ELSEVIER

Meat Science, Vol. 49, No. 2, 145–153, 1998

PII: S0309-1740(97)00129-0 0309-1740/98 $19.00+0.00

Effects of Salt and Temperature on Proteolysis During Ripening of Iberian Ham

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(Received 26 July 1997; revised version received 6 October 1997; accepted 1 November 1997)

ABSTRACT

Fifty-five hams from Iberian pigs were processed using two different dry-curing techniques, traditional and modern. Salt content, non-protein nitrogen and its fractions (peptide, amino acid and volatile basic nitrogen) from Biceps femoris *muscles were quantified. The existence of an overlapping effect of both temperature and salt content on the general non-protein nitrogen production was observed. The most intense proteolytic breakdown took place when higher temperatures were reached during the drying stage. The difference in salt concentration seems to contribute to generating different quantities in the non-protein nitrogen fractions. The inclusion at the end of the cellar stage of a stuffing period would permit increasing the accumulation of free amino acid in high salted hams.* © *1998 Elsevier Science Ltd. All rights reserved*

INTRODUCTION

The development of desirable texture and flavor in high-quality Iberian dry-cured ham requires a long process of about 18–24 months. The traditional processing of this meat product involves dry-salting of hind legs (of about 9–10 kg) in piles with alternate beds of hams and sea salt for 15 days at temperatures below 5°C. Due to current tendency to consume low salted products, in modern process of Iberian ham this salting period is reduced to 9–10 days. When salt diffusion is completed, that usually takes 3 months in the traditional way, the hams are left in drying rooms at environmental conditions during 1–2 months in the summertime. In this stage temperatures are over 20°C, which allows intense drying and fat melting, the so-called 'sweating'. Then, the hams are transferred to a cellar and the ripening continues for a period that generally exceeds 12 months at mild temperatures (12–18°C), there being usually a slight temperature increase at the end of processing during the summer. In modern processing both drying and cellar stages are developed under controlled conditions of temperature and relative humidity. The variations of the climatic conditions could lead to large differences in the sensory characteristics

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of Iberian ham ripened in the traditional way, while more homogenous hams could be obtained in modern processes. Iberian ham matured in both traditional and modern ways have attained high degree of consumer acceptance and have been recognized for Protected Designation of Origin (PDO) by the European Union.

In Iberian ham, the chemical and biochemical changes occurring during the successive steps of processing have been investigated (Antequera *et al.*, 1992; Córboda *et al.*, 1994*a*), as well as the volatile and non-volatile compounds present in cured hams (García *et al.*, 1991; Córdoba *et al.*, 1994*b*). By contrast, no study has so far been reported on the effects of variations of the elaboration process on the proteolysis that take place during ripening, which could be of great importance in the formation of desirable flavor in the final products.

During ripening of Iberian ham and other types of hams (Parma, Serrano, French and Country-style), the proteolytic breakdown of meat proteins results in an increase of non-protein nitrogen concentration (McCain *et al.*, 1968; Cantoni *et al.*, 1974; Buscailhon *et al.*, 1994; Córdoba *et al.*, 1994*a*). These protein changes could be an important source of flavor compounds in dry-cured hams through the involvement of amino acids, small peptides and Maillard-reactions products (Ventanas *et al.*, 1992; Córdoba *et al.*, 1994*b*; Aristoy and Toldrá, 1995). Such modifications in the proteins should be more marked in Iberian hams than in other meat products due to the long ageing time and the high temperatures that characterize some periods of its ripening.

Among the many different types of end products derived from proteins, peptides and amino acids account for the two main components of the perchloric-soluble fraction in the previously mentioned dry-cured hams. Whereas high amounts of free amino acids have been widely recognised as factors capable of enhancing sensory quality of cured hams (Toldrá *et al.*, 1995), an uncontrolled proteolysis has been associated with unacceptable aftertastes which impair the flavor and causes abnormal softness in Italian long-aged heavy hams (Parolari *et al.*, 1994; Virgili *et al.*, 1995). Other authors indicated that some free amino acids, amines and peptides are compounds particularly reported in defective hams (Butz *et al.*, 1974; Toldrá *et al.*, 1990; Arnau *et al.*, 1994). In spite of the influence on the flavor of all these compounds, very little is known about the processing conditions that control their production in dry-cured hams.

