DETERMINATION OF FREE AMINO ACIDS IN DRY-CURED HAMS BY GC-FID - PRELIMINARY RESULTS

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Background

Cured meat products, made from whole pieces of pork, are commonly produced and consumed in different countries, specially from Mediterranean area. Dry-cured ham is a salted meat product, produced by several technological processes and with different times of processing, usually classified in short ripening (4-6 months) and long ripening (≥ 12 months). During the ripening process, the dry-cured ham undergo several biochemical changes, most of them due to proteolysis induced by peptidases (EC 3.4), which are still active after several months of processing [1]. Free amino acids and other compounds, which are generated from proteins and peptides by fragmentation of sarcoplasmatic and myofibrillar proteins, may play an important role in the development of flavour [2] and other organoleptical properties [3] of dry-cured hams. Capillary gas chromatography (GC) can be used for the determination of free amino acids in several matrixes, with a wide range of concentrations. In this communication, it is described a GC-FID procedure for the simultaneous identification and quantification of free amino acids as trimethylsilyl derivatives.

Objectives

The aim of this work was to apply and to study the reliability of a gas chromatographic method for the quantification of free amino acids in dry-cured hams.

Methods

The study was conducted in samples of dry-cured hams obtained from industrial breeds of white pigs and elaborated by a short ripening process. The identification and quantification of free amino acids was carried out by GC-FID following the procedure and chromatographic conditions described previously by Gehrke and Leimer (1971) [4]. Briefly, amino acids were extracted from 2,0 g of *semimembranosus* muscle (SM) of dry-cured ham by homogenization with an ultra-turrax in 20 ml 0.1 M HCl (6 × 10 s @ 18.000 g). Extraction solution was neutralized with 1 M NaOH and deproteinized with ethanol. An accurately known amount of internal standard (decanoic acid) in acetonitrile was added. An aqueous aliquot of total amino acids was concentrated to dryness, redissolved in methylene chloride and evaporated just to dryness to ensure the complete azeotropic removal of water. The derivatisation procedure was carried out at 150 °C during 2.5 hours with bis(trimethylsilyl) trifluoroacetamide (BSTFA). GC analysis was performed on a capillary column (30 m × 0.25 mm × 0.25 μm), from Agilent Technologies, filled with (50 %)-diphenyl-(50 %)-dimethylpolysiloxane (OV-17). The program temperature was from 80 °C to 210 °C, with increases of 5 °C/min.; the injector temperature was 275 °C and detector 300 °C. Helium was used as a carrier gas at a flow rate of 10 ml/min (split 1:5). The identification of the free amino acids was based on the relative retention times and their quantification on the relative areas.

Results and discussion

Figure 1presents a typical chromatogram of amino acids profile of dry-cured ham obtained by GC-FID. The standard curves for some of the free amino acids analysed is showed in figure 2. The mean contents of Asp, Ile, Phe, Tyr and Val in dry-cured hams of short curing process varied between 73.27 to 161.59 mg/100 g of dry-cured hams, as shown in table 1. From the amino acids analysed Phe was the one with the lower contents and Asp was the amino acid with higher values. This technique seems to be appropriate for the identification and quantification of free amino acids in dry-cured hams as was previously suggested by Hortos and Garcia Regueiro [3].

Conclusions

It is concluded that GC-FID is a convenient method for separation, identification and quantification of free amino acids from dry-cured hams.

Pertinent literature

[1] Toldra *et al.* (1992). J. Food Sci., 57 (4), 816-818. **[2]** Garcia *et al.* (1998). *J. Muscle Foods*, 9, 257-266. **[3]** Hortos and Garcia Regueiro (1991). *Proccedings of 7th International Congress of Meat Science and Technology*, Germany, 1047-1050. **[4]** Gehrke and Leimer (1971). *J. Chromatography.*, 57, 219-238.

Acknowledgments

This research was supported by project CIISA/2002/52.Carne-Bioactivos.

