*Fermented and dry products*

## PRE-CURE FREEZING AFFECTS PROTEOLYSIS AND WHITE CRYSTALS FORMATION IN DRY-CURED HAMS

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### INTRODUCTION

Dry-cured ham can be produced from refrigerated or frozen raw material. Previous studies have shown that the curing of frozen hams hardly increases the indices of lipid oxidation in lean meat in comparison with refrigerated raw materials (Kemp *et al.*, 1982; Motilva *et al.*, 1994), however, very few studies have dealt with the proteolysis in these hams. The cured meat undergoes an intense proteolysis due to the action of cathepsins and calpains (Sárraga *et al.*, 1993). As a consequence, peptides, free amino acids and other small nitrogenized compounds are generated. The influence on the smell and taste of dry-cured ham has not yet been known in detail (Buscailhon *et al.*, 1994). The freezing and thawing process which the raw material undergoes is another agent capable of altering the muscular proteins, along with the implicit technological consequences during the curing process.

### OBJECTIVES

The objective of this study is to examine the proteolysis and the white crystals formation in dry-cured ham produced with both frozen and refrigerated raw material.

### MATERIAL AND METHODS

*Raw material*: 20 hams were selected and divided into two groups of 10 according to whether the raw material was refrigerated (R) or frozen (at -18ºC) and thawed (4 days at 4ºC) (F) pre-salting. The quality of the meat was checked according to the pH (507 Crison pH-meter and Ingold Xerolyt electrode) and the electrical conductivity (EC, Pork Quality Meter Intek) in the *semimembranosus* (SM). Hams with pH>6 and EC>5mS were rejected. *Drying and curing of hams*: curing (98% sodium chloride, 1% potassium nitrate and 1% sodium nitrite); salting (3ºC / 98% relative humidity "RH"), 19 days for R hams and 15 days for F hams; washing in cold water (4ºC); post-salting (50 days / 7ºC / 84% RH); drying (165 days / 14 ºC / 75-66% RH); heated drying (26 days / 27ºC / 64% RH) and storage (49 days / 12ºC / 51% RH). *Sampling*: the samples were taken by sectioning the ham at the level of the knee (I), 8cm from the knee. Portion A was taken for physical and chemical analysis The samples came from the SM and *biceps femoris* "BF" muscles. *Phisycal and chemical analysis*: moisture (M) (ISO R-1442 1979); chlorides (Bañón *et al.* 1998); intramuscular fat (IF) (ISO R-1433, 1979); total nitrogen (TN) (ISO R-936, 1979); non-protein nitrogen (NPN) (Penedo, 1989); free tyrosine concentration (TYR) (Pearson 1968) and L*a*b* colour values (Minolta Chroma Meter Reflectance. CIE, 1975). The proteolysis index was expressed as 100*NPN/TN (Flores *et al.*, 1985). The free amino acid composition of the isolated white crystals was determined by liquid chromatography (HP Lithium column. Mondino *et al.*, 1972). *Statistical analysis*: The descriptive statistical techniques and simple variance analysis (Scheefe's mean homogeneity test) were performed with the Statistix 3.5 computer program (Analytical Software).

### RESULTS AND DISCUSSIONS

Table 1 shows the mean values and standard deviations for M, NaCl, NaCl/M ratio, IF NPN/TN and TYR. Significant differences ($p<0.05$) were found between refrigerated hams and frozen hams for the mean values of M, NaCl and NaCl/M (SM and BF), IF (SM) and NPN/NT (BF). The salting and drying were more intense in pre-cure frozen meat. The salt penetration is favoured in those meats with more free water, which appears to increase the amount of solubilized salt on the surface of the ham, which in turn is the main factor regulating the penetration (Sörheim & Gumpen, 1986). For this reason, when the raw material used is frozen it is necessary to reduce the salting time (Flores, 1989).

The proteolytic activity is more intense in the BF muscle, due at the fact that its higher moisture and its lower salt content favour the action of cathepsins and calpains especially during the post-salting stage (Gil *et al.*, 1989). NPN/TN was considerably higher in frozen than in refrigerated hams in the BF. The protein alterations during freezing could explain these results. The denatured proteins are especially sensitive to attack of proteolytic enzymes released following the rupture of cellular structures by the ice crystals (Lawrie, 1977). Consequently, thawed meat provides a more favourable environment for muscular proteases, and this explains the considerably higher rates of proteolysis shown by frozen ham.

TYR was similar in hams produced from refrigerated and frozen raw materials. This circumstance does not correspond to the NPN/TN figures observed in BF since, according to these latter, it would be reasonable to expect a greater release of tyrosin in the frozen product. This anomaly could be related to the large number of crystals found in these hams, above all in BF, while these appear only sporadically in the refrigerated product (see Table 1). In this sense, Arnau *et al.*, (1994) point out that the prior freezing of the raw material significantly increases the incidence of precipitates, although the levels of free tyrosine hardly increase. This finding was found also in our study.

