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Pre-cure freezing affects proteolysis in dry-cured hams

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Abstract

Several parameters (sodium chloride, moisture, intramuscular fat, total nitrogen, non-protein nitrogen, white precipitates, free tyrosine, L^* a^* b^* values and acceptability) related with proteolysis during the curing were compared in dry-cured hams manufactured from refrigerated and frozen/thawed raw material. Pre-cure freezing increased the proteolysis levels significantly ($p < 0.05$) in the zones of the ham where water losses and absorption of salt is slowest. Frozen hams present a high incidence of white precipitates, formed mainly by tyrosine crystals. The colour and acceptability scores are similar in frozen and refrigerated hams. The previous freezing and thawing process accentuates the water losses, salt absorption and proteolysis of the cured meat, although it does not significantly affect the sensory quality of the dry-cured ham.

1. Introduction

Dry-cured ham is a very popular food in Spain, with an annual rate of consumption of 4 kg *per capita* in 1995 (Ferrer, 1996), and is produced from refrigerated or frozen raw material. When the raw material used is frozen it is necessary to reduce the salting time, since the salt penetrates more quickly (Poma, 1987; Flores, 1989). This does not affect the sensory quality of the ham (Poma, 1987; Kemp et al., 1982; Motilva et al., 1994), except for increasing the number of tyrosine crystals in the joint, a phenomenon which can cause certain unfavourable consumer reactions (Arnau et al., 1994). Previous studies have shown that the curing of frozen hams hardly increases the indices of lipid oxidation in lean meat in comparison with frozen raw materials, (Kemp et al., 1982; Motilva et al., 1994), however, very few studies have dealt with the protein changes in these hams and their technological implications.

During the curing process, the meat undergoes an intense proteolysis due to the action of cathepsins and calpains (Sárraga et al., 1993). The proteolytic activity affects the sarcoplasmic and myofibrillar fractions (Penedo, 1989; Córdoba, 1991), and is most intense during the salting and post-salting stages, especially in the muscles situated next to the the backfat (Gill et al., 1989), due to the fact that their high level of moisture and their low salt concentration create favourable conditions for the action of cathepsins and calpains (Baldini et al., 1977). As a consequence of proteilytic activity, peptides, free amino acids and other small nitrogenized compounds are generated (Giolitti et al., 1972; Baldini et al., 1977; Palmia, 1987; Toldrá and Aristoy, 1993; Buscailhon and Monin, 1994; Rodríguez-Núñez et al., 1995). The influence on the smell and taste of dry-cured ham is 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.

The objective of this study is to examine the proteolysis in dry-cured ham produced with both frozen and refrigerated raw material, looking also at the relationship of frozen/refrigerated material to the quality of the finished product.

2. Material and method

2.1. Sampling

In order to obtain the material for the study, 20 hams weighing 11 ± 1 kg were selected, and divided into two groups of 10 according to whether the raw material was refrigerated (2 days at 2–3°C) or frozen (at −26 until −18°C was reached in the centre of the ham) and thawed (4 days at 4°C) pre-salting. The quality of the

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meat was checked according to the pH value and the electrical conductivity (EC) in the *semimembranosus* muscle, and hams with pH > 6 and EC > 5 mS were rejected. The pH was measured with a portable 507 pH-meter (Crison Instruments) using a combined penetration electrode Xerolyt Cat. 10.406 3123 (Ingold Electrodes Inc.) with ranges of operation from 2 to 11 for pH and 0–80°C temperature. The EC was measured with a Pork Quality Meter (Intek) equipped with two cone-tipped steel electrodes 6 mm in diameter, 70 mm long and 15 mm apart. This instrument works with continuous current within a scale of 0–20 mS with 0.5% precision, and with optimal temperatures of between 10 and 40°C.

The curing process was as follows: curing with fine dry salt (98% sodium chloride, 1% potassium nitrate and 1% sodium nitrite); salting with coarse sea salt (3°C/88% relative humidity 'RH'), 19 days for the refrigerated hams and 15 days for frozen hams; washing in cold water (4°C); post-salting (50 days/7°C/84% RH); first drying (105 days/14°C/75% RH); second drying (31 days/22°C/66% RH); heated drying (26 days/27°C/64% RH). Storage (49 days/12°C/51% RH). The temperature and relative humidity during production were registered on an SP3R (Digitron Instrumentation Ltd.) thermohydrograph with working ranges of −20°C to +60°C and 25–90% RH, with precision of 1^{o}C and 5% RH.

