FREE AMINO ACIDS AND BIOGENIC AMINES CONTENS DURING RIPENING OF PORTUGUESE DRY-CURED HAM

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Background

Free amino acids and other non-protein nitrogen compounds may play an important role in the evaluation of the protein breakdown during the ripening of dry-cured hams. Proteolysis yields free precursor amino acids, which may eventually be transformed into biogenic amines through decarboxylation [1,2]. Biogenic amines have been detected in a wide diversity of food products including fish, cheese, wine, vegetables and meat [3]. In the last few years attention has turned to the formation of biogenic amines during production, maturation, storage and decay by biochemical and microbiological shaping of food with high levels of protein. However only a few data for meat and meat products are available [4]. The presence of these compounds may affect the organoleptical characteristics, and they have been proposed as a quality indicator [5]. The biogenic amines assume a relevant role in public health and the main concern is related to their toxicological effects [6].

Objectives

The main aim of this study was to evaluate the free amino acids and biogenic amines contents during an extended ripening process of dry-cured ham elaborated according to the industrial short process (6 months).

Methods

Sixty dry-cured hams from industrial breeds of White pigs were processed into dry-cured hams. After slaughter, hams were refrigerated for 48 h at 0–4°C and cured with a salt mixture and nitrate potassium for 20 days at 3–5°C and 90-95% relative humidity. Then were washed and hung, followed for a ripening stage where the meat product was submitted to different cycles of temperature, humidity and time. Dry-cured ham samples from *Biceps femoris* muscle (BF) obtained at different times of curing process were taken for analysis. Free amino acids were extracted from the samples with 0.1 M HCl and alkalinized with 1M NaOH and analyzed by high-performance liquid chromatography (HPLC). The analytical method involves a pre-column reaction with *o*-phthalaldehyde, after deproteinization, to form fluorescent derivatives with amino acids. These derivatives were quantified by reversed-phase HPLC with fluorescence detection. γ-aminobutiric acid (GABA) was used as internal standard. Biogenic amines (putrescine - Put, cadaverine - Cad, histamine - His, tyramine - Tyr, spermidine - Spd and spermine - Spm) were determined following the high-performance liquid chromatographic method described by Eerola *et al.* (1993). The method includes perchloric acid extraction, derivatisation with dansyl chloride and chromatographic separations with a reversed-phase column, detection at 254 nm (UV) and 1,7-diaminoheptane as internal standard (IS). Statistical analysis of data was carried out by analysis of variance with the Tukey test using GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California USA, www.graphpad.com.

Results and discussion

Figure 1 shows the evolution of free amino acids levels in the *Biceps femoris* muscle. Higher significant ($p < 0.05$) increase in almost free amino acid concentration was observed during the curing process, specially between the sixth and the seventh month. The liberation of free amino acids along processing was not selective and arginine, threonine, lysine and glutamic acid were the predominant free amino acids. A similar profile was observed in Iberian ham [8] and in French dry-cured ham [9]. The French authors explained this phenomenon with the hypotheses of a fall in exopeptidase activities that would result in a rise in the ratio of endopeptidase activities (producing peptides) to exopeptidase activities (producing free amino acids) and a degradation of free amino acids.

In contrast biogenic amines showed wide fluctuations. Tyramine, spermine and cadaverine tended to be present in larger quantities than the amines, putrescine, histamine and spermidine. Hortos and Garcia (1991) reported also dispersed values of biogenic amine and could not relate them with amino acid results. The range of concentrations for each detected amine in samples is presented in Table 1. Spermine was the only amine detected in all samples analyzed and a decrease in spermine average level throughout the ripening extended process was observed. Figure 2 shows typical chromatogram of amines in a dry-cured ham sample. All the amines were well separated and the elution time was less than 25 min. Each amine was identified by the relative retention time values compared to the internal standard.

Conclusions

The quality of dry-cured ham has been directly related to the amounts of free amino acids in the final product. The majority of amino acids showed a greatest concentration on the seventh month of the short elaboration process. The biogenic amine contents never reached the range of toxic levels, even considering that the extension of the ripening time was longer than normal market shelf life for this type of product. From a public health point of view, the levels seemed to be low to produce toxicological effects.

Pertinent literature

[1] Córdoba J. J. *et al.* (1994). *J. Agric. Food Chem.*, 42, 2296-2301. **[2]** Hernández-Jover T. *et al.* (1997). *J. Agric. Food Chem.*, 45, 2098-2102. **[3]** Silla Santos, 1996). *Int. J. Food Microbiol.*, 29, 213-231. **[4]** Treviño E. *et al.* (1997). *Food Chemistry.*, 58 (4), 385-390. **[5]** Veciana-Nogues M. T. *et al.* (1996). *J. Food Prot.*, 59 (11), 1218-1222. **[6]** Draisci R. *et al.* (1998). *Food Chemistry*, 62 (2), 225-232. **[7]** Eerola S. *et al.* (1993). *Journal of AOAC International*. 76 (3), 575-577. **[8]** Flores J. *et al.* (1984). *Rev. Agroquim. Tecnol. Aliment.*, 24 (4), 503-509. **[9]** Buscailhon S. *et al.* (1994). *Meat Science*, 37, 449-456. **[10]** Hortos M. and Garcia Regueiro J.A. (1991). *In Proceedings of the 37th ICoMST Congress* 1991, p.1047-1050. Kulmbach, Germany.

