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Study of shelf life of liver pâté elaborated from Celta pig breed

J.M. Lorenzo*,[1], M.C. García-Fontán*, G. García*, P. Valledor and D. Franco***

*Meat Technology Centre of Galicia, Ourense (Spain)
**Porco Celta Fonsagrada, S.L., Lugo (Spain)
[1]Corresponding author e-mail: jmlorenzo@ceteca.net

Abstract. In the present work, microbial spoilage, lipid oxidation, increase of non-heme iron content and colour changes occurring during refrigerated storage (75 days/4°C) of liver pâtés from Celta pigs were studied. Psychrotrophs and TVC increased during storage reached final values of 6.64 and 7.69 log cfu/g, respectively. *Brochothrix thermosphacta* was detected after 46 days of storage. During refrigerated storage L* values were maintained in a range of 63.8-65.4, whereas redness value decrease during the first point of sampling and after maintained a constant value close to 2.45. On the contrary yellowness value increased in the first 14 storage day, reached a value of 15.3, during the rest of storage period this value decrease until 14.4. Fe-heme content decrease during shelf life period from 17.30 to 13.5 mg Fe-heme/kg liver pâté, whereas Fe-total content presented a inverse relationship, because increase from 110 to 223 mg Fe-total/kg liver pâté. However, this high amount of Fe-total did not seem to be related to oxidative process, because even thought TBAR´S values increase during the firsts 60 days of display period, decreasing in the last sampling point, reaching a final values of 0.015 mg malonaldehyde/kg of liver pâté, this final value was indicate that liver pâté did not undergo important lipid oxidation.

Keywords. Pâté – Celta pig – Refrigeration – Oxidation stability – Non-heme iron.

Étude de la vie utile du pâté élaboré à partir du porc Celta

Résumé. *Dans ce travail, l'étude de divers paramètres a été menée sur du pâté élaboré à partir de porc Celta: il s'agit de la contamination microbienne, l'oxydation lipidique, l'augmentation du fer non héminique et les changements de coloration produits au cours de la réfrigération (75 jours à 4ºC). Les comptages de psychrotrophes et TVC ont augmenté de manière significative (P<0,001) au cours de l'étape de conservation atteignant respectivement des valeurs de 6,64 et 7,69 log ufc/g.* Brochothrix thermosphacta *fut détecté après 46 jours de conservation. Au cours de la conservation à froid, les valeurs de luminosité sont demeurées constantes à 63,8-65,4, tandis que les indices de rouge ont diminué durant le premier point d'échantillonnage, pour se maintenir constants par la suite à une valeur proche de 2,45. En revanche, l'indice de jaune a augmenté au cours des 14 premiers jours de conservation, atteignant une valeur de 15,3 et régressant à un niveau de 14,4 au cours du restant de la période de conservation. La teneur en fer héminique a diminué au cours de la période de vie utile passant de 17,3 à 13,5 mg de fer haem/kg de pâté, tandis que la teneur en fer total a adopté un comportement inverse puisqu'elle a augmenté de 110 à 223 mg Fe-total/kg pâté. Dans tous les cas, la présence de cette quantité de fer total ne semble pas avoir d'influence sur le processus d'oxydation lipidique, car bien que les valeurs de TBAR's aient augmenté au cours de la période de conservation, atteignant 0,015 mg de malonaldéhyde/kg de pâté, elles ne l'ont pas fait de manière assez significative pour pouvoir conclure à une importante oxydation lipidique.*

Mots-clés. *Pâté – Porc Celta – Réfrigération – Stabilité par oxydation – Couleur – Fer non héminique.*

I – Introduction

Apart from microbial spoilage, lipid oxidation is the major factor reducing quality and acceptability of meat and fat products (Morrissey *et al.*, 1998). Lipid oxidation is a complex process whereby polyunsaturated fatty acids are degraded via formation of free radicals, causing flavour, texture, colour and nutritional deterioration of foodstuffs (Gray, 1978). Non-

heme iron (NHI) is considered the most important oxidation promoter in meat systems and, therefore, knowledge of the proportions of the chemical forms of iron is of great importance (Kanner *et al.*, 1991). An increase in the amount of NHI as a result of thermal processes on meat systems has been shown (Lombardi-Boccia, *et al.*, 2002). Miller *et al.* (1994), suggested cooking is not as important as the subsequent refrigerated storage of cooked meats for the release of NHI from myoglobin. The increase of NHI in meats and fish is considered to be a reflection of the decrease of heme iron (HI) as a consequence of the breakdown of the heme molecule during cooking or storage (Gómez-Basauri and Regenstein, 1992a; Gómez-Basauri and Regenstein, 1992b) and this has been linked to the oxidative deterioration of the porphyrin ring of myoglobin (Schricker and Miller, 1983).