The purpose of this study was to investigate the effect of salt concentrations, and temperature of maturing on the release of three non-protein nitrogen fractions: peptide nitrogen, amino acid nitrogen and volatile basic nitrogen.

MATERIALS AND METHODS

Processing of hams

Fifty-five homogeneous thighs of about 10 kg were obtained from Iberian pigs (160 kg live weight) that were grown extensively in pasture with acorns from *Quercus ilex* and *Quercus suber*. The hams were processed in two different plants. The first one was working in a *Traditional* way (processing T), after salting in a pile of salt for 1·5 days kg^{-1}, the remaining time being at low temperature, the drying and ageing steps were made at environmental conditions. The second plant was using a *Modern* system (processing M) with a salting time reduced to 1 day kg^{-1} in order to obtain a less salted product that would meet consumers' demands. In this plant all processing was developed in controlled conditions of temperature and relative humidity.

The evolution of temperature in both plants and the ham salt content are shown in Fig. 1. The processing steps and the number of hams removed for testing at each stage were as follows:

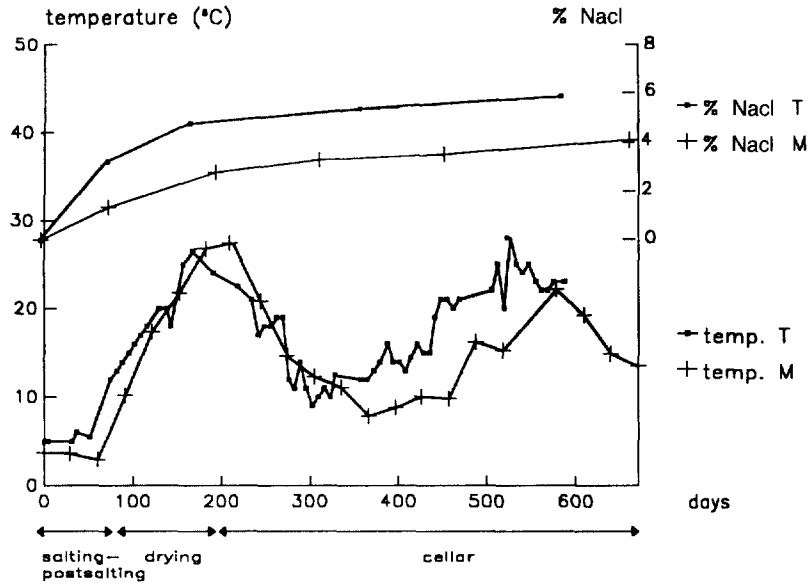

Fig. 1. Environmental temperature and *Biceps femoris* chloride content in the traditional (T) and modern (M) processing of Iberian ham.

	Traditional	Modern
Green State	0 days ($n=5$)	0 days ($n=5$)
Salting-postsalting	75 days ($n=5$)	76 days ($n=5$)
Drying	168 days ($n=4$)	197 days ($n=6$)
Four months cellar		314 days ($n=6$)
Six months cellar	360 days ($n=5$)	
Eight months cellar		456 days ($n=5$)
Fully matured hams	588 days ($n=5$)	665 days ($n=4$)

Entire deep *Biceps femoris* muscles were excised from the hams, minced and homogeneized, and used for analysis.

