# INFLUENCE OF FROZEN LONG-STORAGE DURATION ON PORK QUALITY

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**Abstract – The objective of this study was to investigate the effect of frozen long-storage duration (two years) on pork quality and intramuscular fatty acid composition in loin. The experiment was conducted with 43 entire male pigs (Pietrain×(Landrace×Large White)) which were fed a basal diet without added fat (control diet) or supplemented with different sources of fat: animal fat (1%, 3%), soyabean oil (1%) and calcium soaps of palm oil (1%). In this study only the effect of frozen storage duration is presented. pH values and 24- and 48-h drip loss percentages were higher (P≤ 0.001) in 2-years frozen pork than unfrozen pork, but colour parameters (*a** and *b** values) were lower (P≤0.001) in frozen pork. The percentage of intramuscular fat and total saturated and monounsaturated fatty acids were significantly higher in 2-years frozen pork, whereas this group had the lowest values of polyunsaturated fatty acids. The worst *n-6*/*n-3* and PUFA/SFA ratios values was found in 2-years frozen pork. In conclusion, 2-years frozen storage modified negatively pork characteristics and intramuscular fatty acid profile in this study.**

**Key Words – Fatty acid composition, Frozen pork, Meat quality**

- INTRODUCTION

Freezing is the most frequently used technology to preserve fresh meat during long-term storage. Keeping meat under frozen storage enables the meat industry to (i) adapt its offerings to consumers' demand, (ii) adjust the meat supply to the processing rate, and (iii) transport meat to distant importing countries [1]. Despite the advantages of freezing fresh meat, frozen meat has a stigma because freezing is perceived to reduce meat quality [2], even though this perception is not clearly supported by scientific evidences [3]. Therefore, predicting aspects of pork quality is becoming increasingly important from a nutritional as well as a technological point of view [4]. A thorough understanding of the physical and chemical changes induced by freezer storage and their relation to fresh meat is of utmost importance for the meat industry [5]. The objective of this study was to investigate the effect of frozen long-storage duration (two years) on pork quality and intramuscular fatty acid composition in *Longissimus thoracis et lumborum* muscle.

- MATERIALS AND METHODS

- *Animals and sampling*

Forty three entire males pigs, Pietrain x (Landrace x Large White), were randomly assigned to one of five dietary fat treatments with the individual animal as the experimental unit. All the diets contained the same proportions of raw materials (barley grain, wheat grain and soybean meal 44 % CP), except the proportion of corn grain that was different depending on the percentage of added fat. The five diets differed in their fat sources: 1) Control diet (without added fat); 2) Animal fat (tallow-lard mix) at 1% (AF1); 3) Animal fat at 3% (AF3); 4) Soyabean oil at 1% (SBO1); and 5) Calcium soaps of palm oil fatty acids at 1% (CaSPO1). All the concentrates were isoproteic (17 % crude protein). The pigs were stunned using carbon dioxide and slaughtered at an abattoir at approximately 83.8 ± 6.3 kg carcass weight.

The M. *Longissimus thoracis et lumborum* (LTL) was removed from each carcass 48 h after slaughter. After 24 h at 4 ± 1 °C, the M. LTL was divided in half and the portion more caudal was sectioned into 2 cm-thick steaks for lipid oxidation, fatty acid composition, drip loss and muscle colour measurements. All samples (except those for colour and drip loss) and the portion more cranial of M. LTL were placed in vacuum bags and frozen at -20 °C until meat quality analysis. Two years later, the portion more cranial of M. LTL was thawed and sectioned into 2 cm-thick steaks for lipid oxidation, fatty acid composition, drip loss and muscle colour measurements.

- *pH measurement*

pH values of the LTL were measured using a portable pH meter. Each value was the mean of four random measurements.

- *Instrumental measurement of colour*

A Minolta CM-2002 spectrophotometer was used to measure colour at the surface of a 2 cm-thick LTL chop exposed to air for 2 h. The parameters registered were CIE $L^*$ (lightness), $a^*$ (redness), and $b^*$ (yellowness). Each value was the mean of 10 observations on the same chop.

