CHARACTERIZATION AND EVOLUTION OF THE PEPTIDE FRACTION DURING THE AGEING OF PARMA HAM

Dossena A.[1], Sforza, S.[1], Galaverna, G.[1], Virgili R.[2], Marchelli R.[1]
[1]Dip. di Chimica Organica ed Industriale, Parco Area delle Scienze 17/a, Università di Parma, I-43100, Parma, Italy
[2]Stazione Sperimentale per l'Industria delle Conserve Alimentari, viale Tanara 31/A, I-43100, Parma, Italy

Background

The proteolytic process, originated by endo- and exopeptidases, in long-aged and not-fermented meat products, is undoubtedly the most important physico-chemical modification for the determination of the sensory and rheological features of the end products.
In the last years many studies have been conducted on Parma ham, in order to determine the evolution of the aminoacidic fraction during the ageing and to explain the role of these components on the ham flavor. On the other side, very few is known on the peptide fraction produced during the proteolytic process.

Objectives

A novel method to characterize the low and medium molecular weight peptide content in not fermented meat products has been developed, based on HPLC-MS analysis of the extract obtained by treatment with 0.1N HCl of the homogenized meat product. The extract has subsequently been fractionated by using physical methods, obtaining a complex mixture of amino acid and peptides with molecular weight up to 6000-8000 Da. The method developed has allowed to determine and to track in different hams different peptides derived from the proteolysis of the pig thigh.

Methods

Oligopeptide extraction. 10g of ham sample (biceps femoris) were homogenized with 90 ml of 0.1 M hydrochloric acid and 0.49 ml of a 1 mM aqueous solution of phenylalanyl-phenylalanine (Phe-Phe) was added as internal standard. The resulting solution was homogenized for 2 minutes, then centrifuged at 10000 rpm for 20 min at 5°C. The solution was then subsequently filtered on glass wool, on a paper filter and on 5 μm filters. The resulting liquid phase was extracted three times with ethyl ether. The solution was filtered through a 0.45 μm filter, then filtered again through Amicon Ultrafilters, using an Amicon Micropartition system MPS-1. The filtrate was evaporated under nitrogen and dissolved in 200 μl of an aqueous solution containing 0.2% acetonitrile and 0.1% formic acid.
HPLC analysis. The solution was analyzed by HPLC (Waters Alliance 2690 separation module equipped with a PDA detector Waters 996) with a RP-C18 column (Jupiter Phenomenex, 300Å, 250X4.6 mm) using an elution gradient: eluent A: water, 0.2% acetonitrile, 0.1% formic acid; eluent B: water/acetonitrile (65/35, 0.1% formic acid). The gradient was: 0-15 min. isocratic 99%A, 15-60 min. from 99% to 0%A, 60-69 min. isocratic 0%A, 69-70 min. from 0% to 99%A. Flow 1 ml/min.
Mass spectrometry. The mass spectra, derived either from a direct injection or from an HPLC analysis in the conditions above, were obtained on a Micromass ZMD mass spectrometer equipped with a single quadrupole analyzer (capillary voltage 3kV, cone voltage 30V, positive ion mode).

Results and discussion

The method developed in our research group has been first compared with other methods reported in the literature for the characterization of the peptide fraction of the meat products. In particular, extraction methods which employ TCA and CH_3CN as deproteinizing agent have been considered. It has clearly been shown that the latter methods allow exclusively the recovering of small and medium peptides (up to 500-1000 Da) with hydrophilic characteristics. The method here proposed, using as extracting and not precipitating agent 0.1N HCl, allows the recovering of different lipophilic medium and high molecular weight peptides (up to 6000-8000 Da), which are important in order to characterize in a more complete way the proteolytic process which takes place in the thighs and the associated biochemical processes.
In order to get the mixture containing amino acids and peptides to be analyzed by HPLC, the acidic extract underwent to different filtrations and ultrafiltrations (glass wool, 5 μm filters, 0.45 μm filters, diafiltration with molecular cut-off of 3000Da), which fractionated the complex mixtures, excluding protein and high molecular weight peptides.
The assayed methods (TCA precipitation, CH_3CN precipitation, physical fractionation) have been compared. As shown in Figure 1, the different methods resulted in different HPLC profiles, particularly at the high retention times, where lipophilic peptides elute. These peptides are very important in order to define the sensory and rheological characteristics of the final product.
The methods developed have been applied to the evaluation of the modifications of the peptide fraction in Parma hams during ageing.
At the present time, studies are being conducted in order to use these methods for the quality assessment of typicality and of the ageing time in Parma ham products.