Analytical methods

Moisture was determined following the ISO recommended methods (ISO/1442, 1973).
Salt content was estimated as chlorides, which were extracted with water–ethanol (60:40 v/v) and quantified by the Carpentier Volhard method (AOAC, 1984).
Intramuscular fat was extracted from samples with a chloroform: methanol mixture (2:1) by the method of Folch *et al.* (1957).
pH was measured with a Crison pH-meter 2001 instrument by mixing 10 g of hams with 10 ml of distillate water.
Non-protein nitrogen (NPN) was analyzed in the extracts of muscles made with 0·6 N perchloric acid following the Johnson (1941) method.
Amino acid nitrogen (AN) was determined from the 0·6 N perchloric acid soluble fraction of ham after peptide precipitation with sulfosalicilic acid 10%, according to Moore and Stein (1954).

Peptide nitrogen (PeN) was quantified by the difference between the amino acid nitrogen content after hydrolysis of peptides with 6 N chloride acid of the 0·6 N perchloric acid soluble fraction of hams and the AN previously determined.

Volatile basic nitrogen (VBN) content was determined in the extract of muscles made with 0·6 N perchloric acid using the method of Pearson (1968).

Statistical analysis

The comparison between means from processing T and M was made using Student *t*-test. Differences between stages were performed by the analysis of variance, using the Bonferroni test by StatGraphics 5·0 software package from Statistical Graphics Corp. (Rockville, MD).

RESULTS AND DISCUSSION

The composition of the dry-cured hams (*Biceps femoris* muscle) at the end of the processing is shown in Table 1. Hams processed using both traditional and modern techniques, appeared to have matured normally according to moisture and fat content, pH values, chloride content and NPN. These observations correlate with variations standards for Iberian hams reported by León Crespo *et al.* (1982) and López-Bote *et al.* (1990). As expected, sodium chloride concentrations were greater ($p < 0·05$) in traditional cured hams (processing T), because of more prolonged salt uptake in the pile of salt. In agreement with findings showing that proteolytic activity decreases in dry-cured hams with salt content (Sárraga *et al.*, 1989; Toldrá *et al.*, 1993*a*,*b*; Virgili *et al.*, 1995), the average value of NPN was higher in high moisture and less salted samples. However, the overall effect of salt concentrations on the enzyme-linked protein breakdown was limited, as differences in NPN levels were not significant ($p < 0·05$). The NaCl inhibition appeared counterbalanced by the enhancement of protein cleavage of other processing conditions such as temperature, which was higher in most processing steps of hams with high salt content. In this sense, previous works by Rico *et al.* (1991) and Toldrá *et al.* (1993*a*,*b*) showed that the proteolytic enzymes were found still quite active at the usual water activity values at the end of the processing (0·85–0·90), despite low water activity reducing the activity of cathepsins and other muscle enzymes such as aminopeptidases. In relation to temperature, Toldrá *et al.* (1993*a*, 1993*b*) indicated that these enzymes showed a maximum activity at around 35°C, close to the temperatures of particular phases (drying) during the dry-curing process. Flores *et al.* (1984) also reported higher amounts of NPN in Serrano dry-cured hams when the processing included 15 days at 28–30°C as compared with those kept at 18–22°C. It was also reported by Virgili *et al.* (1995) that NPN was higher in any hams

TABLE 1
Means and Standard Errors of the Composition from *Biceps Femoris* Muscle in Fully Matured Iberian Ham

	Processing T	*Processing M*
Moisture (%)	48·67 ± 0·53	47·28 ± 0·28
Intramuscular fat (%)	12·69 ± 1·29	11·16 ± 1·05
pH	5·94 ± 0·01	5·92 ± 0·08
NaCl (%)	$5·85^{a}$ ± 0·74	$4·09^{b}$ ± 0·16
NPN (mg g^{-1} DM)	23·78 ± 1·12	25·48 ± 1·08

Means with different letters in the same row are significantly different ($p < 0·05$).

