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Flavour differences between northern and southern European cured hams

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In this study two procedures for isolation of volatiles from hams were compared: simultaneous steam distillation-extraction (SDE Likens-Nickerson) and dynamic headspace. Because reliable semi-quantitative data were aimed at, according to our results, SDE extraction should be preferred over dynamic headspace isolation. About 70 volatile components were identified and quantified in several types of ham: southern European dry-cured hams (Serrano, Parma), northern European brine-cured hams and some experimental dry-cured hams with different ripening times. It was shown that the dry-cured hams had a more intense pattern of a whole series of volatiles related to fat oxidation, but also of some compounds related to amino acid degradation. Principal component analysis was used to visualize the relationships between the different ham objects. Some meaningful clusters of hams could be obtained and related to their volatile composition. © 1997 Elsevier Science Ltd

INTRODUCTION

Flavour is an essential parameter for consumer appreciation and the commercial success of meat products. As raw meat has very little smell and taste, flavour is formed in meat by thermal processing (cooking, roasting), fermentation (dry sausages) and/or curing. In the last decade extensive studies have been performed concerning the aroma compounds formed in meat as a result of heat processing and some excellent reviews have been published (Shahidi *et al.*, 1986; Mottram, 1991). Recently, major attention has also been given to aroma development in raw cured hams. Two major meat curing processes may be distinguished, resulting in two main categories of raw hams: dry-cured hams with long ripening and drying periods (seven months or more) and wet-(brine or pickled) cured hams with shorter production periods (three months or less). Brine curing is the major process in northern European countries and produces hams with a typical 'cured' flavour. High quality southern European dry-cured hams, such as the Italian Parma and the Spanish Serrano hams, can be sensory described as imparting an 'aged' flavour. A third type of raw ham, for which smoking is an essential contributor to the flavour character, is beyond the scope of this discussion.

Salt and nitrite are considered to be major ingredients for cured meat flavour. Earlier literature already paid attention to the role of nitrite for curing (Gray *et al.*, 1981; Mottram *et al.*, 1984) and recently Ramarathnam *et al.* used steam distillation-extraction (SDE) and dynamic headspace adsorption for examination of the differences between cured and uncured cooked pork (Ramarathnam *et al.*, 1991, 1993).

Most studies on the flavour of raw hams have been dealing with the dry-curing process. An excellent review of the influence of raw meat properties and processing technology on flavour development in dry-cured hams, including a discussion on the role of the non-volatiles, has been presented by Verplaetse (1994). Important work on the typical products of their country has recently been performed by French, Italian and Spanish workers. In a first study, the group of INRA-Theix, France used vacuum distillation (Berdagué *et al.*, 1991) for isolation of volatiles from ham. However, besides compounds originating from the catabolism of lipids and proteins, several contamination products could also be observed in the chromatograms. The influence of pig crossbreed on the volatile composition (Berdagué *et al.*, 1993) and the time-related changes in volatile compounds during processing (Buscailhon *et al.*, 1993) were studied using dynamic headspace analysis as an isolation procedure. More than 100 volatiles were detected, among which 74 were identified and 52 were quantified by external calibration. From principal component analyses there was some evidence of independence between the compounds related to the oxidation of lipids or the catabolism of amino acids. Buscailhon *et al.* (1994) also studied the relation

between compositional traits, including quantitative data of volatiles, and sensory qualities of French dry-cured hams.

Interesting work on Italian dry-cured Parma ham was performed by the group of Barbieri *et al.* (1992). Volatile compounds were isolated by means of dynamic headspace adsorption on Tenax and were identified by gas chromatography–mass spectrometry (GC–MS), and their mechanism of formation was discussed. The group concluded that a high content of various types of esters was a typical feature of Parma ham with respect to the French and Iberian products, which were characterized by higher amounts of alcohols and aldehydes. Careri *et al.* (1993) related sensory properties of Italian-type dry-cured hams to the chemical data of 26 non-volatile and 122 volatile parameters. They concluded that hams with the highest acceptability scores had high levels of volatile methyl-branched short chain esters and alcohols, and high levels of the non-volatile tyrosine and lysine.

