buffer as described by Laville et al. [10].

The effect of chilling rate and halothane genotype on meat quality parameters and industrial processing yields were estimated with the 8.02 version of SAS (SAS Institute, USA), using the GLM and LSMeans procedures. The FREQ procedure was applied on PSE-like zone class data.

III. RESULTS AND DISCUSSION

Overall the meat quality level (Table 1) is lower than in a previous study [5] conducted on Piétrain sired pigs slaughtered after a 24 hour fasting period including 2 hours resting time. Early post mortem pH (6.25 vs 6.40) and ultimate pH (5.60 vs 5.76) are lower and the PSE-like zone defect rate is higher than expected (44% vs 4%).

Table 1. *Semimembranosus* quality results per PSE-like zone class

	m(sd)	PSE-like zone class				p.=
		1	2	3	4	
pH1[1]	6.25(0.52)	6.32	6.32	6.17	6.13	ns
Temp1[1]	39.9(0.6)	39.7	39.8	39.9	40.0	ns
pH4[2]	5.85(0.25)	6.02_a	5.92_b	5.77_d	5.68_c	***
Temp4[2]	23.8(4.6)	24.0	23.8	23.6	23.4	ns
pH24[2]	5.60(0.15)	5.76_a	5.60_b	5.51_c	5.46_d	***
L*[2]	58.0(6.5)	50.8_a	57.6_b	61.7_c	64.4_d	***
c*[2]	11.6(2.6)	9.2_a	11.7_b	13.2_{bc}	13.7_c	***
h*[2]	0.53(0.12)	0.45_a	0.53_b	0.57_a	0.62_b	***

[1]: measured on right side only; [2]: measured on both sides
* : p<0.05 ;** : p<0.01 ; *** : p<0.0001

The different chilling rates induced an expected variation on Temp4 and pH4 (Table 2), and has no significant effect on *Semimembranosus* ultimate pH (5.59 vs 5.58) as reported by Dransfield et al. [11] and Tomovic et al. [12]. *Semimembranosus* brightness (L*) increased after slow chilling (59.5 vs 57.8), in contrast with Tomovic et al. [12] data showing no significant brightness variation on *Semimembranosus* despite a similar Temp4 gap in chilling rates (7°).

Table 2. Effect of chilling rate on *Semimembranosus* quality

		Carcass side / chilling rate		p.=
		Left / slow	Right / fast	
n=		266	266	
pH4		5.76_a	5.94_b	***
Temp4		27.7_a	19.7_b	***
pH24		5.59	5.58	ns
L*		59.5_a	57.8_b	***
c*		11.92	11.92	ns
h*		0.56	0.53	ns
PSE-like zone class	1 + 2	142 (53%)	158 (59%)	ns
	3 + 4	124 (47%)	108 (41%)	

* : p<0.05 ;** : p<0.01 ; *** : p<0.0001

The chilling effect on the frequency of PSE-like zones frequency described by Vautier et al. [1] was not confirmed in this study (41% and 47%, fast and slow chilling respectively). Applying different chilling rates on both sides of a single carcass certainly reduced bias, which could be suspected in the previous study that showed a 2 points meat percentage difference between the comparative carcass populations (same pH1, pHu and carcass weight).

Halothane genotype produced classical effects on pH4 (5.79 vs 5.91, Nn and NN respectively) but surprisingly pH1 did not differ (6.22 vs 6.25, Table 3). Early pH values were not in agreement with previous studies showing an increase fall in pH for Nn [13], which could possibly be related to the stressful slaughter conditions. Carcass presenting Halothane sensitivity mutation (Nn) showed a higher level of *Semimembranosus* ultimate pH (5.61 vs 5.55) as previously noticed by Aubry et al. [3] (5.71 vs 5.67).

With a limited fasting time and under stressful slaughter conditions, halothane mutation has a massive effect on the frequency of PSE-like zone defects with a 2 to 10 times higher rate depending on the group of pigs followed (61% vs 15%, Nn and NN, respectively).