The samples were taken by sectioning the ham at the level of the knee (I), 8 cm from the knee (II) and 8 cm from the butt (III) (Fig. 1). Portions A and C were taken for physical and chemical analysis, and portion B was for sensory analysis. The samples for physical and chemical analysis came from 4 different zones of the ham, representing external or superficial muscles ('EXT'), intermediate or middle muscles ('MID'), internal or deep muscles ('INT'), and muscles from the butt ('BUT'). The colour was measured at cutting level II and the thickness of the adipose panicle was measured at cutting level III. The joints for sensory analysis were machine-cut transversally across the muscle fibres, and were approximately 2 mm thick, 4 cm long and 2.5 cm wide. The samples were divided into 3 groups according to their degree of moisture.

3. Methods

In order to determine the moisture content, the sample (5 g) was dried at 105°C for 24 h (6760 sterilizer, Heraeus Inst.; basic BP scales, Sartorius AG) (ISO R-1442, 1979). The chlorides were determined following the modified Carpenter-Volhard method (ISO 1841, 1979). The sample (0.5 g) was mineralized at 200°C with nitric acid (2 ml) and 5% potassium permanganate (10 ml) (Wistreich, 1959), there being present an excess of silver nitrate 0.1N (10 ml) which was valued by electrometry with hydrochloric acid 0.1N (Titrino SM 702, Metrohm AG Instruments Ltd.) using a silver electrode (Metrohm n° 6 04 04100).

The intramuscular fat (IF) was extracted using the Soxhlet method (ISO R-1433, 1979). The sample was

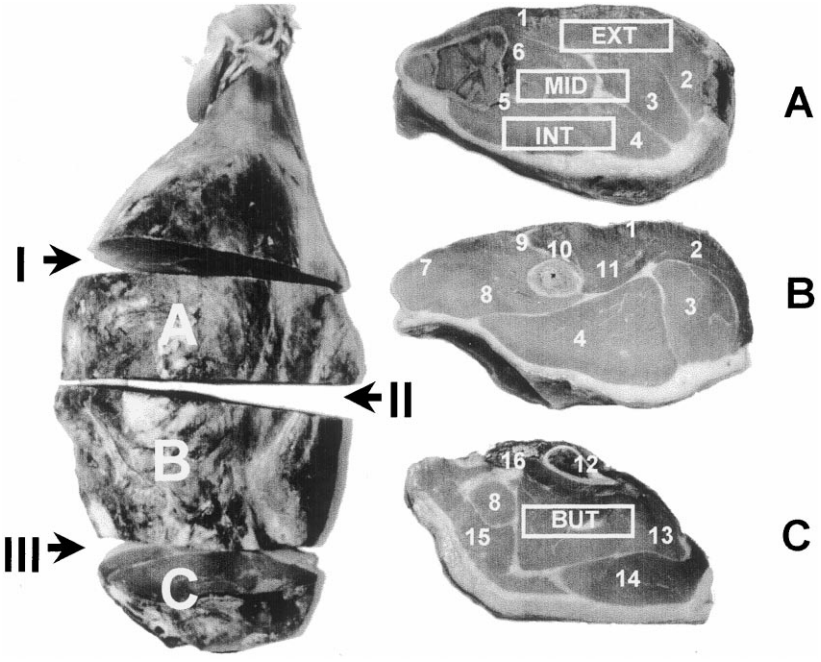

Fig. 1. Dry-cured ham sampling: cross-sections and sample location. *Muscles*: 1. Gracilis. 2. Semimembranosus. 3. Semitendinosus. 4. Biceps femoris. 5. Flexor digitorum. superficialis. 6. Gastrocnemii. 7. Tensor faciae latae. 8. Quadriceps femoris. 9. Sartorius. 10. Pectineus. 11. Adductor. 12. Obturatorii externi. 13. Gluteus profundi. 14. Gluteobiceps. 15. Gluteus medius. 16. Iliopsoas.

dehydrated (2 g) and subjected for 75 min to a 40–60°C petroleum ether circuit at 80°C (Soxtec System HT2 extractor, Tecator Ltd).

The total nitrogen (TN) was determined following the Kjeldahl method (ISO R-936, 1979). The sample (0.25 g) was digested (426 digestor, Büchi Labortechnik) with sulphuric acid (10 ml) and a catalyst (1 g) (4% copper sulphate (II) penta hydrate, 3% selenium and 86% potassium sulphate), distilled with 40% sodium hydroxide (75 ml) for 5 min (Büchi 323 distiller), collected in an excess of boric acid and valued with hydrochloric acid 0.1N (pH electrode Metrohm n° 6 02 03100).

In order to obtain the non-protein nitrogen fraction (NPN), the sample (2 g) was first homogenized with water (50 ml) and 20% tri-chloroacetic acid (15 ml), and then filtered (Penedo, 1989). The results were expressed as 100*NPN/TN (Flores et al., 1985).