Spanish workers studied the volatile composition and the lipid oxidative changes in long-ripened dry-cured hams from Iberian pigs (Antequera *et al.*, 1992; Lopez *et al.*, 1992). These pigs are produced using an extensive feeding system based on acorns and grass. They concluded that, as a result of the high fat content, hydrolytic and oxidative changes in the lipid fraction during processing played a major role in the flavour formation of these products. Garcia-Regueiro and Diaz (1994) compared volatile compounds in dry-cured hams produced from heavy and light Large White pigs. According to Vidal-Aragon *et al.* (1994), the dynamic headspace of altered dry hams showed an increase of ketones, alcohols and especially of sulfur compounds, which were important for off-flavour formation.

In our work, emphasis was on the aroma composition of raw hams and it is beyond the scope of this publication to discuss the extensive work that has been performed concerning the non-volatiles (salt, lipids, proteins, amino acids) influencing taste and texture characteristics of ham, or to discuss the numerous studies dealing with the biochemical changes, proteolytic or lipolytic, during drying/ripening of dry- or brine-cured raw ham. Some excellent studies also discussed the enzyme systems promoting proteolysis, increasing non-protein-nitrogen and the formation of non-volatile amino acids, which are important precursors of a whole series of volatile aroma compounds (Toldra, 1992; Sarraga, 1992).

The objective of this study was to compare flavour differences, with particular reference to the volatile composition, between brine- and dry-cured hams. As typical dry-cured products two commercial Spanish Serrano hams and one Italian-type Parma ham were selected. Also three short-ripened (2, 4 and 6 months' production time) local dry-cured products were included in the study. The four brine-cured hams in this study were locally produced from pigs of Belgian, Dutch and Spanish origin. Being aware of the fact that gas chromatographic patterns are largely influenced by isolation procedures, we found it necessary to use both a 'total volatile' analysis procedure (Likens-Nickerson extraction) and a headspace technique, based on adsorption of volatiles on Tenax.

In fact, this study was part of a long-term project concerning the use of starter cultures to stimulate the ripening process of dry-cured hams and to produce high quality hams within shorter production times. Up to now, very little information is available in literature concerning the use of starter cultures in dry-cured hams.

MATERIALS AND METHODS

Materials

For the purpose of this study two commercial Serrano hams, manufactured by conventional technology and ripened for nine months, were obtained from a Spanish producer (Code: Serrano 1 and 2). We were informed that for the production of these hams higher temperatures than conventional were used in the final stage of the ripening process. The conventional dry-curing process has been described by Toldra (1992). As a third reference product for the southern European dry-cured hams, an Italian Parma ham was purchased locally (code: Parma).

Experimental dry-cured hams were produced by a Belgian manufacturer from commercial available cross-breed pigs (Belgian Landrace×Pietrain), slaughtered at 90 kg. Analyses of the hams were performed after ripening for 2, 4 and 6 months (code: Drycur 2 M, Drycur 4 M and Drycur 6 M). The dry-curing process consisted of two saltings with, in total, 40 g NaCl, 150 ppm $NaNO_2$ and 50 ppm KNO_3 per kg of raw ham. Salting was followed by a resting period of 70 days at 2–4°C and 75–85% relative humidity. During ripening the temperature was gradually increased from 2 to 20°C, while relative humidity changed from 80 to 70%. The final weight loss of the ham was 30%.

The brine-cured hams used in this study were produced by a Belgian manufacturer and four hams were selected: one ham was a classical commercial production (code: Wetcur C), and three hams were experimental products, produced according to the classical brine-curing process from Belgian (code: Wetcur B), Dutch (code: Wetcur D) and Spanish pigs (code: Wetcur S). Brine-cured hams were produced in a tank curing process which lasted 12 days and used a brine composition of 500 ppm KNO_3, 400 ppm $NaNO_2$ and 19% NaCl. After resting for two weeks at 2–3°C and 90% relative humidity, the hams were pressed for three days at 6°C (pressure of 0.16 kg cm^{-2}), followed by another resting period of two weeks at 5–6°C and 80% relative humidity. Following a ripening period at 65% relative humidity, the hams were dried until a weight loss of 25%. The total process took about three months.

