AMINO ACID ANALYSIS IN FRESH PORK AND DRY-CURED HAM BY HPLC OF PHENYLISOTHIOCYANATE DERIVATIVES

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ABSTRACT

One of the most important biochemical changes in ham during the dry-curing process consists in a substantial increase in its free amino acids concentration. This increase may result in an enhance of the natural characteristic taste of dry-cured hams.

Precolum phenylisothiocyanate (PITC) derivatization combined with reverse phase liquid chromatography has been tested for free amino acids analysis in muscle from both fresh pork and dry-cured ham. Preliminar chromatograms obtained by using a waters pico-tag amino acid analysis with a Supelcosil™ LC-18-DB column showed the presence of an unidentificated peak coeluting with the arginine and taurine peaks. Furthermore, a reagent peak also coeluted with ornithine and tryptophane. These problems could be overcome by introducing some modifications which consisted in changes in column temperature (50 ºC instead of 46 ºC), pH of mobile phase (6.75 instead of 6.4) and gradient conditions (including flow rate gradient in the initial six minutes step).

The 21 amino acids identified in meat were succesfully separated and their quantitation completed. A noticeable increase in the free amino acid concentration along the dry-curing process has been detected. In fact, the concentration of free amino acids in dry cured hams is extremely high as compared to raw meat with special increases in glutamic acid (1368.6 vs 63.6 mg/100 g Protein), arginine (887.1 vs 84.2 mg/100 g Protein), alanine (1032.1 vs 198.8 mg/100 g Protein), valine (827.5 vs 50.5 mg/100 g Protein), leucine (937.0 vs 42.2 mg/100 g Protein) and lysine (2008.6 vs 40.9 mg/100 g Protein).

INTRODUCTION

A characteristic texture and flavor are developed in hams during the dry-curing process. An intense proteolysis has been reported (Flores et al, 1984; Toldrá et al, 1991). The final result of the observed proteolytical changes in meat proteins and peptides are free amino acids which can have an important role in the flavor (Kato et al, 1989). The conditions for free amino acids extraction from hams and sample deproteinization have been already studied (Aristoy and Toldrá, 1991) but some problems remain in the chromatographic separation.

The objective of this study is the analysis of free amino acids in hams along a complete dry-curing process, by HPLC of the precolumn phenylisothiocyanate derivatization method.

MATERIALS AND METHODS

Preparation of amino acid extracts: Samples of M. Biceps femoris were removed at different stages of a typical spanish dry-curing process: Raw (time 0), post-salting (t= 2 months) and drying (5 and 7 months). Three hams were assayed at each stage.

The amino acids extraction was performed by homogenizing 4g of ham previously diluted 1:5 (samples t=0 and t=2), 1:7.5 (sample t=5) and 1:10 (sample t=7) with 0'1 N HCL in a stomacher homogenizer for 8 min and centrifuged at 10,000 g for 20 min (Aristoy and Toldrá, 1991). Supernatant was filtered through glass wool and deproteinized by adding 2.5 volumes of acetonitrile. After centrifugation (10,000 g, 5 min), the supernant was derivatized according to Bidlingmeyer et al (1987). Hydroxiproline, aminobutyric acid and norleucine were used as internal standards in similar levels to those expected in the respective samples.

Chromatographic separation: Samples were analyzed in a 1050 Hewlett Packard liquid chromatograph equipped with a multiwavelength UV detector (254 nm). The column was a Supelcosil™ LC-18 DB, 250 x 4.6 mm (5um particle size) protected with a LC-18 Pelliguard packed guard column (200 x 2.1 mm). The temperature was controlled to 50±1ºC.

Chromatographic conditions consisted of a solvent system of two eluents: (A) 0.14 M sodium acetate containing 0.5 ml/l of triethylamin (TEA) and adjusted to pH 6.75 with glacial acetic acid, and (B) 60:40 of acetonitrile:water. To achieve the separation, the following flowrate and solvent composition gradient was performed: Initial to 1 ml/min, 10%B; 6 min linear change to 0.8 ml/min and 12.5% B; 32 min linear to 58% B; 33 min step to 1 ml/min and 100% B; wash for 8 min and reequilibrate at 10%B during 16 min before a new injection.