- *Drip loss*

A 2-cm-thick chop was weighed and placed on a supporting mesh in a sealed plastic container. After a storage period of 24 and 48 hours at 4 ± 1°C, the samples were taken out of the container, dabbed lightly on filter paper and weighed again. Drip loss was expressed as a percentage of the initial weight, based on Honikel [6].

- *Lipid oxidation*

Lipid oxidation was measured by the 2-thiobarbituric acid method of Pfalzgraf *et al.* [7]. The TBA-reactive substances (TBARS) values were calculated from a standard curve of malondialdehyde, and expressed as mg malondialdehyde/kg sample.

- *Intramuscular fat and fatty acid analysis*

Intramuscular fat (IMF) was extracted from the muscle according to the Bligh & Dyer [8] method and quantified as the weight percentage of wet muscle tissue. The samples were extracted according to [8] to determinate composition in fatty acids from intramuscular fat and 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.

*Statistical analysis*

All data were statistically analysed by the general linear model (GLM) procedure of IBM SPSS version 19 (IBM SPSS, 2010). The model included dietary fat supplementation and frozen

storage duration as main effects and also their interaction. However, only the results related to the effect of frozen storage duration were presented when the interaction was not significant. Duncan's post hoc test was used to assess differences between mean values when P≤0.05.

- RESULTS AND DISCUSSION

Meat quality results, which include pH values, colour measurements and drip loss, are presented in Table 1. The pH value was higher (P≤0.001) in 2-years frozen than unfrozen pork. Microorganisms and endogenous enzymes degrade meat proteins, produce ammonia and organic sulphides and amines, which could have increased pH [9]. There was no (P>0.05) effect of frozen storage duration on *L** values, but *a** and *b** values were significantly (P≤0.001) lower in 2-years frozen pork. Similarly, Hansen *et al.* [10] reported that redness was lower for 30 months-frozen chops than for fresh chops and they did not find significant differences in lightness. Both 24- and 48-h drip loss percentages were higher (P≤0.001) in 2-years frozen than unfrozen chops.

Table 1 Means and standard deviation (sd) of meat quality parameters.

| | unfrozen pork | | 2-years frozen pork | | Sign. |
|---|---|---|---|---|---|
| | x | sd | x | sd | |
| pH values | 5.51a | 0.13 | 5.65b | 0,15 | *** |
| *L** | 45.92 | 2.84 | 45.04 | 4.02 | ns |
| *a** | 6.48b | 1.72 | 4.05a | 1.66 | *** |
| *b** | 12.55b | 1.58 | 9.05a | 1.68 | *** |
| 24-h Drip loss | 1.79a | 0.61 | 5.04b | 1.71 | *** |
| 48-h Drip loss | 2.54a | 0.85 | 6.18b | 1.95 | *** |

Different letters in the same row indicate significant differences among mean values; ns = P>0.1; *** = P≤0.001.

There was a significant interaction between dietary fat supplementation and frozen storage duration in the content of lipid oxidation (Table 2). Regarding the oxidative behaviour in unfrozen pork, chops from pigs fed control and animal fat diets had greater (P≤0.01) TBARS values than chops from pigs fed CaSPO1 diet. However, no significant differences (P>0.05) were observed for lipid oxidation values between diets in 2-years frozen pork. There was not an important increase in TBARS values during frozen storage. Those results agreed with Hansen *et al.* [10], who found that TBARS values were not significant higher for frozen chops compared to fresh samples.

Table 2 Dietary fat supplementation-frozen storage duration interaction for the content (TBARS values) of mg of malondialdehyde/kg of M. LTL.

| | unfrozen pork | | 2-years frozen pork | | Sign. |
|---|---|---|---|---|---|
| | x | sd | x | sd | |
| Control | 0.097c,z | 0.020 | 0.081y | 0.016 | t |
| AF1 | 0.104c,z | 0.020 | 0.085y | 0.014 | * |
| AF3 | 0.091bc, z | 0.016 | 0.075y | 0.020 | t |
| CaSPO1 | 0.073a,y | 0.005 | 0.095z | 0.017 | * |
| SBO1 | 0.080ab | 0.010 | 0.083 | 0.011 | ns |
| Sign. | ** | | ns | | |

Control: without fat supplemented; AF1: 1% animal fat supplemented; AF3: 3% animal fat supplemented; SBO1: 1% soyabean oil supplemented; CaSPO1: 1% calcium soaps of palm oil fatty acids supplemented.
Different letters in the same column indicate significant differences among mean values of diets: a, b, c; different letters in the same row indicate significant differences among mean values of frozen storage duration: y, z; ns = P>0.1; t = P<0.1; * = P≤0.05.