The content of free tyrosine (TYR) was determined following the method of Pearson (1968). The absorbance of the sample (1 ml of de-proteinized filtrate, 10 ml of sodium hydroxide 0.5 N and 1 ml of Folin-Ciolcateau solution) was measured at a wavelength of 660 nm (DU 65 spectrophotometer, Beckman RIIC Ltd.).

The free amino acid composition of white crystals was determined by liquid chromatography using a 200×4.6 mm HP Lithium 4152 column (amino acid analyser Pharmacia LKB, Alpha Plus). The crystals (2 g approximately) were isolated, gringed and dessicated. 0.05 g of crystal taken at random was deproteinized with 5% sulfosalicylic acid (50 ml), homogenized for 1 h at 4°C and filtered through a 0.22 μm pore-size filter membrane (Millipore), adjusting the pH of the filtrate to 2.2 with 0.3 M lithium hydroxide (Mondino et al., 1972).

The colour was measured by reflection (Chroma Meter II Reflectance CR-200, Minolta Ltd.). This colour meter works with diffuse light at an angle of observation of 0°, the light being projected onto the surface of the meat through a window with a diameter of 8 mm. The results were expressed in the L^* a^* b^* values of the CIELab system (CIE, 1975).

For the sensorial study, 212 panelists chosen at random rated the acceptability of the dry-cured ham following a mixed hedonic scale (5: very good; 4: good; 3: average, 2: poor; 1: very poor) in a sensorial test carried out in accordance with UNE 87004 (1979).

3.1. 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).

4. Results and discussion

4.1. Proteolysis

Just as in the case of other factors, such as temperature, the action of water or the pH value, the proteolytic activity during the curing process is regulated by the concentration of salt in the ham, which has a curbing influence on such activity (Sárraga et al., 1989; Toldrá et al., 1992). Table 1 shows the mean values and standard deviations for the moisture, NaCl and IF content. The highest moisture content was found in INT, followed by MID, BUT and EXT. The muscles situated next to the skin and the backfat seem to retain more water after curing than the muscles not covered by skin (Córdoba, 1991; Pineda and Carrascosa, 1993; Arnau et al., 1995). The highest concentration of NaCl was found in BUT, a zone characterized by rapid salt penetration and a high level of dehydration (Arnau et al., 1995), followed by INT, MID and EXT. The highest IF was found in MID, followed by BUT, EXT and INT. Frozen ham showed a significantly higher level ($p < 0.05$) of NaCl compared to refrigerated ham in the 4 zones studied. This contrasted with the lower figures for moisture content, as described by Motilva et al. (1994): this seems surprising given the shorter salting time and higher IF. As a result, 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 (Sorheim and Gumpen, 1986).

Table 1
Moisture, NaCl and intramuscular fat for pre-cure refrigerated (R) and frozen/thawed (F) dry-cured hams

	Moisture (g kg^{-1})			NaCl (g kg^{-1})			Intramuscular fat (g kg^{-1})		
	R	F	F-R	R	F	F-R	R	F	F-R
	M ± S.D.	M ± S.D.		M ± S.D.	M ± S.D.		M ± S.D.	M ± S.D.	
INT	628.7 ± 8.3	604.7 ± 14.2	−24.0*	54.1 ± 4.9	73.9 ± 9.5	19.8*	25.0 ± 10.5	31.4 ± 11.2	6.4
MID	605.6 ± 17.3	581.9 ± 21.5	−23.7*	52.4 ± 4.5	72.2 ± 9.5	19.8*	30.4 ± 12.3	43.8 ± 24.4	13.3
EXT	488.3 ± 20.2	465.2 ± 23.6	−23.1*	46.4 ± 4.5	60.6 ± 8.2	14.2*	27.7 ± 8.9	40.3 ± 13.1	12.6*
BUT	541.8 ± 16.3	502.2 ± 18.4	−39.6*	60.4 ± 7.1	79.0 ± 11.4	18.6*	27.0 ± 6.1	42.0 ± 12.0	15.0*
Mean	566.1 ± 13.4	538.5 ± 13.2	−27.6*	53.3 ± 4.6	71.4 ± 9.2	18.1*	27.5 ± 7.8	39.3 ± 13.3	11.8

*Means are significantly different ($p < 0.05$).

