INFLUENCE OF CROSSBREEDING AND FROZEN STORAGE ON FATTY ACID COMPOSITION OF PORK PACKAGED IN MODIFIED ATMOSPHERE

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Abstract – The aim of this study was to investigate the behaviour of pork from pigs from two different crossbreedings packaged in modified atmosphere (70% O_2/30% CO_2), following one year frozen storage and thawing, on fatty acid composition throughout 13 days of display at 4±1 °C. The study was conducted with 20 female pigs from two different crossbreeding schemes: Pietrain (P) × (Landrace (LR) × Large White (LW)) and P × (LR × Duroc (D)). Pre-frozen chops from both maternal lines had higher intramuscular fat (IMF) content and saturated fatty acids (SFA) percentage. However, the polyunsaturated fatty acid (PUFA) percentage did not show any differences between pre-frozen and fresh chops in LR×D line; meanwhile, they were lower in pre-frozen in LR×LW line. Display did not present significant effects on fatty acid profile (expressed as a percentage). When results were expressed as mg/100g muscle, pre-frozen chops from the two crossbreedings had a greater content of total SFA. However, only pre-frozen pork from LR×D line had a greater amount of monounsaturated fatty acid (MUFA), PUFA, n-6 and n-3 than fresh pork. Also, the content of C18:1*n*-6, C20:4*n*-6, C22:5*n*-3, C22:6*n*-3 and PUFA, such as *n*-6 and *n*-3, slowly decreased throughout display.

Key Words – Display, Duroc breed, IMF content.

I. INTRODUCTION

Traditionally, pig production in Spain has been based on crossing Landrace × Large White dams with lean sire lines, such as Pietrain [1]. However, only a few researches have focused on the effect of the inclusion of Duroc breed in maternal line in crossbreeding among white breed to improve meat quality without decreasing lean growth. On the other hand, freezing is the most frequently used technology to preserve fresh meat during long-term storage, enabling its nutritive value to be maintained. Keeping meat under frozen storage enables the meat industry to (i) adapt its offer to consumers' demand, (ii) adjust the meat supply to the processing rate, and (iii) transport meat to distant importing countries [2]. In addition, modified atmosphere packaging (MAP) is a common means of retail sale display in supermarkets, and is used to maximise meat shelf-life and maintain an attractive fresh appearance [3].

Lipids of pork can suffer alterations during frozen storage, owing to lipolysis and oxidation, which results in quality deterioration. Changes in fatty acid composition, particularly in the phospholipid fraction, provide a measure of the degree of lipolysis and oxidation in stored meats [4]. Therefore, the aim of this study was to investigate the behaviour of pork from pigs from two different crossbreedings (including 0% or 50% of Duroc genes in the maternal line) packaged in modified atmosphere, following one year frozen storage and thawing, on fatty acid composition.

II. MATERIALS AND METHODS

A. Animals and sampling

The experiment was conducted with 20 female pigs from two different crossbreeding schemes: Pietrain (P) × (Landrace (LR) × Large White (LW)) and P × (LR × Duroc (D)). During the experiment, all pigs were subjected to the same feeding and management. The pigs were stunned using carbon dioxide and slaughtered at an abattoir at approximately 90.8 ± 4.9 kg carcass weight.

The M. *Longissimus thoracis et lumborum* (LTL) was removed from each carcass immediately after quartering. After 48 h at 4±1 °C in a cooling

chamber, the M. LTL was divided in halves and the caudal portion was sectioned into 2 cm-thick boneless pork chops and packaged in polystyrene tray sealed with a polyethylene and polyamide laminate film, using a packaging machine. The modified atmosphere (MA) used was 70% O_2 and 30% CO_2. The cranial portion of M. LTL were placed in vacuum polyethylene-polyamide bags, and stored at -20 °C ± 2 °C in the dark in a freezer. Twelve months later, the cranial portion of M. LTL was thawed in tap water for four hours before the vacuum was broken, and sectioned into 2 cm-thick boneless pork chops and packaged in the previous MA composition. All the packs were kept at 4°C±1°C and standard supermarket lighting conditions (14 h at day) during 13 days of storage time. Intramuscular fat and fatty acid analysis were performed on day 0, 4, 7, 10 and 13.