Sample treatment

In order to obtain representative samples for analyses, hams were homogenized on arrival at the lab. Slices of 1 cm thickness were cut and the external fat was removed. The slices were further divided into cubes of about 1 cm^3. The cubes were mixed thoroughly and portions of 100 g were placed in polyethylene bags and stored in the freezer at −20°C until the time of analysis.

Isolation of volatiles

The volatile constituents of all ham samples were isolated using a 'total volatile analysis' procedure, based on a simultaneous SDE (Likens-Nickerson). For some ham samples a dynamic headspace procedure, based on adsorption of volatiles on Tenax, was also used.

Likens-Nickerson extraction

In an air-conditioned room, 100 g of frozen ham cubes were ground in a domestic blender and suspended in 600 ml of water in a 1 l flask. The sample flask was attached to a modified Likens-Nickerson apparatus. In a 100 ml round-bottom flask, 50 ml of chromatographic grade dichloromethane and 15 μg of dodecane as internal standard were added. Additionally, 10 ml of dichloromethane was added to the Likens-Nickerson solvent return loop. Both solvent and sample mixture were heated to boiling with heating mantles and allowed to reflux for 4 h. After cooling to ambient temperature, the dichloromethane fractions of the solvent flask and the return loop were collected. Concentration of the combined fractions to 200 μl was performed in a Kuderna Danish and a Micro Kuderna Danish concentrator (Alltech Associates Inc., USA). For each ham sample triplicate isolations of volatiles were performed.

Dynamic headspace

The ham volatiles were isolated by a dynamic headspace procedure using glass tubes (16 cm×4 mm i.d.) filled with Tenax TA (90 mg, 20–35 mesh), as adsorption traps. The traps were purchased from Chrompack and preconditioned at 220°C for 3 h. Frozen ham cubes were ground in a domestic blender and 40 g was immediately put into a cylindrical glass extractor (height 18 cm; diameter 6 cm). For removal of air contaminants the sampling flask was flushed with helium during 5 min before installing the Tenax trap. In the following step the volatiles were extracted in a helium stream (60 ml min^{-1}) at 60°C during 25 min and adsorbed on Tenax TA. Additionally, the adsorption trap was backflushed with helium (30 ml min^{-1}) during 7 min at ambient temperature for removal of water and to avoid blocking of the cold trap during thermal desorption. The volatiles were injected into the GC–MS apparatus using a thermal desorption-cold trap injector as described later. Quantification of volatiles was performed using an external standard mixture of nonane, decane and undecane.

Gas chromatography–mass spectrometry

For GC–MS analyses two different sets of apparatus were used. The Likens-Nickerson concentrates were analysed by injection of 1 μl on a HP 5890 gas chromatograph coupled to a HP 5971A MSD mass spectrometer (Hewlett-Packard, USA). The GC was equipped with a capillary fused silica column (HP PONA crosslinked methyl silicone, 50 m×0.2 mm i.d., 0.5 μ film thickness). Carrier gas was helium (1 ml min^{-1} flow rate) and the column temperature was initially maintained at 40°C for 5 min and subsequently programmed from 40 to 250°C at a rate of 5°C min^{-1}, where it was held for 13 min. Split injection (1:5 split ratio) was used and the injector and transfer lines were respectively maintained at 250 and 280°C. The mass spectra were obtained by electron impact at 70 eV. The chromatograms were recorded by monitoring the total ion current in the 40–260 mass range and with a solvent delay of 6.8 min. Identification of the volatiles was based on comparison of the spectra with the spectra of the NBS49K library and of a self-made library, and was assisted by Kovats indices. Semi-quantitative determinations of the volatile constituents were calculated by relating the peak areas to the peak area of dodecane as internal standard and were expressed as ng g^{-1} ham (ppb). Mean values for the individual constituents were calculated from triplicate analyses of each ham.