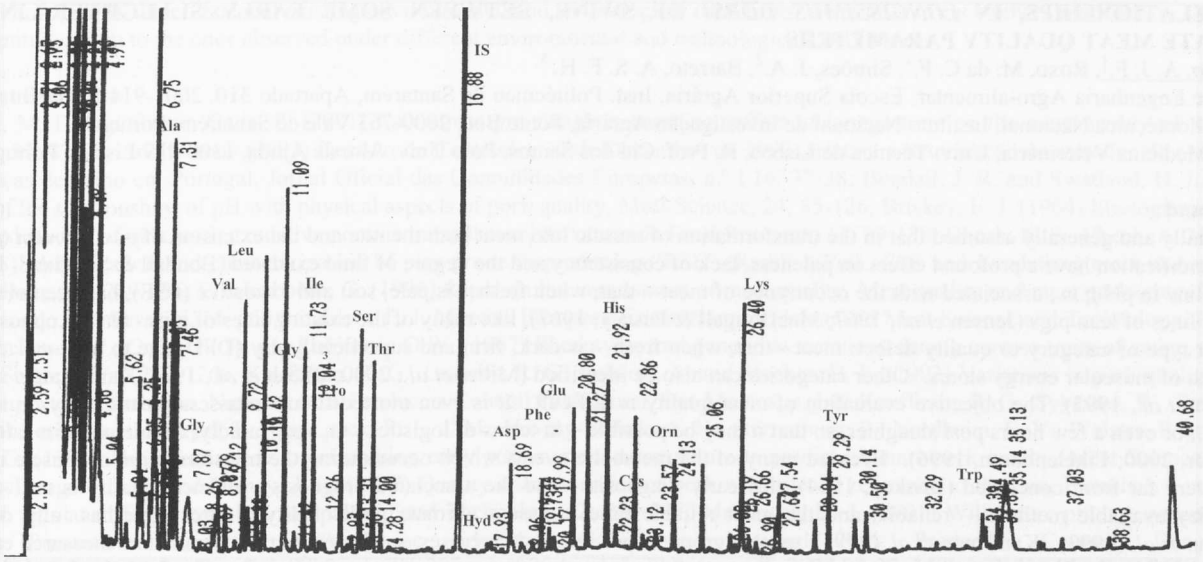

Figure 1. Typical GC-FID chromatogram of free amino acids from *semimembranosus* muscle of dry-cured ham. The identification of peaks is as follows: Ala (7.31); Gly1 (8.26); Val (9.44); Leu (10.89); Ile (11.73); Gly2 (11.82); Pro (12.93); Ser (13.31); Thr (13.56); I.S. (decanoic acid, 16.88); Hyp (17.93); Asp (18.69); Phe (19.99); Glu (20.80); His (21.92); Cys (22.86), Orn (24.13); Lys (26.40); Tyr (29.29); Trp (37.03).

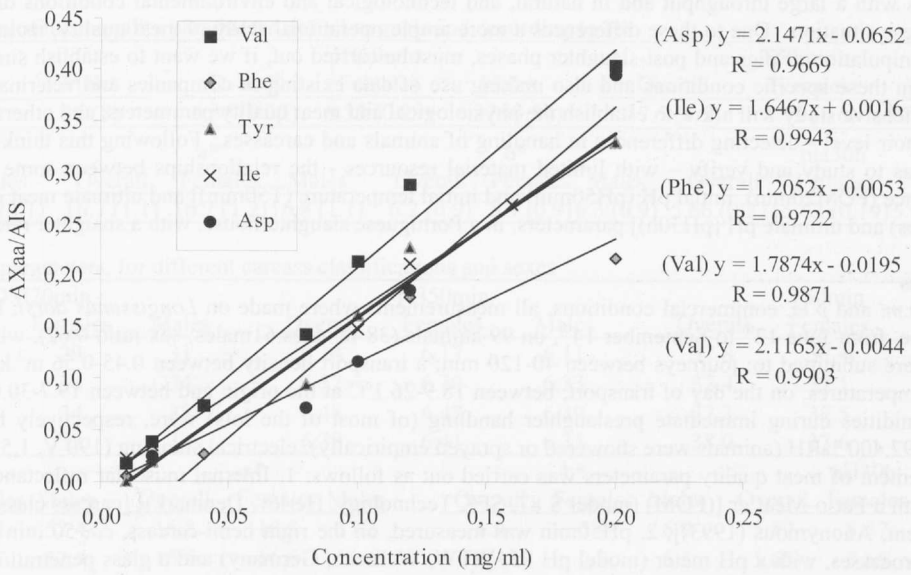

Figure 2. Linear regression equation (Y=aX+b) of some free amino acids analysed. The coefficient of linear correlation of each curve is displayed (R).

Table 1. Contents of some the free amino acids analysed from *semimembranosus* muscle of dry-cured hams processed by a short ripening process.

DRY-CURED HAMS	FREE AMINO ACIDS (mg/100 g)				
Sample id.	Asp	Ile	Phe	Tyr	Val
24 SM	128.68	156.38	–	106.64	–
26 SM	190.05	230.52	38.70	146.63	187.16
28 SM	146.44	146.22	–	113.17	21.80
29 SM	110.41	185.16	–	128.66	–
30 SM	176.08	–	44.36	174.35	–
37 SM	145.57	39.26	97.47	105.81	12.57
40 SM	235.81	258.77	113.91	182.80	120.98
41 SM	170.37	165.14	–	134.93	16.48
47 SM	150.96	83.06	71.94	75.39	191.26
Mean	**161.59**	**158.06**	**73.27**	**129.82**	**91.71**
S.E.	12.31	25.38	14.61	11.46	34.97

S.E. – standard error of mean.