EVOLUTION OF FREE AMINO ACIDS DURING RIPENING OF IBERIAN CURED HAM WITH LOW SALT CONCENTRATION.

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BACKGROUND

The ham from Iberian pigs is a meat cured product of high sensorial quality with a first-rate consumer acceptance. The extensive system of feeding of these pig based on acorns and grass, and the prolonged technological processing of the hams covering 18-24 months give the final product sensorial characteristic not to be found in any other type of cured ham.
The traditional processing of this hams involves salting period legs for 15 days at temperatures below 5ºC, followed by a post-salting of controlled increasing temperatures and decreasing relative humidity. When salt diffusion is completed, the hams are left in drying rooms at environmental conditions during 1-2 months in the summertime and 12 additional months in a cellar environment.
Due to actual tendency to consume low salted products, in modern process of Iberian ham the salting period is reduced to 9-10 days.
During ripening of Iberian ham proteolytic breakdown of meat proteins results in an increase of free amino acids that could be an important source of flavor compounds in dry-cured hams. Whereas high amounts of free amino acids have been widely recognised as factors enable to enhance sensory quality of cured hams (McCain et al., 1968; Córdoba et al., 1994; Toldrá et al., 1995), an uncontrolled proteolysis has been associated with unacceptable aftertaste which impair the flavor and causes abnormal softness in Italian long aged heavy hams (Parolari et al., 1994; Virgili et al, 1995).

OBJECTIVE

The purpose of this study was to determine free amino acids release during processing of Iberian ham with low salt concentration.

METHODS

Processing of the hams

Fifteen hams obtained from Iberian pigs, which were fattened in pasture with acorns as the basic food source. Five hams were taken before processing. The other ten hams were processed in a local industry, with a salting time reduced to a 1day/Kg. In this plant all process was developed in controlled conditions of temperature and relative humidity. The step included in the sampling procedure and the number of hams removed for testing at each stage were as follow: Green state, n=5; Drying n=6; Fully ripened n=4.
Samples of *Biceps femurs* and *Semimembranosus* muscles were taken and analysed for moisture, NaCl and free amino acids were identified and their content measured.
Moisture was determined following the ISO recommended methods (ISO/1442).
NaCl was estimated as chlorides, which were extract with water ethanol (60:40 v/v) and quantified by the Carpentier Volhard method by titration with $AgNO_3$-NH_4CNS (AOAC, 1984).
Free amino acids. Free amino acids were identified and their content measured by HPLC, according with Córdoba *et al.*, 1994, using a Beckman HPLC with Spherisorb-ODS-II column (5 µm, 250 x 4.6 mm), and UV detection system at 254 nm.

RESULTS AND DISCUSSION

All free amino acids detected in the green stage increased significantly ($p<0.05$) during ripening in both the *semimembranosus* and the *biceps femoris* muscle, being this increase more intense in drying stage (Fig. 1) . The relatively high temperatures reached at this stage probably stimulated proteolysis. A higher increase of free amino acids in drying stage have been also observed in Iberian ham (Córdoba et al., 1994; Martín et al., 1998).Temperature reached during drying of Iberian ham seems to stimulate proteolytic activity of catepsine D and exopeptidases of tisular and microbial origin leading to amino acids release. Therefore, catepsine D and certain exopeptidases present in raw meat remain active during the whole production process (Toldrá et al., 1992; Verplaetse, 1994), which are activated when temperature of processing is increased. Thus, the increase of temperature during drying of Iberian ham play a main role in the proteolysis that take place in this kind of hams. In cellar stage there was also increase in free amino acids, but was always lower that it observed in drying stage. In this stage a decrease of proteolytic activity seems take place, and also should be noted that some free amino acids are characterised by their intense reaction with reducing compounds in the Maillard condensations (Ashorr and Zent, 1984; Wong and Stanton, 1989). Also, the loss of phenyalanine, valine and isoleucine could indicate that some Strecker degradation reactions were taking place (Ventanas et al., 1992).
Glutamic acid, alanine, lisine, leucine and arginine were the free amino acids that show higher amount in final product (Fig 1). These free amino acids have also been reported as those more predominant in Iberian ham (De Prado, 1988; Córdoba et al., 1994b), serrano dry cured hams (Toldrá et al., 1992) and Parma ham (Careri et al., 1993).
Most of the free amino acids analysed showed lower amounts of those reported in Iberian hams with more salt concentration (Córdoba et al., 1994b; Martín et al., 1998a). Thus, in the low salted ham took place a lower proteolytic activity than that cited by the forme author in high salted hams. This fact is apparently in a contradiction with published data reporting proteolytic inhibition with salt (Sárraga et al., 1989). However, unlike other proteinases, both exopeptidases and aminopeptidases (enzymes directly responsible of amino acids release) remain active through the hams processing (Toldrá et al,. 1992). Therefore, the main parameter which regulates the free amino acids formation over the range of salt concentration from the present work (3.86-6.36%D.M) and those reported by Martín et al., 1998b in salted hams (4.97-7.77%DM) is the temperature. Thus, although temperature in drying stage of the processing followed in the hams analysed was very similar to that reported in high salted hams, temperature of the cellar was always lower in our hams. The inclusion at the end of the cellar stage of a stuffing period with temperature close to 25ºC could permit to increase the accumulation of free amino acids in low salted hams
When free amino acids concentration of the two muscle analysed (*Biceps femoris* and *Semimembranosus*) were compared, a higher amount of Asp, Glu, Gly, Val, Ile and Leu were observed in *Biceps femoris* which showed lower salt concentration that *Semimembranosus* muscle. In this case since temperature is the same for two muscles, salt concentration seems to play the main role