For dynamic headspace analyses a HP G1800A GCD mass spectrometer (Hewlett-Packard, USA) equipped with a TCT thermal desorption-cold trap injector (Chrompack, The Netherlands) was used. The aroma compounds were thermally desorbed from the Tenax trap at a temperature of 210°C for 5 min under a helium flow of 10 ml min^{-1}. The desorbed volatiles, cryofocused in a silica capillary cooled at −110°C, were directly injected into the column by quickly heating the silica capillary to 200°C and maintaining this temperature for 5 min. Separation of the volatiles was performed on an HP-1 crosslinked methylsilicone gum (50 m×0.32 mm i.d., 1.05 μm film thickness) with a helium flow of 1.3 ml min^{-1}. The oven temperature was held at 34°C for 5 min and then programmed from 34 to 250°C at a rate of 5°C min^{-1}, where it was held for 10 min. Chromatograms were obtained by monitoring the total ion current in the 10–425 mass range without using a solvent delay. Semi-quantitative determinations were performed by using an external standard mixture of 0.12 μg of nonane, decane and undecane. The peak areas of the aroma compounds were related to the mean area of the three standard compounds. Semi-quantitative data of the volatiles released under the conditions described before were expressed as ng g^{-1} ham (ppb).

Statistical analysis

Mean and standard deviations were calculated for all quantified volatiles. The relationships between the different hams and the various volatiles were visualized by principal component analysis.

RESULTS AND DISCUSSION

It is generally recognized that the procedure selected for isolation of the volatiles from a food product is a crucial aspect of objective measurement of its aroma characteristics. We found it necessary to use both a headspace technique, which took into account the release of volatiles from the food matrix, and a 'total volatile' procedure, based on simultaneous SDE.

Dynamic headspace analyses

A typical dynamic headspace GC–MS analysis of a Serrano ham under the previously described conditions is presented in Fig. 1. The peak numbering is in accordance with the numbering in Table 1, which represents semi-quantitative data of a Serrano ham (Serrano 2) and of an experimental dry-cured ham ripened for six months (Drycur 6 M). About 60 constituents were identified in the headspace analyses of dry-cured hams, of which saturated and unsaturated aldehydes, ketones, alcohols, furans and sulfur compounds should be the major contributors to the flavour. Furthermore, some aromatic and aliphatic hydrocarbons, which might arise from contamination or could be secondary degradation compounds of lipid autoxidation, occurred in the chromatogram. Because of their relatively high threshold value (Devos *et al.*, 1990), hydrocarbons probably had no significant contribution to the flavour of the raw hams.

Aldehydes were the major class of volatiles that occurred in the headspace chromatograms. From a biochemical point of view, an important distinction could be made between aldehydes originated from lipid oxidation and aldehydes formed from amino acid degradation. Linear saturated (C_5 to C_{10}), unsaturated (C_6 to C_{11}) and polyunsaturated aldehydes (2,4-nonadienal and the isomeric 2,4-decadienals), with more than 5 carbon atoms are typical fat degradation compounds, formed by lipolysis-autoxidation mechanisms (Belitz & Grosch, 1987). Otherwise, butanal, methyl-branched aldehydes (2-methylpropanal, 2-methylbutanal and 3-methylbutanal) and phenylacetaldehyde are probably related to proteolysis and amino acid degradation. Because of their low threshold values and distinctive odour characters (e.g. rancid, sweet, floral, pungent notes), the aldehydes should be major contributors to ham flavour.

The monofunctional ketones identified in this study were methylketones, of which 2-propanone occurred in the highest concentration. As 2-propanone is used frequently for cleaning glassware, probably part of the 2-propanone concentration originated from lab-air contamination. Among the bifunctional ketones, 3-hydroxy-2-butanone might be important as a contributor to buttery notes.

