INFLUENCE OF CROSSBREEDING AND FROZEN STORAGE ON OXIDATIVE AND MICROBIAL STABILITY 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 colour and lipid oxidation, exudative losses and microbial counts throughout 13 days of storage 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)). Fresh chops from pigs from LR×D maternal line had greater *a and lower *L** values than chops from the rest of treatments. Pre-frozen chops from both maternal lines had higher exudative loss percentages than fresh chops. The metmyoglobin percentage was higher in fresh and pre-frozen chops from LR×D line. Fresh chops had greater psychrotrophic viable (PVC) and Enterobacteriaceae (EE) counts than pre-frozen during the first days of storage. However, both PVC and EE counts of fresh chops slowly increased throughout display; meanwhile, PVC counts of pre-frozen chops dramatically increased until day 10. In conclusion, pre-frozen chops the from two crossbreeding had a shorter shelf-life than fresh chops due to higher microbial growth. exudative losses and colour deterioration.**

Key Words – colour, Duroc breed, lipid oxidation.

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].

Therefore, a thorough understanding of the physical and chemical changes induced by frozen storage, crossbreeding and display and their relation to fresh meat is of utmost importance for the meat industry from a technological point of view. The objective 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 colour stability, lipid oxidation, exudative losses and microbial counts.

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 trays 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 was 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. Physical-chemical and microbiological analyses were performed on day 0, 4, 7, 10 and 13.

B. Instrumental measurement of colour

A Minolta CM-2002 spectrophotometer was used to measure colour at the surface of a 2-cm-thick LTL chop after opening the trays and exposing them to air for 2 h. The parameters registered were L^* (lightness), a^* (redness) and b^* (yellowness). Each value was the mean of ten observations on the same chop.

The relative content of metmyoglobin (MMb) were calculated from the reflectance curve according to Krzywicki (1979) [4] using 730 nm as maximum wavelength.

C. Exudative loss

After the appropriate MAP storage duration, LTL chops were removed from trays and excess moisture removed before being weighed. Exudative loss was expressed as a percentage of the initial chop weight (24 h postmortem).

D. Lipid oxidation

Lipid oxidation was measured by the 2-thiobarbituric acid (TBA) method [5]. TBA-reactive substances (TBARS) values were calculated from a standard curve of malondialdehyde and expressed as mg malondialdehyde per kg sample.

E. Microbiological analyses

Total aerobic psychrotrophic viable counts (PVC) and Enterobacteriaceae count (EE) were studied throughout display. Two sterile cotton swabs moistened in 0.1% peptone water were used for swabbing 10 cm^2 of meat surface, delimited by a sterile stainless steel template. Swabs were stirred thoroughly in 10 ml of 0.1% peptone water. Serial 10-fold dilutions were prepared by diluting 1 ml in 9 ml of 0.1% peptone water. PVC were determined by pour plate methods in Plate Count Agar (Merck) and plates were incubated to 7 days at 10 °C. For EE, violet red bile dextrose agar (Merck) with a double layer was used and plates were incubated for 48 h at 30 °C.

F. 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

Fresh chops from LR×D maternal line had ($P \leq 0.001$) the lowest L^* values, while fresh chops from LR×LW had ($P \leq 0.001$) the lowest b^* values (Table 1). The lightness (L^*) of chops in MAP increased throughout display (Table 2). However, the yellowness (b^*) of chops increased between day 0 and day 4 of storage, but remained stable thereafter. Hansen *et al.* [6] reported that pre-frozen chops had significantly higher b^* values than fresh chops, and the b^* values were found to be rather stable during the chill storage for both of them.

There was a significant interaction ($P \leq 0.05$) among treatments and display on a^* values (Fig.1). Fresh chops from pigs from LR×D maternal line had greater a^* values than chops from the rest of treatments during all the storage. It could be due to an increased muscle haem (red pigment) concentration for the Duroc suggesting more red oxidative fibres [7]. On day 4, fresh chops from

both maternal lines and pre-frozen chops from LR×LW line had the greatest a* values throughout display, while pre-frozen chops from LR×D line obtained the greatest one on day 7. Hansen *et al.* [6] reported that redness was lower for 30 months-frozen pork chops than for fresh chops. These authors explained that the lower redness of the frozen pork may have been caused by processes occurring in the frozen state such as myoglobin cold denaturation.

Table 1. Effect of treatments on pork quality parameters 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***		
L*	49.7b	50.6b	47.7a	50.2b	***	0.32
b*	8.0a	8.9b	9.3b	8.7b	***	0.12
Exudative loss (%)	4.7a	6.8b	3.9a	6.8b	***	0.20
% MMb	13.8a	12.8a	18.2b	17.0b	*	2.51
TBARS	0.22a	0.25b	0.24b	0.24b	t	0.02

LR: Landrace; LW: Large White; D: Duroc. Different letters in the same row indicate significant differences among mean values: t = $P<0.1$; * = $P\leq0.05$; *** = $P\leq0.001$.