Differences between unfrozen and 2-years frozen pork were significant (Table 3) when comparing the percentage of intramuscular fat (IMF) and concentrations of most individual

fatty acids in the IMF. The intramuscular fat of loin was significantly (P≤0.001) higher in 2-years frozen than unfrozen pork. It could be due to the concentration of fat in muscle by water losses that occur after thawing. On the other hand, the proportion of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 *n*-9) and total saturated (SFA) and monounsaturated fatty acids (MUFA) were significantly higher in 2-years frozen pork. However, proportions of *n*-6 polyunsaturated fatty acid (PUFA), such as linoleic acid (C18:2) and arachidonic acid (C20:4), and proportions of *n*-3 PUFA, such as α-linolenic acid (C18:3) and eicosapentaenoic acid, EPA (C20:5), were significantly lower after freezing. Therefore, the total PUFA percentage was lower in 2-years frozen pork. The muscle content of phospholipids (PL) is relatively constant and contains mainly polyunsaturated fatty acids [11]. Awad *et al.* [12] found that a considerable decrease in the PL content was evident as the frozen storage time of muscle progressed. This drop can be accounted for the enzymic hydrolysis of PL concentration during the frozen storage and it could be related to this drop in total PUFA percentage.

Table 3 Fatty acid composition of intramuscular fat (% of total fatty acids) in the M. *Longissimus dorsi*.

| | unfrozen pork | | 2-years frozen pork | | Sign. |
|---|---|---|---|---|---|
| | x | sd | x | sd | |
| IMF (%) | 2.17a | 0.53 | 3.01b | 0.99 | *** |
| C12:0 | 0.066a | 0.01 | 0.073b | 0.01 | *** |
| C14:0 | 1.11a | 0.12 | 1.26b | 0.15 | *** |
| C16:0 | 22.72a | 0.82 | 23.54b | 0.87 | *** |
| C16:1 | 3.31 | 0.50 | 3.33 | 0.41 | ns |
| C18:0 | 10.50a | 0.81 | 11.19b | 0.82 | *** |
| C18:1 *n*-9 | 39.86a | 2.47 | 41.32b | 2.24 | ** |
| C18:1 *n*-7 | 4.45 | 0.36 | 4.34 | 0.28 | ns |
| C18:2 *n*-6 | 10.50b | 2.39 | 9.39a | 2.25 | * |
| C18:3 *n*-6 | 0.087b | 0.02 | 0.074a | 0.02 | ** |
| C18:3 *n*-3 | 0.40b | 0.06 | 0.36a | 0.08 | *** |
| C20:1 *n*-9 | 0.73 | 0.08 | 0.75 | 0.07 | ns |
| C20:2 *n*-6 | 0.34 | 0.06 | 0.34 | 0.06 | ns |
| C20:2 *n*-3 | 0.11b | 0.03 | 0.08a | 0.03 | *** |
| C20:3 *n*-6 | 0.35b | 0.08 | 0.25a | 0.07 | *** |
| C20:3 *n*-3 | 0.07 | 0.01 | 0.07 | 0.02 | ns |
| C20:4 *n*-6 | 2.23b | 0.70 | 1.58a | 0.65 | *** |
| C20:5 *n*-3 | 0.12b | 0.04 | 0.08a | 0.03 | *** |
| C22:5 *n*-3 | 0.38b | 0.11 | 0.27a | 0.09 | *** |
| C22:6 *n*-3 | 0.16b | 0.05 | 0.10a | 0.04 | *** |
| ∑SFA | 35.17a | 1.42 | 36.82b | 1.49 | *** |
| ∑MUFA | 48.90a | 3.16 | 50.29b | 2.70 | * |
| ∑PUFA | 14.78b | 3.36 | 12.62a | 3.12 | *** |
| ∑*n*-6 | 13.52b | 3.10 | 11.65a | 2.90 | ** |
| ∑*n*-3 | 1.25b | 0.26 | 0.96a | 0.23 | *** |
| P/S ratio | 0.42b | 0.10 | 0.35a | 0.09 | *** |
| *n*-6/*n*-3 ratio | 10.79a | 0.53 | 12.15b | 0.49 | *** |

Different letters in the same row indicate significant differences among mean values; ns = P>0.1; * = P≤0.05; ** = P≤0.01; *** = P≤0.001.