Table 3. Halothane genotype effects on *Semimembranosus* quality

		Halothane genotype		p.=
		NN	Nn	
n=		198	332	
pH1[1]		6.25	6.22	ns
Temp1[1]		39.8	39.9	ns
pH4[2]		5.91_a	5.79_b	***
Temp4[2]		23.7	23.7	ns
pH24[2]		5.55_a	5.61_b	***
L*[2]		58.6	58.6	ns
c*[2]		12.0	11.8	ns
h*[2]		0.54	0.54	ns
PSE-like zone class	1 + 2	168 (85%)	130 (39%)	***
	3 + 4	30 (15%)	202 (61%)	

[1]: measured on right side only; [2]: measured on both sides
*: p<0.05 ;**: p<0.01 ; ***: p<0.0001

For hams with no PSE-like zone defects, the protein solubility is similar for both internal and external sides of the *Semimembranosus* (55.8 vs 54.6 mg/g, Table 4). This level is lower for the internal side of *Semimembranosus* (48.1 vs 53.7 mg/g) when the defect is present, confirming the sarcoplasmic protein denaturation of PSE-like zones, revealed by Laville et al. [10]. Local sampling of the *Semimembranosus* indicates that the denaturation is limited to the inside part of the muscle, which corresponds to the subjective evaluation practiced for the PSE-like zone grading of hams. Local variations are observed with a similar scheme for chemical analysis of glycogen metabolism. The glycogen content (39.0 vs 44.9 µmol/g.) and GP content (160.1 vs 172.2 µmol/g.) are lower on the inside of the *Semimembranosus* muscle for 3+4 class hams. These results indicate that the PSE-like zone defects may be associated with local metabolism disorders which need further investigation. No significant local difference in GP or glycogen level was reported between NN and Nn. On the other hand, the levels of glycogen, lactate and GP for external side are higher for hams with PSE-like zone defects, in agreement with the ultimate pH values measured on the external side of the *Semimembranosus*.

The cooking yields (83.7 vs 86.6), the cohesion defects rate (28.0 vs 18.6) and the "paste-like" defects rate (35.3 vs 26.0) revealed a lower meat quality for hams presenting a 3+4 PSE-like zone defect grade (Table 5, 3+4 vs 1+2 respectively). This data is in agreement with previous results [6] showing a reduction in the cooking and global slicing yields for defective hams. Despite their higher ultimate pH level's, Nn halothane genotype carriers showed a reduced cooking yield (84.7 vs 86.5, Nn and NN respectively). The documented relationship between pHu and cooking yield [14] seems to be dismissed when applied to a heterogeneous halothane genotype population slaughtered under inappropriate conditions.

Table 4. Chemical characterisation and protein solubility of *Semimembranosus*

		Side	Prot. sol. (mg/g)	Gly.	Lact.	PG
				(µmol/g)		
PSE-like zone class	1 + 2 (n=56)	ext.	55.8_a	38.4_a	78.4_{ac}	155.2_{ad}
		int.	54.6_a	36.4_a	76.6_a	149.4_a
	3 + 4 (n=40)	ext.	53.7_a	44.9_b	82.4_b	172.2_b
		int.	48.1_b	39.0_a	82.0_{bc}	160.1_{cd}
p. =			C*** S*** CxS*	C*** S*** CxS*	C***	C*** S**

*: p<0.05 ;**: p<0.01 ; ***: p<0.0001
S=side; C=PSE-like zone defect class

Table 5. Effect of PSE-like zone class, halothane genotype and chilling rate on processing yields.

		Cooking yield	Cohesion defect	Paste-like defect	Hole defect
		(%)			
PSE-like zone class	1 + 2	86.6_a	18.6_a	26.0_a	20.4
	3 + 4	83.7_b	28.0_b	35.3_b	25.8
Halothane genotype	NN	86.5_a	20.1	27.8	19.8
	Nn	84.7_b	24.2	32.5	24.3
Chilling rate	slow	85.5	17.6_a	29.0	19.2_a
	fast	85.3	27.5_b	32.2	25.7_b
p. =		C*** H**	C* R*	C*	R*

*: p<0.05 ;**: p<0.01 ; ***: p<0.0001
C=PSE-like zone defect class; H=halothane genotype; R=chilling rate

Globally, the chilling rate has no significant effect on major processing yields such as the cooking yield (29.0 vs 32.2) and the "paste-like" defect rate (29.0 vs 32.2). Whereas, a slower chilling rate induced a reduction of cohesion

defects (17.6 vs 27.5) and hole defects (19.2 vs 25.7).

