FORMATION OF PEPTIDES IN ITALIAN DRY-CURED HAM DURING PROCESSING

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Background

Traditional dry cured ham from Southern Europe is considered a high quality product due to the flavour. Development of the desired mature flavour requires a long, costly processing time (Toldra et al. 1993). Intense proteolysis has been observed during the dry curing process (Molina and Toldra, 1992) as a consequense of the active endogenous proteolytic enzymes (Toldra et al. 1993). In general a decrease in peptide size is observed (Rodriguez-Nunez et al. 1995) during processing.
In the recent years interest in the field of flavour analysis of dry cured hams has mainly been directed towards volatile compounds. However, the flavour from the non volatile water soluble fraction has received less attention. Peptides generated by enzymatic action on proteins in meat may be important for the flavour. Yamasaki and Maekawa (1978) found a peptide with delicious taste from beef treated with papain.

Objectives

The aim of the present study was to elucidate by means of HPLC which peptides appear to be important for the development of mature flavour in dry cured hams. Knowledge about the composition of the mature flavour could be of interest for other meat products.

Methods

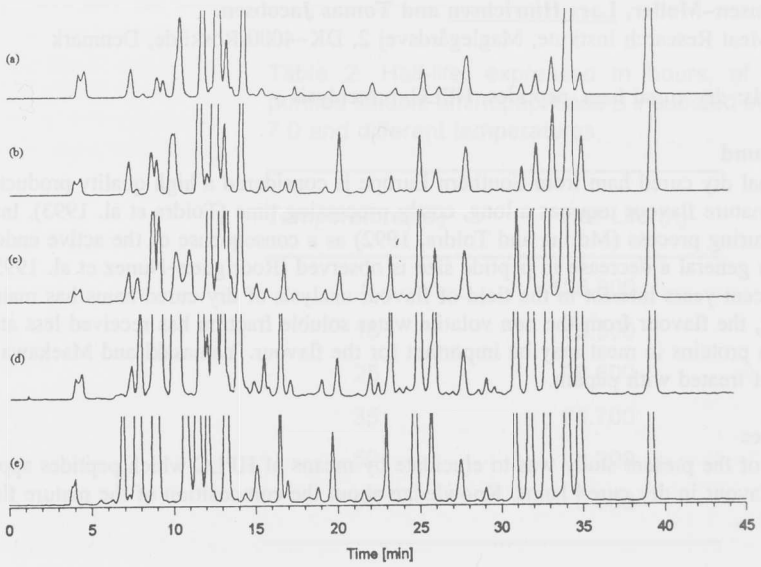
Products. 29 Parma hams were purchased at a factory in Langhirano, Italy. The hams were processed according to the traditional processing scheme based on 25 d of dry salting at 1-3°C, resting for 90 d at 1-4°C, drying and primary ripening for 90 d at 15-20°C at RH 60-90 %. Pork fat containing pepper was then smeared on cut surfaces followed by final ripening for 160 d at 17-18°C and post ripening for additionally 120 d as described by Virgili and Parolari (1991). Samples for analysis were taken at specific stages in the process: Five hams prior to salting (3 d), five hams after salting (25 d), five hams after drying (125 d), five hams after first ripening (211 d), five hams after second ripening (fully matured product) and three hams after post ripening (485 d).
Sampling. The hams were derinded leaving 1 cm fat on the hams. Representative samples for analysis were then taken in the cushion part, which included the biceps femoris, semimembranosus and semitendinosus muscles. Samples were stored in sealed vacuum bags at -20°C for no longer than 2 months.
Sample pretreatment. 3.00 ml 25 mM sodium phosphate buffer pH 6.0 containing the internal standard (0.20 mM of d-Leu-Tyr) was added to 0.50 g meat. After homogenization the homogenate was centrifuged for 5 min. at 14.000 g and 5°C. 400 µl of the supernatant was transferred to a purified Microcon 3 ultrafiltration unit. The filter was centrifuged at 10.000 g for 1 hour. 25 µl of the filtrate was diluted with 225 µl water and substantiated for derivatization. 15 µl of a 10 mM AQC (Cohen and Michaud, 1993) dissolved in MeCN and 50 µl of 0.2 M sodium borate buffer pH 8.8 were mixed with 50 µl sample extract. After 10.0 min. at 60°C, 1.5 µl of acetonitrile/acetic acid (1:1; v/v) was added. 5 µl of the derivate was injected in the HPLC system.
High-performance liquid chromatography. A Hitachi system consisting of an AS-4000 autosampler, a L-6200 gradient pump, a F-1080 detector and a D-6000 HPLC-manager equipped with a Hypersil BDS (3 µm, 60 mm x 4,6 mm I.D.) column (Hewlett Packard) operated at 40°C was used. Two mobile phases were used *A*: 0.2 M potassium phosphate buffer pH 6.0/ water/triethylamine (125:875:1; v/v/v) and *B*: MeCN/water (800:200; v/v) with the gradient profile: 0-52 min. 98% A - 70 % A; 52.1-52.5 min. 70% A - 20% A, 52.6-55.6 min. 20% A; 55.7-56,7 min. 20 - 98% A. The flow was 1.5 ml/min. For detection fluorescence was measured with excitation at 245 nm and emission at 395 nm. In this way only NH-containing substances were detected. Relative retention times were calculated as retention time of unknown x 100/retention time of internal standard.