held at 18°C than in their counterparts at 15°C. The existence of an overlapping effect of both temperature and salt on the general NPN production can be assessed from the evolution of NPN along the processing of hams in the two plants (Fig. 2). The NPN levels seemed to progress according to temperature. During salting and postsalting the release of NPN was lower in the hams from processing M, because in this plant the rise of temperature was delayed. In contrast, a more intense proteolysis breakdown took place in the following stage in these hams due to the longer drying stage, specially when temperature of processing was higher (Fig. 1). While the hams were kept in the cellar, the NPN levels remained at a fairly constant level in processing M. On the other hand, the NPN amount observed in hams from processing T is consistent with the superior temperature measured in this plant during the complete cellar stage. It is also noteworthy, in hams from processing T, that the increase of NPN is related with the sudden rise of temperature observed during the latest months of ripening. The NPN production did not follow the same progression as the sodium chloride content measured in the hams. NPN increased in both processes at a high rate during salting, and increased again markedly during the drying period, in spite of salt concentration being always higher than 3·5%. This increase could be related to the relatively high temperatures (up to 25°C) reached during this particular step in the processing of Iberian hams. Although several authors have reported a high rate of NPN production after salting in Parma ham (Bellati *et al.*, 1985) and Serrano ham (Flores *et al.*, 1984), none of the above works describe an increase of NPN as high as the one we observed during drying. Despite this, in the hams from processing T, with high salt content during the drying, proteolysis is clearly less active, as evidence by lower NPN levels ($p < 0{\cdot}05$). However, the higher temperature at the end of processing in high salted hams leads to a major increase in NPN levels in Parma hams. Thus, although the rate of increase of NPN was different in the two process studied, the overall NPN values were indeed very close at the end of ripening.

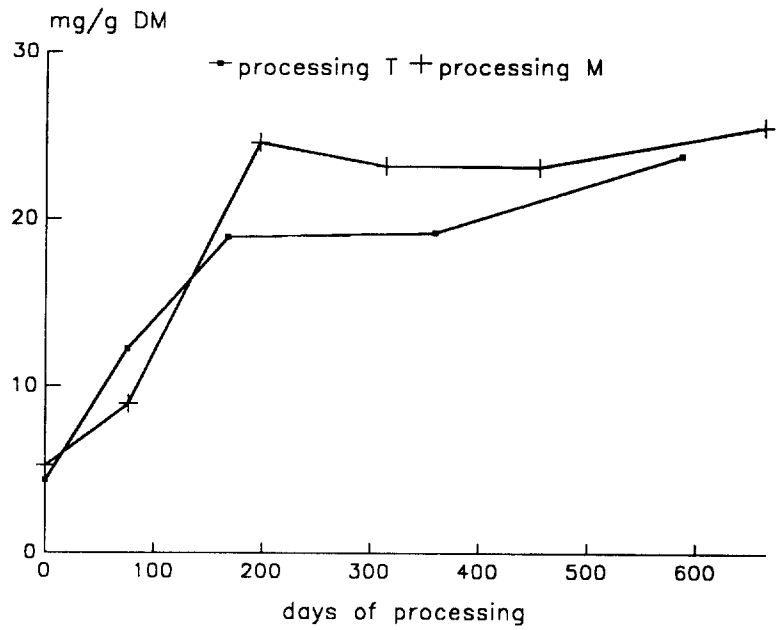

Fig. 2. Levels of non-protein nitrogen during the traditional (T) and modern (M) processing of Iberian ham.

The evolution of the three non-protein nitrogen fractions (AN, PeN and VBN) during both processes of ham maturing is shown in Figs 3 and 4. In process T, as expected, the most intense proteolytic breakdown took place during the drying stage, when a higher temperature of processing was reached. Also, during the latest months of ageing the AN and VBN levels rose significantly ($p < 0{\cdot}05$) when the temperature reached values up to 20–25°C. This significant increase in AN was apparently due exclusively to the formation of free amino acids at the expenses of peptides. However, the rate of AN formation at the end of the processing period could have been greater than observed due to its degradation to volatile derivatives as revealed by both the increase of VBN and the presence of Streeker aldehydes derived from amino acids in the volatile fraction of Iberian ham (García *et al.*, 1991). The increase of VBN at the end of processing T could be related to the enhancement of the activity of microorganisms growing on the hams, when a new increase of temperature takes place. In this sense, haloterant organisms, such as Micrococcaceae and moulds, have been reported in advanced stages of Iberian ham process (Rodríguez *et al.*, 1994; Nuñez *et al.*, 1996).