Chemical analysis: Protein contents in ham was determined by official Standard method based on Kjeldhal semimicro determination (Presidencia del Gobierno, 1979).

RESULTS AND DISCUSSION

A high variety of chromatographic conditions for the phenylthiocarbamyl free amino acid derivatives separation have been reported in the literature (Ebert, 1986; Bidlingmeyer et al, 1987; Cohen and Strydom, 1988; Sarwar and Botting, 1990). These conditions mainly depend on the column and the kind of samples. When some of these conditions were applied to analyze ham samples in a Supelcosil column, special difficulties appeared in the separation of two groups of peaks; one formed by arginine, taurine and an unknown peak and the other by tryptophane, ornithine and a reagent peak. In order to optimize the separation, different ionic strength and pH of the aqueous solvent, gradient conditions and column temperature were assayed. The conditions for an optimal separation were those described in the Materials and Methods section. A typical chromatogram of dry-cured ham is shown in figure 1. As can be observed, only 32 minutes are required for a complete separation of the 21 free amino acids existing in the ham.

Figure 1.- Separation of phenylthocarbamyl aminoacids of dry-cured ham (t= 7 months): 1 asp, 2 glu, 3 OHpro (IS), 4 asn, 5 ser, 6 gln, 7 gly, 8 his, 9 tau, 10 arg, 11 thr, 12 ala, 13 pro, 14 ∝aba (IS), 15 tyr, 16 val, 17 met, 18 ile, 19 leu, 20 Nleu (IS), 21 trp, 22 orn, 23 lys. UNK, unknown peak. Rg, reagent peak.