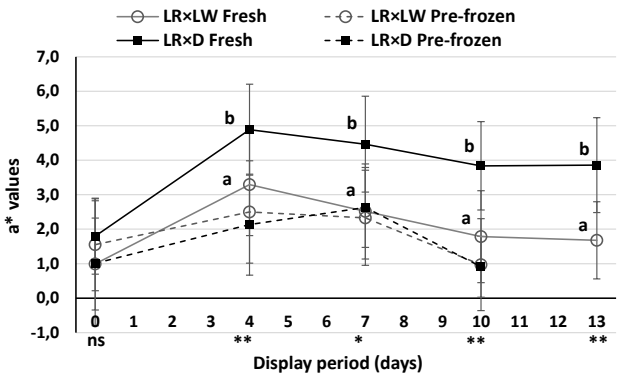

Figure 1. Evolution of *a** values of fresh and pre-frozen chops from pigs from LR×LW and LR×D maternal lines throughout MAP display. Different letters at the same day of storage indicate significant differences among mean values; ns = $P>0.1$; * = $P\leq0.05$; ** = $P\leq0.01$.

Exudative losses increased ($P\leq0.001$) throughout MAP display until day 10 (Table 2), and pre-frozen chops from both maternal lines had higher ($P\leq0.001$) exudative loss percentages than fresh chops (Table 1). On the other hand, the metmyoglobin (MMb) percentage was higher ($P\leq0.05$) in fresh and pre-frozen chops from LR×D maternal line and gradually increased ($P\leq0.001$) until the last days of storage.

There was only a slight tendency for TBARS values to be lower in fresh chops from LR×LW line than the rest of treatments (Table 1). All treatments started with very low values of about 0.08 mg malonaldehyde/kg sample, which remained stable during the first four days of display; yet, on day 7 of storage, TBARS values slowly increased until the last day (Table 2). The increasing O_2 concentrations in packaging atmospheres caused a corresponding enhancement of oxidation and, therefore, a decrease of shelf-life due to discoloration development [8], such as lipid oxidation as MMb formation.

Table 2. Effect of display (days) on pork quality parameters in M. LTL: mean and standard errors of the means (SEM).

	0	4	7	10	13	Sign.
n	***40***	***40***	***40***	***40***	***20***	
L*	46.9a	48.8b	50.1bc	51.4c	50.8c	***
b*	7.6a	9.1b	9.2b	8.7b	8.8b	***
Exudative loss (%)	--	4.7a	5.6ab	5.9b	5.4ab	***
% MMb	3.9a	8.6a	16.0b	21.0bc	23.3c	***
TBARS	0.08a	0.13a	0.25b	0.42c	0.36c	***

Different letters in the same row indicate significant differences among mean values: *** = $P\leq0.001$. Standard errors of the means (SEM): the same values than table 1.

There were significant differences between fresh and pre-frozen chops in PVC counts during the first seven days of display, having fresh chops greater counts than pre-frozen ones (Fig. 2). However, PVC counts of fresh chops slowly increased throughout display, while PVC counts of pre-frozen chops dramatically increased until day 10. Greer *et al.* [9] found that the lag phase of bacterial growth in frozen/thawed pork was shorter than for fresh meat, On the other hand, EE counts were greater again in fresh chops than pre-frozen throughout MAP display until day 10. EE counts of fresh chops remained stable during all the days of display. On the contrary, no EE counts were found in pre-frozen chops during the first four days of display. These counts increased from 4 to 7 days

and later remained stable until the last days. During freezing, microbial spoilage is effectively terminated as the microbes become dormant. Unfortunately, they regain their activity during thawing [10].

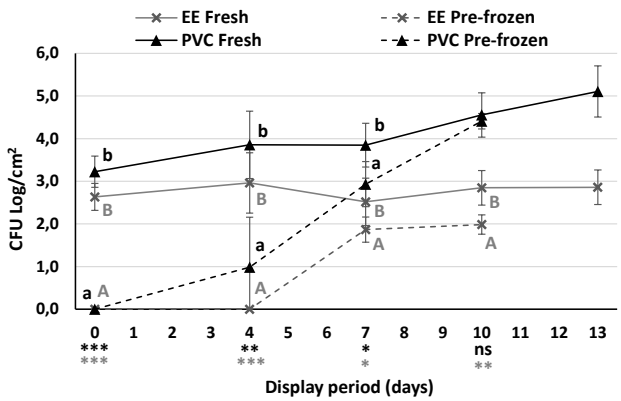

Figure 2. Evolution of total aerobic psychrotrophic viable (PVC) and Enterobacteriaceae (EE) counts of fresh and pre-frozen chops throughout MAP display. Different letters at the same day of storage indicate significant differences among mean values; ns = $P>0.1$; * = $P\leq0.05$; ** = $P\leq0.01$; *** = $P\leq0.001$.

IV. CONCLUSION

Pre-frozen chops had higher or equal L^* and b^* values, exudative losses and lipid oxidation content than fresh chops independently of crossbreeding. However, LR×D maternal line presented greater a^* values in fresh chops and higher MMb percentages in both fresh and pre-frozen chops than LR×LW line during all the storage. In addition, pre-frozen chops had lower PVC counts in the first days of display than fresh chops, but they increased dramatically reaching the counts of fresh chops on day 10. However, EE counts had lower values in pre-frozen than fresh chops throughout display. In conclusion, pre-frozen chops from the two crossbreedings had a shorter shelf-life than fresh chops due to higher microbial growth, exudative losses and colour deterioration.

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