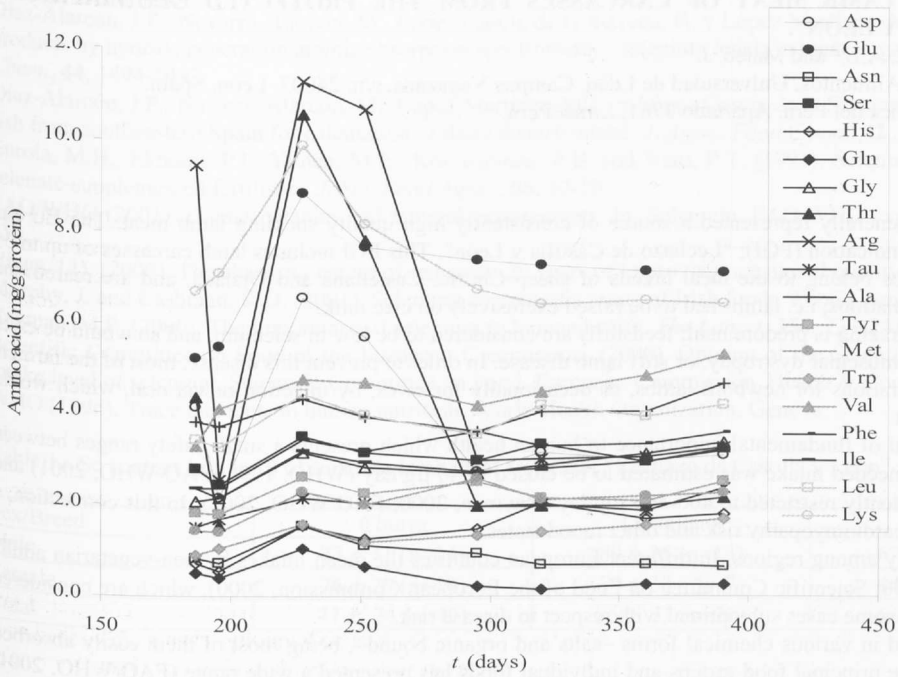

Figure 1. Amino acid concentration evolution along a ripening extended process from *Biceps femoris* muscle (BF) of dry-cured ham.

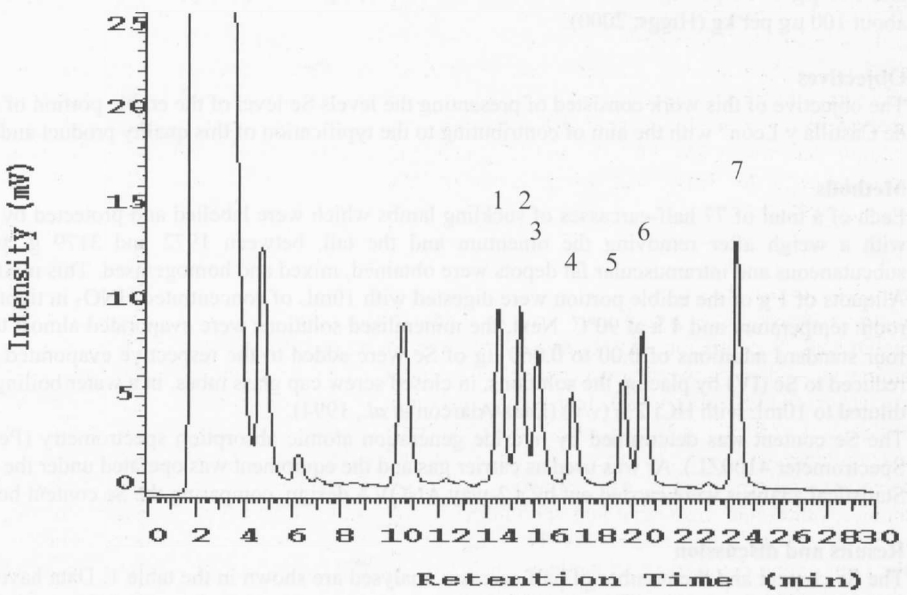

Figure 2. Chromatogram of dansyl derivatives of biogenic amines in a dry-cured ham sample. Peak identification and retention time: (1) Put, 14.47; (2) Cad, 15.34; (3) His, 16.09; (4) IS, 17.51; (5) Tyr, 19.59; (6) Spd, 20.38; (7) Spm, 24.24.

Table 1. Biogenic amine contents in *Biceps femoris* muscle of dry-cured ham.

	[Amine] (mg/kg)					
t/day	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine
187	n.d.[a] – 3.89	b.loq[b] – 73.41	n.d. – 28.29	n.d. – 3.16	1.58 – 7.08	36.42 – 49.15
196	n.d. – 3.84	0.73 – 28.03	n.d. – 4.62	n.d. – 6.60	1.11 – 8.56	25.68 – 48.55
228	n.d. – 2.61	n.d. – 23.70	1.07 – 5.78	n.d. – 15.25	b.loq– 7.71	30.05 – 50.05
252	n.d. – 2.45	b.loq – 56.79	n.d. – 3.90	n.d. – 133.84	n.d. – 11.03	32.74 – 44.76
295	n.d. – 21.32	0.83 – 227.07	n.d. – 6.20	n.d. – 83.74	n.d. – 13.38	35.77 – 57.06
320	b.loq – 15.42	7.64 – 61.34	n.d. – 2.01	n.d. – 52.32	n.d.	41.11 – 47.21
360	b.loq – 1.18	6.81 – 8.96	1.08 – 2.02	n.d.	n.d.	36.75 – 44.88
390	1.82 – 9.94	7.37 – 13.60	0.52 – 0.79	n.d.	n.d.	23.59 – 35.21

[a] not detected; [b] below limit of quantification (b.loq).