The colour of meat products is another important quality attribute that influences consumer acceptance, and a brown-gray colour is preferred for cooked products (Cornforth, 1994). Colour changes in cooked products during refrigerated storage have been linked to oxidation phenomena, and several factors such as the characteristics and amount of fat, the packaging method and the presence of antioxidants have been reported as being influential (Jo and Ahn, 1999).

Liver pâté is a traditional product for which there has been an increasing demand by European consumers in the last 15 years (Rosmini *et al.*, 1996). Liver pâtés contain high amounts of fat and iron, and therefore, oxidative deterioration of liver pâtés during refrigeration is expected. The differences between pâtés from Celta and white pigs in terms of their fatty acid composition and antioxidative status are expected to influence their susceptibility to oxidative deterioration during refrigerated storage.

The aim of the present work was to study the microbial changes of liver pâtés from Celta pigs during refrigerated storage as assessed by lipid oxidation, increase in the amount of NHI and colour deterioration.

II – Materials and methods

1. Experimental design

For the manufacture of the pâtés (1.5 kg), muscles and adipose tissues from Celta pigs were used. In the recipe the ingredients were as follows per 100 g of product: 27 g liver, 26 g adipose tissue, 26 g muscle, 16 g chestnut, 2 g sodium caseinate, 2 g sodium chloride. The procedure for the manufacture of the pâtés has been described by Estévez *et al.* (2004). Liver pâtés were packed in glass containers prior to thermal treatment (80°C/30′). After the containers were allowed to cool at room temperature, they were stored in the dark at 4°C for 75 days from the day of the manufacture (day 0). Liver pâtés were analysed at days 0, 14, 48, 60 and 75 for lipid oxidation, concentration of NHI, instrumental colour and microbial counts. After each of the refrigeration stages, instrumental colour was measured on the surface of the pâtés and then they were stored at −80°C until the analytical measurements were carried out.

2. Analytical methods

A. Microbial analyses

In each liver pâtés unit, after aseptically removing and discarding the outer plastic, 10 g of the product were aseptically taken and homogenized with 90 ml of sterile 0.1% peptone water also containing 0.85% NaCl and 1% Tween 80 as emulsifier, at 40-45°C for 2 min in a Masticator blender (IUL Instruments, Barcelona, Spain), thus making a 1/10 dilution. Successive decimal dilutions were prepared by mixing 1 ml of the previous dilution with 9 ml sterile 0.1% peptone water.

Phychrotroph microflora was enumerated in Standard Plate Count Agar (PCA) agar (Merck), after incubation at 7°C for 10 d; *Enterobacteriaceae* in violet red bile dextrose (VRBD) agar

(Merck) after incubation at 37°C for 24 h; *Staphylococcus aureus* in Baird Parker agar (Merck) + Egg Yolk Tellurite Emulsion (Biokar Diagnostics) incubated at 37°C for 24 h and Sulfite reducing clostridia in Perfringens Selective Agar (SPS) agar (Merck) after incubation at 44ºC for 24 h. Presence or absence of *Salmonella* was investigated by Enzyme Linked Fluorescent Assay (ELFA), VIDAS®-SLM protocol was carried out according to the procedures recommended by the manufacturer. From each sample and on each culture medium, 1 ml of each dilution was inoculated in duplicate on plates and mixed before solidification. Plates of VRBD agar were covered with a layer of the same culture medium before incubation. After incubation, plates with 30-300 colonies were counted.

B. Iron analysis

HI was measured according to the methodology of Hornsey (1956) with the next expressions (Merck, 1989):Hematin (µg hematin/ g muscle) = Absorbance × 342.44 and HI (mg/100 g meat) = (Hematin x 8.82)/100. Total iron (TI) content was measured following the methodology proposed by Lorenzo *et al.* (2003) and NHI was calculated as difference between TI and HI content.

C. Colour measurement

A portable colorimeter (Konica Minolta CR-400 Osaka, Japan) was used to measure liver pâtés colour in the CIELAB space (CIE 1978). (lightness, L*; redness, a*; yellowness, b*)

D. Measurement of TBARs

Lipid stability was evaluated in the liver pâtés using the method proposed by Vyncke (1975) with the modification that samples were incubated at 96 ºC in a forced oven (Memmert UFP600, Germany, Schawabach). Results are expressed as (mg malonaldehyde / kg of fresh meat).

III – Results and discussion

Figure 1 shows microbial spoilage evolution of TVC, psychrotrophs, LAB, enterobacteriaces and *Brochothrix thermosphacta.* All microbial populations increased during storage at 4ºC, reached final values of 7.69, 6.64, 6.74, 5.54 and 3.6 log cfu/g, respectively. *Brochothrix thermosphacta* was detected after 46 days of storage.