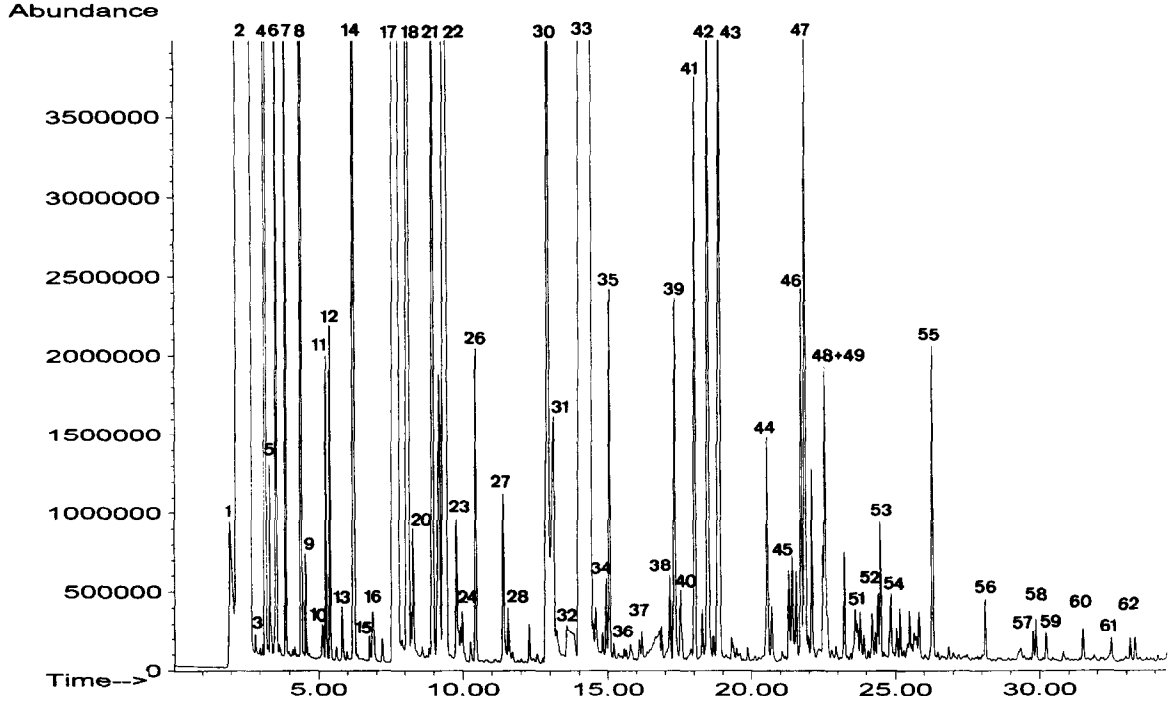

Fig. 1. Dynamic headspace GC–MS analysis of a dry-cured Spanish ham. Peak numbering is in accordance with the numbering in Table 1.

Table 1. Semi-quantitative data and coefficients of variation (CV) of volatiles, isolated by dynamic headspace, in Serrano 2 and Drycur 6 M (expressed as ng released compound g^{-1} ham)