The presence of white precipitates is an alteration in dry-cured ham which is closely related to proteins in the literature. The study of the nitrogen present in the precipitates shows that 67% of the TN corresponded to NPN, which was primarily formed by tyrosine (Table 2), as has been suggested by Arnau *et al.*, (1996). However, the increased proteolysis found in the frozen ham compared to refrigerated ham does not seem, at first sight, to be significant enough to explain the higher number of hams affected. This fact leads us to think that other factors may regulate the formation of precipitates, such as the rupture of tissue membranes during freezing, which would favour the nucleation and growth of tyrosine crystals (Nyvlt, 1971). This would explain the presence of proteins in the precipitates.

## CONCLUSSIONS

Pre-cure freezing increases the proteolysis levels significantly in the zones of the ham where water losses and absorption of salt is slowest (BF muscle).

Frozen hams present a high incidence of white precipitates, formed mainly by tyrosine crystals.

The previous freezing and thawing process accentuates the water losses, salt absorption and proteolysis of the dry-cured ham.

## REFERENCES


Arnau, J., Gou, P., Guerrero, L. (1994). *J Sci Food Agri* **66**, 279. Arnau, J., Guerrero, L., Hortós, M., García-Regueiro, J.A. (1996) *J Sci Food Agric* **70**, 449. Bañón, S., Gil, M.D., Granados M.V. and Garrido, M.D. (1998) *Z LebensmUnters For* **206,** 88. Buscailhon, S., Berdagué, J. L., Bousset, J., Cornet, M., Gandemer, G., Touraille, C., Monin, G. (1994). *Meat Sci* **37**, 229. Flores, J., Bermell, S., Nieto, P. (1985) *Rev Agroquim Tec* **25** (3), 400. Flores, J (1989). *Avances en la tecnología del jamón curado*. 1. IATA. Valencia. Gil, M., Arnau, J., Sárraga, C. (1989). *Proc 35th ICoMST*, 734. Kemp, J. D., Langlois, B. E., Johnson, A. E. (1982). *J Food Protect* **45**, 244. Lawrie, R. A. (1977) *Ciencia de la carne*. Acribia Zaragoza. Lide, D.R. (1991) *Handbook of Chemnistry and Physics*. CRC Press. USA. Mondino, A., Bongiovanni, G., Fumero, S., Rossi, L. (1972). *J Chrom,* **74,** 255. Motilva, M.J., Toldrá, F., Nadal, M.I., Flores, J. (1994) *J Food Sci* **59** (2), 303. ISO Norms (1979) Determination of moisture (R-1442), lipids (R-1443) and proteins (R-1841). Nyvlt, J. (1971) *Industrial crystalisation from solutions.* Butterworth, London. Pearson, D. (1968) *J Sci Food Agric* **19**, 366. Penedo, J. C. (1989) Ph.D. thesis, Univ of Córdoba. Sárraga, C., Gil, M., García-Regueiro, J. A. (1993). *J Sci Food Agric* **62**, 71. Sorheim, O., Gumpen, S. A. (1986). *Proc 32nd EMMRW* **2**, 295.


**Table 1.** Drying-salting and proteolysis parameters for pre-cure refrigerated (R) and frozen-thawed (F) dry-cured hams.

| | *Semimembranosus* | | | *Biceps femoris* | | |
|---|---|---|---|---|---|---|
| | R | F | | R | F | |
| | M±SD | M±SD | | M±SD | M±SD | |
| Moisture % | 48.83±2.02 | 46.52±2.36 | * | 62.87±0.83 | 60.47±1.42 | * |
| NaC l% | 4.64±0.45 | 6.06±0.82 | * | 5.41±0.49 | 7.39±0.95 | * |
| NaCl / M% | 9.52±0.97 | 13.05±1.85 | * | 8.61±0.08 | 12.26±1.83 | * |
| IF % | 2.77±0.89 | 4.03±1.31 | * | 2.50±1.05 | 3.14±1.12 | |
| NPN/TN% | 23.3±3.2 | 23.6±2.4 | | 30.6±2.9 | 35.2±4.50 | * |
| TYR | 1.77±0.33 | 1.75±0.18 | | 1.51±0.26 | 1.65±0.25 | |
| Crystals | 0 | 8 | | 2 | 10 | |

*Means are significantly different (P<0.05)
Moisture (M), Intramuscular Fat (IF), Non Protein/Total Nitrogen (NPN/TN) and free Tyrosine concentration (TYR).
Number of hams with white crystals (crystals).

**Table 2.** Free amino acid composition of the white crystals.

| | M ± SD (%) | S |
|---|---|---|
| Aspartic acid | 1.58 ± 0.14 | 5 |
| Threonine | 2.73 ± 1.46 | 97 |
| Serine | 2.05 ± 0.45 | 422 |
| Glutamic acid | 3.08 ± 0.32 | |
| Glycine | 1.97 ± 0.41 | 251 |
| Alanine | 3.62 ± 0.04 | 167 |
| Valine | 2.39 ± 1.32 | 58 |
| Isoleucine | 1.51 ± 0.63 | 34 |
| Leucine | 1.76 ± 0.11 | 23 |
| Tyrosine | 70.54 ± 0.09 | 0.5 |
| Phenylalanine | 5.91 ± 2.04 | 29 |
| Lysine | 1.81 ± 0.54 | 6 |
| Arginine | 1.07 ± 0.16 | 181 |

S: solubility in water at 25°C (g / kg) (Lide *et al.*, 1991).