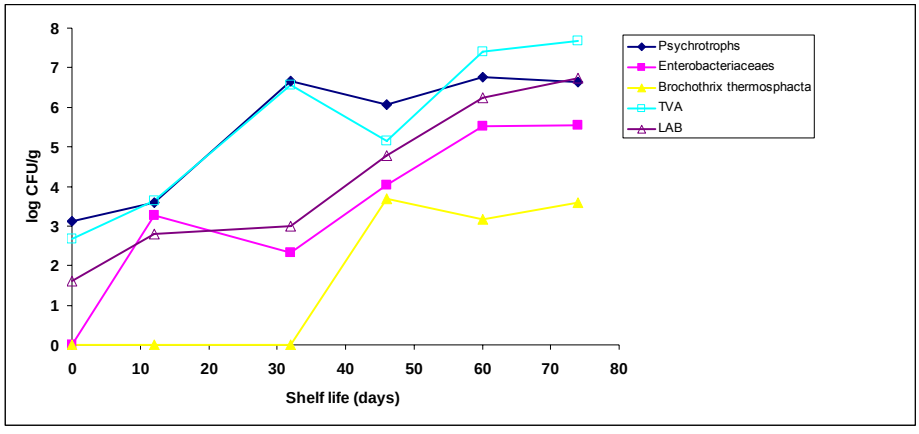

Fig. 1. Evolution of microbial groups (TVC, psychrotrophs, LAB, enterobacteriaces and *Brochothrix thermosphacta*) on liver pâté from Celta pig under refrigeration storage.

Table 1 shows colour characteristics evolution of liver pâté. During refrigerated storage L* values was maintained in a range of 63.8-65.4, whereas redness value decrease during the first point of sampling and after maintained a constant value close to 2.45. On the contrary yellowness value increased in the first 14 storage day, reached a value of 15.3, during the rest of storage period this value decrease until 14.4. Compared to pâtés from Iberian pigs of Estévez and Cava (2004) pâtés from our work presented a darker colour (64.52 *vs* 65.48) with less redness (8.43 *vs* 2.68) and higher yellowness (12.14 *vs* 14.39) during 90 days of storage in similar conditions. Obviously differences in the recipe, colour characterises of the meat and adipose tissue, feeding regime and breed explain these differences.

Table 1. Evolution of colour parameters, ashes, total iron, non-heme and heme iron and lipid oxidation of liver pâté from Celta pig under refrigeration storage

Colour characteristics	Shelf life (days)				
	0	14	48	60	75
Luminosity (L*)	63.84	63.68	64.15	63.93	65.48
Redness (a*)	3.85	2.60	2.40	2.21	2.68
Yellownes (b*)	12.48	15.33	14.55	14.78	14.39
Ashes-Fe content					
Ashes (%)	1.76	2.04	2.12	2.06	2.22
TI (ppm)	110.8	177.0	201.0	178.1	223.0
NHI (ppm)	93.5	164.7	188.8	163.6	209.5
HI (ppm)	17.3	12.3	12.2	14.4	13.5
Lipid oxidation					
TBARS (mg MDA/kg pâté)	0.149	0.136	0.179	0.318	0.015

NHI content increased during refrigerated storage, from 93 to 209 mg/kg pate from day 0 to day 75, whereas HI content decrease during shelf life period from 17.30 to 13.5 mg /kg liver pâté and TI content presented a inverse relationship, because increase from 110 to 223 mg TI/kg liver pâté (Table 1). Results suggest that some disruption of the porphyrin ring could have occurred during storage that led to the release of iron. For Gómez-Basauri and Regenstein (1992a) and Miller *et al.* (1994) the increase of NHI during refrigeration of meat is a reflection of the degradation of HI. Damage in the porphyrin ring during cooking or storage has been suggested to cause the breakdown of heme molecule and the release of iron from globin (Gómez-Basauri and Regenstein, 1992a). The degradation of HI would reduce the nutritional value of the pâtés in terms of bioavailability of iron, since HI is more available than NHI (Hunt and Roughead, 2000).

However, this high amount of TI did not seem to be related to lipid oxidative process, because TBARS of liver pâtés increasing during 60 days of refrigerated storage, decreasing in the last 15 days of shelf life to a close value to 0. This final value indicates that liver pâté did not undergo important lipid oxidation or also it could be explained by disappearance of primary oxidation products. Secondary peroxidation products such peroxides, could be present in the liver pâté but was not measured.

IV – Conclusions

According to this study, lipid oxidation, and the increase of NHI during refrigerated storage of liver pâtés could not be closely related. Colour changes seem not to be linked to oxidative processes and microbial counts. This previous results represent a starting point for the promotion elaborated meat products from this endangered pig breed.

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