Pertinent literature

- Marchelli R., Sforza S., Virgili R. Occurrence and sensory correlations of peptides in parma ham. In *"Flavour and Fragrance Chemistry"*, 2000, Kluwer Academic Publishers, 45-53.
- Sforza, S., Pigazzani, A., Motti, M., Galaverna, G., Dossena, A., Porta, C., Virgili, R., Marchelli, R. Oligopeptides and free amino acids in Parma hams of known cathepsin B activity. *Food Chemistry*, 2000, *75*, 267-273.
- Virgili R., Schivazappa C., Parolari G., Bordini C.S., Degni M. Proteases in fresh pork muscle and their influence on bitter taste formation in dry-cured ham. *Journal of Food Biochemistry*, 1998, *22*, 53-63.
- Virgili R., Parolari G., Schivazappa C., Soresi Bordini C., Borri, M. Sensory and texture quality of dry-cured ham as affected by endogenous cathepsin B activity and muscle composition. *Journal of Food Science*, 1995, *60*, 1183-1186.
- Aristoy M.C., Toldrà F. Deproteinization techniques for HPLC amino acid analysis in fresh pork muscle and dry-cured ham. *Journal of Agriculture and Food Chemistry*, 1991, *39*, 1792-1795.

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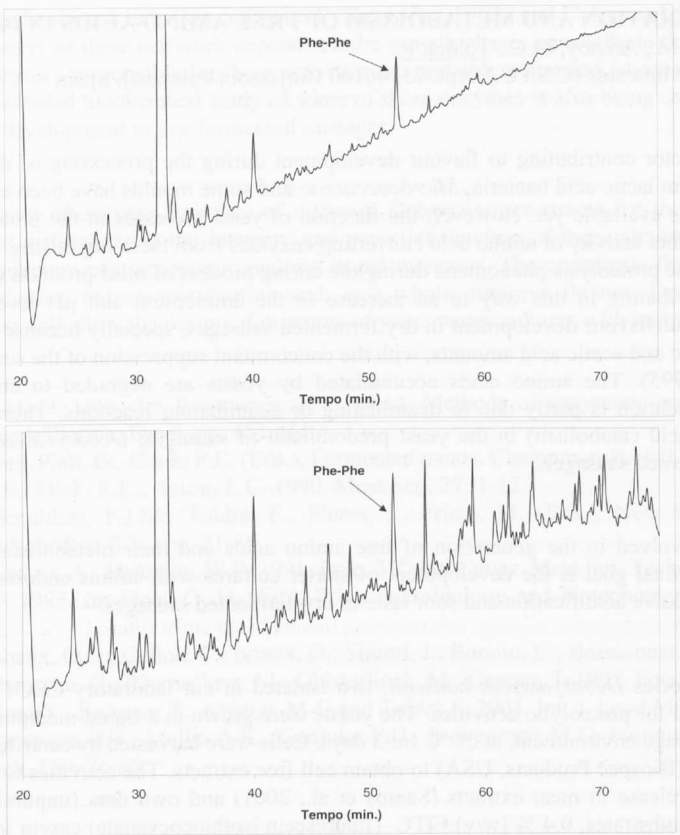

Figure 1. HPLC profiles of a peptide fraction extracted from Parma ham by using as deproteinizing agent TCA (top) or by using subsequent filtrations (bottom).