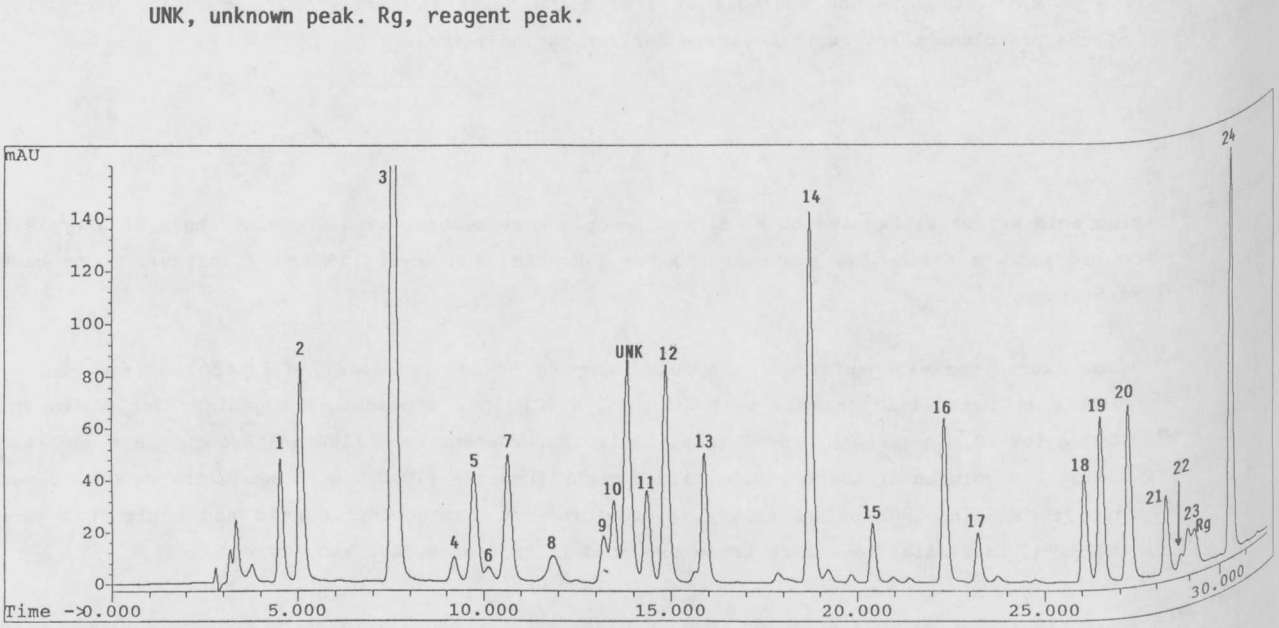

TABLE I: EVOLUTION OF FREE AMINOACIDS CONTENTS ALONG THE PROCESSING OF DRY-CURED HAM

Aminoacids (mg/100g of protein)*	Raw (t=0)	Post salting (t=2 months)	Mid-drying (t=5 months)	End drying (t=7 months)
Aspartic acid	13.1	125.0	546.3	736.6
Glutamic acid	63.6	357.3	1007.8	1368.6
Asparagine	21.0	105.0	147.5	173.6
Serine	41.4	262.3	540.9	700.3
Glutamine	346.8	245.5	230.5	164.5
Glycine	118.9	245.1	462.5	604.5
Histidine	78.2	196.8	348.3	454.7
Taurine	416.6	360.9	189.2	215.1
Arginine	84.2	338.6	541.7	887.1
Threonine	67.9	275.0	549.8	732.8
Alanine	198.8	460.0	778.0	1032.1
Proline	94.9	224.1	671.8	661.9
Tyrosine	39.6	209.1	387.8	471.0
Valine	50.5	245.9	654.1	827.5
Methionine	23.9	145.0	243.3	341.5
Isoleucine	30.8	169.1	469.1	589.4
Leucine	42.2	310.0	669.9	937.0
Phenylalanine	48.0	194.1	356.8	544.5
Tryptophane	11.7	35.0	81.5	88.8
Ornithine	13.8	35.2	69.2	111.3
Lysine	40.9	443.2	1049.1	2008.6
Total	1846.8	5125.0	9964.8	13652.0

*Protein contents (g/100g ham): 22.1 for t=0, 22.3 for t=2, 25.9 for t=5 and 26.6 for t=7.

This method was then used to follow the evolution of free amino acids along the processing of dry-curing ham. The results, at different stages, are shown in Table I. Three internal standards (hydroxiproline, aminobutyric acid and norleucine) at three different concentrations were used to facilitate the amino acids quantitation because of the great range of concentrations found in each sample and among stages. There is a very noticeable increase of all the free amino acids, except glutamine and taurine, along the dry-curing process. A small increase in free amino acid levels has been also observed when storing pork meat for 8 days at 4ºC (Kato et al, 1989). In our case, a substantial increase in amino acids concentration has been already observed during the post-salting stage (see table I) which was kept at 4ºC. Dry-cured hams have an extremely high concentration of free amino acids when compared to raw ham (7.4 times). Major changes were observed for glutamic acid (1368.6 vs 63.6 mg/100 g Protein), arginine (887.1 vs 84.2 mg/100 g Protein), alanine (1032.1 vs 198.8 mg/100 g Protein), valine (827.5 vs 50.5 mg/100 g Protein), leucine (937.0 vs 42.2 mg/100 g Protein) and lysine (2008.6 vs 40.9 mg/100 g Protein). Glutamine and taurine, which are two of the major amino acids in raw ham, were the only two slightly decreasing along the processing. According to table I, it seems that free amino acids are continuously generated so that it is difficult to conclude which stage is most important for this increase. In any case, it is reasonable that muscle aminopeptidases, which have been shown to be active in the dry-cured ham conditions (Toldrá et al, 1991), would have an important participation in the observed liberation of free amino acids.

CONCLUSIONS

Chromatographic conditions for an optimal separation of phenylthiocarbamyl amino acids from raw and dry-cured ham have been optimized.

There is a noticeable increase in the concentration of free amino acids along the processing of dry-cured ham, specially in glutamic acid, arginine, alanine, valine, leucine and lysine.

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