B. Intramuscular fat and fatty acid analysis

The fat was extracted in chloroform-methanol (1:1 v/v), with 2,6-ditert-butyl-4-methylphenol (BHT) (1 g/10 ml methanol) as antioxidant [5]. One milliliter of chloroform phase was used to assess the percentage of intramuscular fat (IMF) by drying at 100 °C for 20 min; the results were expressed as the weight percentage of wet muscle. The rest was evaporated in a sand bath under nitrogen gas at 50 °C. The methyl esters from fatty acids (FAMES) were analysed in a gas chromatograph HP-6890 II, with a capillary column SP-2380 (100 m x 0.25 mm x 0.20 µm), using nitrogen as the carrier gas. Fatty acid composition was quantified using nonadecanoic acid (C19:0) as the internal standard.

C. Statistical analysis

All data were statistically analysed by the general linear model procedure of IBM SPSS version 22 (2013). The model included treatments (LR×LW or LR×LD maternal lines and pre-frozen or fresh) and display as main effects and their interaction. Duncan's post hoc test was used to assess differences between mean values when $P \leq 0.05$.

III. RESULTS AND DISCUSSION

Display did not present significant effects on fatty acid profile (expressed as a percentage) (data not shown). In contrast, differences among treatments were significant (Table 1) when comparing concentrations of most individual fatty acids in the IMF. Pre-frozen chops from both maternal lines had higher ($P \leq 0.001$) intramuscular fat (IMF) content, stearic acid (C18:0) and the sum of total saturated fatty acids (SFA) percentages than fresh chops from each maternal lines. This increase of IMF content could be due to a relative increase of fat concentration in muscle due to water losses that occur after thawing. However, there were no differences in the proportion of oleic acid (C18:1*n*-9) and monounsaturated fatty acids (MUFA) between pre-frozen and fresh chops.

Table 1. Effect of treatments on intramuscular fat content and fatty acid composition (% of total fatty acids) in M. LTL: mean and standard errors of the means (SEM).

	LR×LW Fresh	**LR×LW Pre-frozen**	**LR×D Fresh**	**LR×D Pre-frozen**	**Sign.**	**SEM**
n	***50***	***40***	***50***	***40***		
% IMF	1.52a	1.96b	2.04b	2.51c	***	0.05
C16:0	21.52a	22.89b	22.99b	22.98b	***	0.08
C18:0	9.98a	11.19b	10.99b	11.97c	***	0.10
C18:1*n-9*	36.06ab	35.90a	37.57c	37.37bc	*	0.24
C18:2*n-6*	16.21c	15.28bc	13.78a	14.18ab	***	0.23
C18:3*n-3*	0.36b	0.37b	0.33a	0.38b	**	0.00
C20:4*n-6*	3.38c	2.69b	2.24a	1.90a	***	0.08
C22:5*n-3*	0.40c	0.34b	0.28a	0.25a	***	0.01
C22:6*n-3*	0.09c	0.06b	0.06b	0.05a	***	0.00
∑SFA	33.41a	36.10b	35.99b	37.02c	t	0.17
∑MUFA	43.42ab	43.33a	45.083b	44.64ab	***	0.28
∑PUFA	22.48c	20.58b	18.24a	18.34a	***	0.34
∑*n-6*	20.63c	18.95b	16.84a	16.98a	***	0.31
∑*n-3*	1.21c	1.06b	0.95a	0.92a	***	0.02
PUFA/SFA	0.68c	0.57b	0.51a	0.50a	***	0.01
n-6*/*n-3	17.16a	18.04bc	17.73ab	18.41c	**	0.11

LR: Landrace; LW: Large White; D: Duroc. Different letters in the same row indicate significant differences among mean values: t = $P<0.1$; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.