IV. CONCLUSION

In comparison with previous work, the intended degrading conditions of slaughter induced an increased rate of PSE-like zones. For a high level of defects within a population, the chilling rate did not influence the frequency of PSE-like zone defects for hams. Moreover, except for the *Semimembranosus* pH4 and L*, the chilling differences tested did not produce any raw or processed meat quality modifications. Halothane mutation genotype (Nn) induced a massive effect on the frequency of PSE-like zones (x4) for pigs slaughtered in stressful conditions and with a reduced fasting time. Local differences in sarcoplasmic protein solubility were observed on external and internal *Semimembranosus* samples from affected (1+2 class) and non-affected (3+4) hams, indicating local protein denaturation. GP and glycogen content were also found to be lower on the internal side of the Semimembranosus. The latter needs further metabolic investigations to explore the relationship between local glycolytic disorder and the frequency of PSE-like zones.

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REFERENCES

1. Vautier A., Gault E., Lhommeau T., Le Roux A., Martin JL., and Vendeuvre JL. 2010. Carcass chilling and pork quality: effects on drip loss, texture measurements and “pse-like zones” hams frequency. 56th ICoMST. Jeju, Korea.
2. Minvielle, B., Boulard, J., Vautier, A., & Houix, Y. (2003). Viandes déstructurées dans la filière porcine: Effets combinés des durées de transport et d’attente sur la fréquence d’apparition du défaut. 35ème JRP.
3. Aubry A., Ligonesche B., Guéblez R., D. Gaudré D. 2000. Comparaison de porcs charcutiers NN et Nn pour les performances de croissance, carcasse et qualité de viande, et l’aptitude à produire du jambon cuit. 32ème JRP.
4. Fujii, J., Otsu, K., Zorzato, F., Deleon, S., Khanna, V. K., Weiler, E., O'Brien, P., & MacLennan, D. H. (1991). Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science, 253, 448−451.
5. Vautier, A., Boulard, J., Bouyssière, M., Houix, Y., & Minvielle, B. (2008). Prediction level of meat quality criteria on ‘‘PSE-like zones” defect of pork’s ham. 54th ICoMST, Cape Town, South Africa.
6. Vautier A., Bozec A., Gault E., Lhommeau T., Martin JL., and Vendeuvre JL. 2011. Visible spectroscopy and redox potential as alternatives of ultimate pH for cooking yield prediction. 57th ICoMST, Ghent, Belgium.
7. Hugenschmidt G., Hadorn R., Scheeder M., Wenk C. 2009. Effect of early and ultimate pH in raw ham on destructured zones in cooked cured ham. 55th ICoMST, Copenhagen, Denmark.
8. Dalrymple, R. H., & Hamm, R. (1973). A method for the extraction of glycogen and metabolites from a single muscle sample. Journal of Food and Technology, 8, 439–444.
9. Monin, G., & Sellier, P. (1985). Pork of low technological quality with a normal rate of muscle pH fall in the immediate postmortem period: The case of the Hampshire breed. Meat Science, 13, 49−63.
10. Laville, E., Sayd, T., Sante-Lhoutellier, V., Morzel, M., Labas, R., Franck, M., Chambon, C., Monin, G. Characterisation of PSE zones in semimembranosus pig muscle. Meat Science. 2005, 70, 167-172.
11. Dransfield E., Ledwith M.J., Taylor A.A. (1991). Effect of electrical stimulation, hip suspension and ageing on quality of chilled pig meat. Meat Science 29:129-139.
12. Tomovic M.V., Petrovic L., Dzinic N.R. (2008). Effects of rapid chilling of carcasses and time of deboning on weight loss and technological quality of pork Semimembranosus muscle. Meat Science 80:1188-1193.
13. Laville E., Sayd T., Terlouw C., Blinet S., Pinguet J., Fillaut M., Glénisson J., Chérel P. 2009. Differences in Pig Muscle Proteome According to HAL Genotype: Implications for Meat Quality Defects. J. Agric. Food Chem. 57: 4913–4923.
14. Jacquet B., Sellier P., Runavot J.P., Brault D., Houix Y., Perrocheau C., Gogué J., Boulard J. (1984). Prédiction du rendement technologique de la fabrication du jambon de Paris à l’aide de mesures prises à l’abattoir. 16ème JRP.