Table 2
Non protein/total nitrogen (NPN/TN), free tyrosine concentration (TYR) and precipitates incidence for pre-cure refrigerated (R) and frozen/thawed (F) dry-cured hams

	NPN/TN (%)			TYR (g kg^{-1})			Precipitates[a]		
	R	F	F-R	R	F	F-R	R	F	F-R
	M ± S.D.	M ± S.D.		M ± S.D.	M ± S.D.		N	N	
INT	30.6 ± 2.9	35.2 ± 4.5	4.6*	1.51 ± 0.26	1.65 ± 0.25	0.14	2	10	8
MID	30.3 ± 2.3	33.7 ± 2.9	3.4*	1.50 ± 0.19	1.59 ± 0.25	0.09	1	10	9
EXT	23.3 ± 3.2	23.6 ± 2.4	0.3	1.77 ± 0.33	1.75 ± 0.18	−0.02	0	8	8
BUT	26.9 ± 3.2	26.9 ± 3.1	0.0	1.54 ± 0.25	1.62 ± 0.15	0.08	1	10	9
Mean	27.8 ± 2.5	29.8 ± 2.8	2.0	1.58 ± 0.22	1.65 ± 0.17	0.07	1 ± 0.7	10 ± 0.9	8.5

*Means are significantly different ($p < 0.05$).
[a] Number of hams with precipitates.

Table 2 shows the mean values and standard deviations for NPN/TN and TYR. Significant differences ($p < 0.05$) were found between refrigerated hams and frozen hams for the mean values of NPN/TN (INT and MID). NPN/TN was considerably higher in frozen than in refrigerated hams in the internal zones (INT and MID). The protein alterations during freezing could explain these results, since the intramuscular water produces a certain denaturation upon crystalization, due to a double mechanism of ionic strength and translocation of the intra-extracellular water: in addition, 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.

4.2. Free tyrosine and tyrosine crystals

The presence of white precipitates is an alteration in dry-cured ham which is closely related to the changes in its proteins. The precipitates can appear in crystalline form, or as a white veil which covers the surface of the joint, this last case being more frequent in vacuum-packed slices. The release of tyrosine during the curing process, as in the case of other aminoacids, has an enzymatic origin (Toldrá and Etherington, 1988; Sárraga et al., 1993). It depends on the factors which regulate the cathepsin and calpain activity, although tyrosine is less soluble in water than other amino acids found in meat, having a special tendency to form precipitates when the moisture content of the ham falls during the curing process. According to our results, TYR was similar in hams produced from refrigerated and frozen raw materials. This circumstance does not correspond to the NPN/TN figures observed since, according to these latter, it would be reasonable to expect a greater release of tyrosine in the frozen product. This anomaly could be related to the large number of crystals found in these hams, above all in INT and MID, while these appear only sporadically in the refrigerated product (see Table 2). 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 also found in our study.

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 3), as has been suggested by other authors (Butz et al., 1974; Schneider, 1979; Comi et al., 1981; Silla et al., 1985; 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.

Table 3
Free amino acid composition of the white precipitates

	M ± S.D. (%)	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^{-1}) (Lide, 1991).

4.3. Colour

The normal colour of dry-cured meats depends on three factors: the concentration of pigment in the tissue, the degree of nitrosated pigment conversion and the condition of the proteins in the meat (Jay and Fox, 1994). The results obtained indicate that L^* was similar for both types of ham (R:39.4 ± 2.9; F:39.2 ± 1.5), while a slight lowering could be observed in a^* (R:11.6 ± 4.0; F:9.3 ± 1.2) and b^* (R:5.5 ± 5.8; F:2.8 ± 0.7) in the frozen product compared to the refrigerated product. Following on from this, the pre-freezing of meat should not affect the colour of dry-cured ham, since the nitric oxide is able to form strong co-ordinated covalent links with the heminic iron, independently of state of oxidization or the globin structure (Jay and Fox, 1994). On this subject, Lawrie (1977) points out that the stability of the red colour of cured bacon is increased when the nitrosomyoglobin is converted into myohemochromogenic nitric oxide, a pigment whose globulin fraction is unbalanced by the action of the curing agents, such as salt or temperature. So, it seems that the same agents which alter the colour of fresh meat favour colour retention in dry-cured products.

4.4. Acceptability

The sensory quality of dry-cured ham depends on a large number of chemical compounds, many of them still unknown, most of which come from the oxidation of lipids (Buscailhon et al., 1994). Taste is the main organoleptic attribute contributing to the acceptability of the ham, whilst salt seems to be relatively unimportant (León et al., 1984; Rovira et al., 1996). The panelists taking part in the experiment reported that they normally ate dry-cured ham between 'a few times per week' and 'a few times per month'. The ham was considered to be between average and good, both in the case of the frozen product (3.6 ± 0.8) and in the case of the refrigerated product (3.4 ± 0.7). In the light of this, it seems that the freezing of the raw material hardly influences the consumer's opinion of the product, and, consequently, the differences in salting, drying, proteolysis and appearance would not seem to have any significant effect on the quality of dry-cured ham. On this point, other authors agree that the sensorial quality of dry-cured ham is retained when the raw material is frozen (Kemp et al., 1982; Motilva et al., 1994), and, according to some, it may even be better (Poma, 1987).

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