On the other hand, polyunsaturated fatty acid (PUFA), *n*-6, *n*-3 and some long chain fatty acid (C20:4*n*-6 and C22:5*n*-3) percentages did not show any differences between pre-frozen and fresh chops in LR×D line; meanwhile, they were lower in pre-frozen than fresh chops in LR×LW line. Therefore, PUFA percentage decreased during one year frozen storage, producing a relative increase of SFA percentage, but only in LR×LW line.

Hernández *et al.* [4] found a decrease in PUFA percentage of the phospholipid (PL) fraction after 6 months of frozen storage in pork; in particular, linoleic and α-linolenic acids, and nonpolar lipid fatty acids remained unchanged. This general decrease in phospholipid PUFA could be accounted for the enzymic hydrolysis of PL concentration during frozen storage, since lipolytic enzymes remained active at freezing temperatures. Hydrolysis of PL occurred during frozen storage of muscle giving rise to the formation of free fatty acids and water-soluble decomposed phospholipids.

In addition, IMF content, C18:0 and SFA were higher in pork from LR×D line than in pork from LR×LW maternal line. The LR×LW line had higher PUFA, *n*-6 and *n*-3 proportions and PUFA/SFA ratio than LR×D line. This suggests that increasing Duroc genes in maternal line results in increasing IMF, which agreed with Blanchard *et al.* [6]. Cameron and Enser [7] showed that increasing IMF content tended to increase concentrations of SFA and MUFA (which are mainly found in neutral lipids), but decreased the concentration of PUFA (which are mainly found in PL), which was confirmed by our results.

Treatments (Table 2) and display (Table 3) influenced the amount of most individual fatty acids (expressed as mg/100 g muscle). The pigs from LR×D dam line produced lipids with higher ($P \leq 0.001$) content of C16:0, C18:0, C18:1*n*-9, C18:2*n*-6 and C18:3*n*-3 and showed, consequently, a higher level of SFA, MUFA and PUFA. Those results could be related to the highest IMF percentage found in that dam line.

Pre-frozen chops from the two crossbreedings had a greater ($P \leq 0.001$) content of C16:0, C18:0, C18.2*n*-6, C18:3*n*-3 and SFA than fresh chops. However, only pre-frozen pork from LR×D line had greater amounts of C18:1*n*-9 and total MUFA, PUFA, *n*-6 and *n*-3 than fresh pork. No differences were found among treatments in the long chain fatty acid studied. Previously, we have reported that IMF was higher in pre-frozen than in fresh chops thus causing a relative increase of the neutral lipids (NL) fraction due to the water losses that occur after thawing. These NL are composed mainly of triacylglycerols (TG), which are located in the adipocytes along the muscle fibres and in the interfascicular area, and their content in the muscle is strongly related to the total fat content [8]. Meanwhile, PL contain mainly long chain PUFA, which are strictly controlled in order to preserve membrane properties and maintain relatively constant their content, which agreed with our results.

Table 2. Effect of treatments on fatty acid composition of intramuscular fat (mg/100 g muscle) in M. LTL: mean and standard errors of the means (SEM).

	LR×LW Fresh	**LR×LW Pre-frozen**	**LR×D Fresh**	**LR×D Pre-frozen**	**Sign.**	**SEM**
n	***50***	***40***	***50***	***40***		
C16:0	199.0a	258.6b	298.1b	374.8c	***	9.7
C18:0	91.9a	125.7b	142.6b	195.7c	***	5.2
C18:1*n-9*	339.6a	412.7ab	492.8b	612.9c	***	16.9
C18:2*n-6*	142.9a	166.2b	175.8b	224.7c	***	4.8
C18:3*n-3*	3.4a	4.1b	4.4b	6.1c	***	0.2
C20:4*n-6*	28.6	28.7	27.5	29.2	ns	0.5
C22:5*n-3*	3.4	3.6	3.4	3.9	ns	0.1
C22:6*n-3*	0.8	0.7	0.8	0.8	ns	0.0
∑SFA	308.6a	407.4b	466.6b	604.5c	***	15.8
∑MUFA	409.3a	497.7ab	591.3b	731.3c	***	20.1
∑PUFA	196.6a	223.4ab	231.5b	289.6c	***	5.9
∑*n-6*	180.7a	205.8ab	213.8b	268.2c	***	5.5
∑*n-3*	10.5a	11.4a	12.0a	14.7b	***	0.3