The fatty acid ratios, which are related to human health, are shown too in Table 3. The 2-years frozen pork had the lowest PUFA/SFA ratio and produced meat with less nutritional value (for human consumption), under the nutritional guidelines for PUFA/SFA > 0.4 [13]. Also, the *n*-6/*n*-3 ratio was higher after freezing. It could be due to the *n*-3 fatty acids are more easily oxidized [14] and its drop would be much greater than the *n*-6 fatty acids in frozen pork.

- CONCLUSION

Meat quality parameters were significantly altered by freezing of pork during two years, causing an increase in pH values and drip losses and a decrease in the colour measurements ($a^*$ and $b^*$ values). The intramuscular fatty acid profile also was affected during frozen storage resulting in a decrease in the percentage of PUFA, which modified the remaining percentages and made lipid profile less healthy for human consumption.

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REFERENCES

- Estévez, M., Ventanas, S., Heinonen, M. & Puolanne, E. (2011). Protein carbonylation and water-holding capacity of pork subjected to frozen storage: effect of muscle type, premincing, and packaging. Journal of Agricultural and Food Chemistry 59: 5435-5443.
- Lagerstedt, A., Enfält, L., Johansson, L., & Lundström, K. (2008). Effect of freezing on sensory quality, shear force and water loss in beef M. longissimus dorsi. Meat Science 80: 457-461.
- Pietrasik, Z., & Janz, J. A. M. (2009). Influence of freezing and thawing on the hydration characteristics, quality, and consumer acceptance of whole muscle beef injected with solutions of salt and phosphate. Meat Science 81: 523-532.
- Hallenstvedt, E., Øverland, M., Rehnberg, A., Kjos, N. P. & Thomassen, M. (2012). Sensory quality of short- and long-term frozen stored pork products. Influence of diets varying in polyunsaturated fatty acid (PUFA) content and iodine value. Meat Science 90: 244-251.
- Bertram, H. C., Andersen, R. H. & Andersen, H. J. (2007). Development in myofibrillar water distribution of two pork qualities during 10-month freezer storage. Meat Science 75: 128-133.
- Honikel K.O (1998) Reference methods for the assessment of physical characteristics of meat. Meat Science 49: 447-457.
- Pfalzgraf A., Frigg M. & Steinhart, H. (1995). Alpha- tocopherol contents and lipid oxidation in pork muscle and adipose tissue during storage. Journal of Agricultural and Food Chemistry 43: 1339-1342.
- Bligh E. G. & Dyer, W. J.(1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37: 911-914.
- Devine, C. E., Graham, R. G., Lovatt, S., & Chrystall, B. B. (1995). Chapter 2: Red meats. In L. E. Jeremiah, Freezing effects on food quality (pp. 51–86).
- Hansen, E., Juncher, D., Henckel, P., Karlsson, A., Bertelsen, G. & Skibsted, L. H. (2004). Oxidative stability of chilled pork chops following long term freeze storage. Meat Science 68: 479-484.
- Cameron, N. D. & Enser, M. B. (1991). Fatty acid composition of lipid in Longissimus dorsi muscle of Duroc and British Landrace pigs and its relationship with eating quality. Meat Science 29: 295-307.
- Awad, A., Powrie, W. D. & Fennema, O. (1968). Chemical deterioration of frozen bovine muscle. Journal of Food Science 33: 227-234.
- Department of Health (1994). Nutritional aspects of the cardiovascular disease. Report of health and social subjects No. 46. London: Her Majesty's Stationery Office.
- Lopez-Bote, C. J., Rey, A. I., Sanz, M., Gray, J. I. & Buckley, D. J. (1997). Dietary vegetable oils and α-tocopherol reduce lipid oxidation in rabbit muscle. The Journal of Nutrition 127: 1176-1182.