in proteolytic activity.
In conclusion both salt concentration and temperature of processing should be considered as the main factors that influenced proteolytic activity in the final product.

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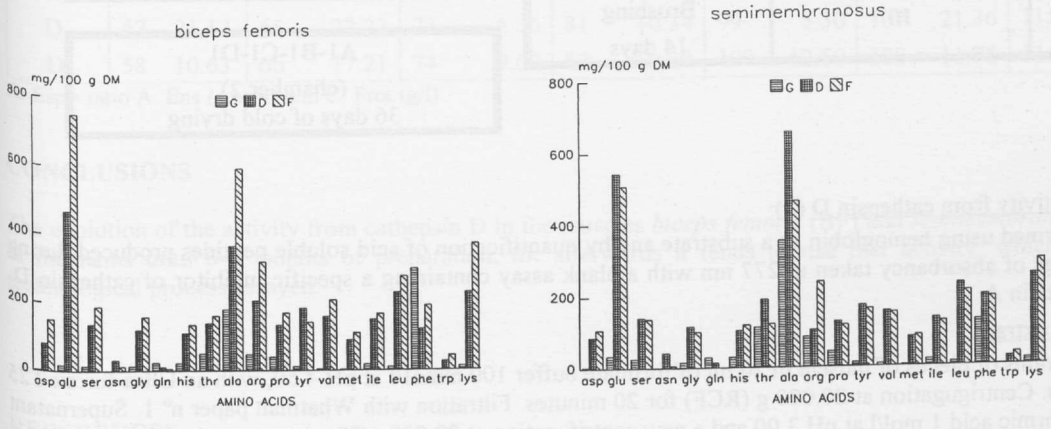

Figure 1. Changes in free amino acids in *biceps femoris* and *semimembranosus* muscles during ripening of Iberian hams: G (green ham), D (drying), F (fully ripened).

Table 1. Variations of different parameters in *Biceps femoris* and *Semimembranosus* muscles during ripening of Iberian hams.

Etapas	Moisture (%)		salt (% ClNa/ DM)	
	biceps femoris	semimembranosus	biceps femoris	semimembranosus
FR	$^{1}72.21 \pm 0.478$	$^{1}73.43 \pm 0.920$	$^{1}0.00 \pm 0.00$	$^{1}0.00 \pm 0.00$
SE	$^{2}59.54 \pm 1.967^{a}$	$^{2}49.58 \pm 1.742^{b}$	$^{2}5.52 \pm 0.164^{a}$	$^{2}4.23 \pm 0.149^{b}$
PF	$^{3}49.86 \pm 0.731^{a}$	$^{3}34.71 \pm 2.021^{b}$	$^{3}6.32 \pm 0.276^{a}$	$^{3}3.86 \pm 0.114^{b}$

Means with different letters in the same row are significantly different ($P<0.05$).
Means with different numbers in the same column are significantly different ($P<0.05$).