LR: Landrace; LW: Large White; D: Duroc. Different letters in the same row indicate significant differences among mean values: ns = $P>0.1$; *** = $P \leq 0.001$.

There were no significant ($P>0.05$) differences in the content of C16:0, C18:0, C18:1*n*-9 and total SFA and MUFA throughout MAP display. In contrast, the content of C18:1*n*-6, C20:4*n*-6, C22:5*n*-3, C22:6*n*-3 and PUFA slowly decreased throughout display. There is a close relationship between lipid oxidation and the composition of fatty acids. Lipid oxidation is a complex process in which molecular oxygen reacts with unsaturated fatty acids, particularly with PUFA [9]. Specially, the n-3 fatty acids are more easily oxidised, owing to the fact that unsaturated fatty acids react more rapidly, and the more double bonds that a fatty acid contains, the more reactive it is [10]. Therefore, the decrease in the content of PUFA throughout display could be due to an oxidation thereof.

Table 3. Effect of display (days) on fatty acid composition of intramuscular fat (mg/100 g muscle) in M. LTL: mean.

	0	**4**	**7**	**10**	**13**	**Sign.**
n	***40***	***40***	***40***	***40***	***20***	
C16:0	306.7	279.9	281.3	283.4	212.9	ns
C18:0	146.9	137.3	138.9	141.6	103.3	ns
C18:1*n-9*	501.0	458.6	463.0	467.3	361.2	ns
C18:2*n-6*	201.1c	177.bc	174.2bc	172.0b	132.4a	**
C18:3*n-3*	5.1b	4.4b	4.5b	4.4b	3.2a	t
C20:4*n-6*	33.1c	28.3b	27.4ab	26.9ab	24.4a	***
C22:5*n-3*	4.2c	3.6b	3.5ab	3.3ab	3.0a	***
C22:6*n-3*	0.9b	0.7ab	0.7a	0.7a	0.6a	**
∑SFA	480.8	441.8	445.2	450.4	335.2	ns
∑MUFA	601.0	550.2	556.0	560.3	435.0	ns
∑PUFA	267.1c	234.7b	230.2b	227.2b	180.1a	**
∑*n-6*	246.7c	216.6b	212.5b	209.8b	165.4a	**
∑*n-3*	13.8c	12.2bc	11.9b	11.6b	9.7a	**

Different letters in the same row indicate significant differences among mean values: ns = $P>0.1$; t = $P<0.1$; ** = $P\leq0.01$; *** = $P\leq0.001$. Standard errors of the means (SEM): the same values than table 2.

IV. CONCLUSION

Pre-frozen chops had higher IMF and total SFA content than fresh chops independently of crossbreeding; therefore, they suffered a relative increase of fat concentration in muscle due to the water losses that occur after thawing. However, the total PUFA percentage, such as *n*-6 and *n*-3, decreased during one year frozen storage only in the crossbreeding with higher PUFA percentage, LR×LW maternal line. In addition, pre-frozen pork from the crossbreeding with higher IMF content, LR×D line, had greater amount of total MUFA, PUFA, *n*-6 and *n*-3 than fresh pork, but there were no differences in the long chain fatty acid studied.

Display did not affect fatty acid profile when results were expressed as percentage. However, the content (expressed as mg/100g of muscle) of C18:1*n*-6, C20:4*n*-6, C22:5*n*-3, C22:6*n*-3 and PUFA decreased slowly throughout display probably due to an oxidation thereof.

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