Results and discussion

The action of endogenous and microbial enzymes within meat leads to the formation of low molecular weight substances, which by themselves contribute to the flavour or serve as intermediates in further reactions. All such reactions will generate an alternating pattern of low molecular weight substances dependent on the specificity of the enzymes and processing conditions. In figure 1a-e typical chromatograms of extracts from raw meat (fig. 1a), after post salting (fig. 1b), after first (fig. 1c) and second ripening (fig. 1d) and finally after post ripening (fig. 1e) are shown. Indeed the amount and number of low molecular weight nitrogenous compounds increase during processing. The derivatization procedure used does not distinguish between peptides and free amino acids. Therefore some peaks in the chromatograms are free amino acids rather than peptides. This is demonstrated in figure 2, where a post ripened ham is compared to the most common free amino acids. As the free amino acids are the ultimate product of proteolysis, they will be present in the highest amount compared to the peptides, which dynamically are formed and broken down to amino acids.
To recognize the latent patterns of peptide generation, the peak hights originating from non-amino acids were exposed to principal component analysis. The peak hights were scaled by the square root in order to get data within the same range before principal component analysis. The 2 first principal components described 49 % and 15 %, respectively, of the total variation in the data (results not shown). 5 groupings were observed, group I with raw hams (3 d), group II with hams after salting (25 d), group III with hams after drying (125 d), group IV with hams after first (211 d) and second ripening (365 d) and finally group V with post

ripened hams (485 d). Groups I–IV were essentially described by the first principal component, whereas group V was described by the second principal component. In figure 2 the characteristic peaks (1–11) are indicated on the chromatogram.

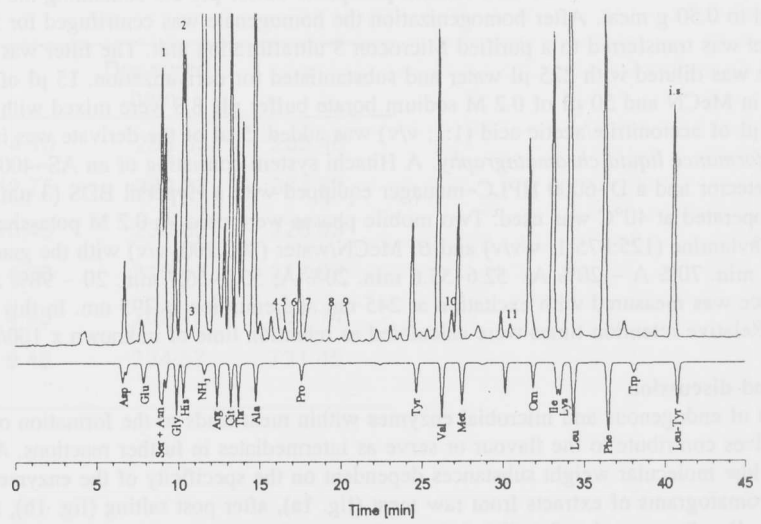
The raw hams and hams after salting were characteristic by a low content of the compounds with relative retention times of 24.7 (2) , 41.1 (6), 43.7 (8), 66.6 (10) and 74.9 (11), whereas hams after first and second ripening had a high content of these compounds. Hams after drying had an intermediate content of these compounds. Also the post ripened hams were characteristic by a high content of these compounds and in addition by a characteristic content of compounds with relative retention times of 18.4 (1), 27.3 (3), 30.3 (4), 40.0 (5), 42.1 (7) and 46.7 (9).



Figur 1. *Representative chromatograms of N–containing compounds during processing: Raw hams (a), after secondary salting (b), after drying (c), after final ripening (d) and after post ripening (e).*

The present observations indicate a relationship among processing and formation of low molecular weight nitrogenous substances. The first 365 d of processing the peptides with relative retention time 24.7 (2), 41.1 (6), 43.7 (8), 66.6 (10) and 74.9 (11) are formed (group I–IV) and the post ripening leads to the formation of additional peptides with relative retention times of 18.4 (1), 27.3 (3), 30.3 (4), 40.0 (5), 42.1 (7) and 46.7 (9). The peptide generation seems to be 2–phased, the first phase incuding raw meat, hams after salting, hams after drying and hams after first and second ripening, and a second phase including the post ripened hams. This is in accordance with previous observations in which a similar phase division was observed based on the content of volatile compounds (Hinrichsen and Pedersen, 1995). Rodriguez–Nuñez et al. (1995) observed a more intense formation of peptides below 2700 Da during the first 3.5 months of processing of Spanish Serrano ham, but was unable to detect a change in the peptide formation late in the ripening. This is not in accordance with the presented results and may be due to different technologies as these authors applied a more crude separation technique, which may cover differences in the peptide patterns.

Identification of the 11 characteristic peaks i.e. by LC–MS will be necessary in order to verify the proteinaceous origin of these compounds.



Figur 2. *Chromatogram of N–containing peaks in 10 pooled samples of ham compared to an amino acid standard. Non–amino acid peaks, which increase during ripening, are indicated by numbers (1–10).*

Acknowledgements

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