During processing M, an activation occurred in these low-salted hams by high temperatures of the drying step, as evidenced by the strong increase of AN and PeN. AN reached the maximum levels and later its concentration levelled off during ripening. In contrast to the large decrease of PeN and the increase of AN during the cellar stage in processing T, no relevant changes in both PeN and AN amounts were detected during this period in processing M. These observations are in accordance with the overall NPN evolution in this plant.

The fully matured hams from processing T contained higher amounts of amino acids and VBN, and peptides were more abundant in the samples from processing M (Table 2). The higher abundance of AN in high salted hams is apparently in contradiction with published data reporting proteolytic inhibition with salt (Sárraga *et al.*, 1989). However,

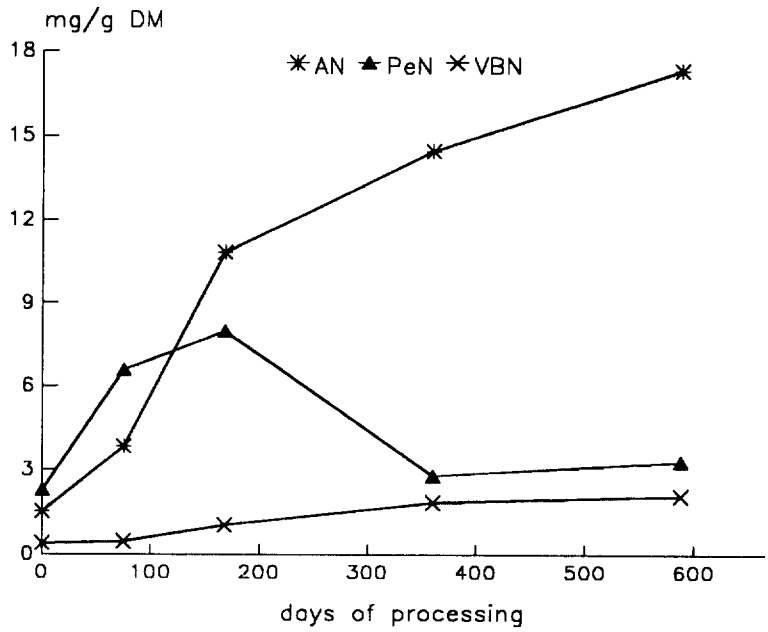

Fig. 3. Levels of amino acid nitrogen (AN), peptide nitrogen (PeN) and volatile basic nitrogen (VBN) during the traditional (T) processing of Iberian ham.

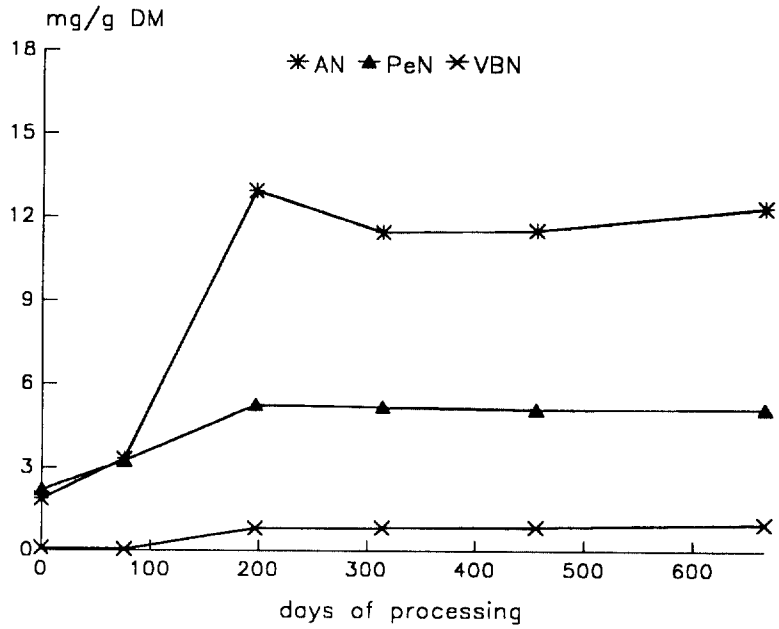

Fig. 4. Levels of amino acid nitrogen (AN), peptide nitrogen (PeN) and volatile basic nitrogen (VBN) during the modern (M) processing of Iberian ham.

unlike other proteinases, both exopeptidases and aminopeptidases (enzymes directly responsible of amino acids release) remain active through the ham's life span (Toldrá *et al.*, 1992). Thus, the main parameter which regulates the AN formation in hams over the range of salt concentrations studied is temperature. As showed in Fig. 3, AN production only took place during the warm steps (drying period in both batches, and cellar only in processing T). Thus, the inclusion at the end of the cellar stage of a stuffing period with temperatures close to 25°C could permit an increase in the accumulation of free amino acids in these high-salted hams.

The observed enhancement of PeN in hams with lower salt levels (processing M) agrees with findings showing that cathepsin activity decreases upon salt addition (Sárraga *et al.*, 1989). These inhibition effects of NaCl on proteinase-induced reactions are evident from the intermediate to the final steps in high-salted hams (Fig. 3). One may speculate why the level of salt had no marked effects on the amino acid concentration whereas the accumulation of peptides is depressed. Two explanations could be proposed. *In vitro* experiments have shown that salt can be a powerful inhibitor for most of the proteinases, which release large fragments from protein, in particular cathepsin D and cathepsin H (Rico *et al.*,

TABLE 2

Means and Standard Errors of Amino Acid Nitrogen (AN), Peptide Nitrogen (PeN) and Volatile Basic Nitrogen (VBN) from *Biceps Femoris* Muscle in Fully Matured Iberian Ham

	Processing T	*Processing M*
AN (mg g^{-1} DM)	$17{\cdot}28^{a} \pm 0{\cdot}51$	$12{\cdot}30^{b} \pm 1{\cdot}43$
PeN (mg g^{-1} DM)	$3{\cdot}29 \pm 0{\cdot}42$	$5{\cdot}13 \pm 0{\cdot}83$
VBN (mg g^{-1} DM)	$2{\cdot}08^{a} \pm 0{\cdot}13$	$0{\cdot}99^{b} \pm 0{\cdot}04$

Means with different letters in the same row are significantly different ($p < 0{\cdot}05$).

1990,1991). It was also reported by Toldrá and Etherington (1988) that a decrease in such proteinase activity occurs during curing time. However, cathepsin D seems to be the main contributor to myosin cleavage during the ripening of Iberian ham (Córdoba *et al.*, 1994*a*). Beside, it has been described that certain exopeptidases present in raw meat remain very active during the whole production process (Toldrá *et al.*, 1992; Verplaetse, 1994). On the other hand, it is possible that the marked insolubilization of meat proteins observed by Córdoba *et al.* (1994*a*) during the processing of Iberian hams interfered selectively with the enzymatic activity. The process of protein aggregation in which the myofibrillar proteins are involved is favoured by both salt concentrations (greater than 0·4 M) and high temperature (Samejima *et al.*, 1985). Therefore, these critical conditions could be met in the high-salted samples after post-salting, causing difficulties to release the peptides during the subsequent ripening steps. If low salt processing is used, the structure of the myofibrillar substrate can remain substantially unaltered and may suffer extensive proteolysis when activated by temperature.

From these results it can be concluded that the conditions of processing (temperature and salt concentrations of the hams) could determine the compounds released form protein breakdown during the dry-curing process.

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