No.	Volatile compounds	Serrano 2	CV Serrano 2	Drycur 6 M	CV Drycur 6 M
1	Air	n.q.	—	n.q.	—
2	Water	n.q.	—	n.q.	—
3	Ethanol	0.21 ± 0.14	68.5	0.26 ± 0.04	16.3
4	2-Propanone + propanal	10.25 ± 2.49	24.3	9.09 ± 0.66	7.3
5	2-Propanol	0.54 ± 0.21	38.7	1.15 ± 0.12	10.5
6	Pentane	6.58 ± 1.60	24.3	3.03 ± 1.12	36.9
7	Dichloromethane	6.70 ± 2.88	43.0	4.06 ± 1.52	37.5
8	2-Methylpropanal	10.87 ± 3.17	29.2	2.56 ± 0.81	31.5
9	1-Propanol	0.61 ± 0.06	9.9	1.19 ± 0.58	48.7
10	Diacetyl	n.q.	—	n.q.	—
11	Butanal	2.27 ± 0.56	24.7	0.92 ± 0.43	47.1
12	2-Butanone	2.21 ± 0.50	22.5	1.04 ± 0.65	62.5
13	2-Butanol	0.41 ± 0.01	1.4	0.78 ± 0.43	55.7
14	Hexane	11.86 ± 2.27	19.2	12.05 ± 4.46	37.0
15	Tetrahydrofuran	n.q.	—	n.q.	—
16	2-Methyl-1-propanol	0.56 ± 0.09	16.6	1.11 ± 0.53	48.0
17	3-Methylbutanal	57.35 ± 25.45	44.4	14.43 ± 6.26	43.4
18	2-Methylbutanal	20.45 ± 8.49	41.3	3.92 ± 1.07	27.4
19	Benzene	0.28 ± 0.04	12.9	0.29 ± 0.12	42.2
20	1-Butanol	1.02 ± 0.18	17.3	0.91 ± 0.50	55.5
21	2-Pentanone	9.92 ± 3.54	35.7	9.77 ± 0.62	6.3
22	Pentanal + unknown	24.91 ± 0.95	3.8	12.51 ± 3.76	30.1
23	2-Pentanol	1.11 ± 0.30	26.7	1.38 ± 0.55	40.0
24	3-Hydroxy-2-butanone	0.32 ± 0.04	11.3	0.38 ± 0.13	35.8
25	2-Ethylfuran	0.39 ± 0.05	13.6	0.42 ± 0.11	25.6
26	Heptane	2.45 ± 0.85	34.5	2.17 ± 2.01	95.0
27	3-Methyl-1-butanol	1.23 ± 0.30	24.0	4.27 ± 1.34	31.5
28	Dimethyl disulfide	1.59 ± 1.24	78.0	1.72 ± 0.35	20.6
29	2-Pentenal	n.q.	—	n.q.	—
30	Toluene	11.48 ± 2.51	21.8	12.25 ± 4.73	38.6
31	1-Pentanol	5.14 ± 2.38	46.4	3.95 ± 0.35	9.0
32	2-Hexanone	0.99 ± 0.09	8.7	0.74 ± 0.15	20.2
33	Hexanal	195.3 ± 80.5	41.2	104.5 ± 39.2	37.5
34	Butyl acetate	0.44 ± 0.09	20.2	0.84 ± 0.35	42.1
35	Octane	2.72 ± 0.51	18.9	2.90 ± 2.75	95.0
36	2-Octene	n.q.	—	n.q.	—
37	2-Hexenal	0.22 ± 0.04	16.4	0.32 ± 0.12	38.2
38	Ethyl benzene	0.52 ± 0.26	51.1	0.86 ± 0.35	41.1
39	1-Hexanol	3.38 ± 1.29	38.3	2.59 ± 1.23	47.5
40	Xylene	0.62 ± 0.28	44.2	1.32 ± 0.66	50.4
41	2-Heptanone	4.48 ± 1.85	41.2	2.75 ± 1.27	46.1
42	Heptanal	7.90 ± 3.00	38.0	4.85 ± 2.02	41.6
43	2-Butoxyethanol + 2,6-dimethylpyrazine	n.q.	—	n.q.	—
44	2-Heptenal + benzaldehyde	1.82 ± 0.65	36.0	1.56 ± 0.59	37.7
45	Dimethyl trisulfide	0.92 ± 0.49	53.6	0.85 ± 0.15	17.6
46	2,3-Octanedione	2.52 ± 1.13	44.8	7.44 ± 2.91	39.1
47	1-Octen-3-ol	7.14 ± 1.36	19.0	5.86 ± 2.57	43.9
48	2-Pentylfuran	1.01 ± 0.25	24.5	0.97 ± 0.23	23.3
49	Octanal	2.46 ± 0.84	34.2	2.64 ± 1.05	40.0
50	1,2,3-Trimethylbenzene	n.q.	—	0.54 ± 0.30	55.0
51	Phenylacetaldehyde	0.42 ± 0.19	45.4	0.62 ± 0.26	42.5
52	Limonene	0.32 ± 0.11	33.9	0.73 ± 0.44	60.1
53	2-Octenal	0.89 ± 0.29	32.7	0.87 ± 0.42	48.2
54	3,5-Octadien-2-one	0.51 ± 0.08	16.0	0.80 ± 0.55	68.9
55	Nonanal	2.34 ± 0.85	36.3	5.43 ± 4.35	80.2
56	2-Nonenal	0.46 ± 0.18	38.6	0.55 ± 0.30	55.8
57	Decanal	0.20 ± 0.03	14.2	1.51 ± 0.11	7.5
58	2,4-Nonadienal	0.33 ± 0.09	25.9	0.34 ± 0.16	48.6
59	Dimethyl tetrasulfide	0.25 ± 0.07	27.7	—	—
60	2-Decenal	0.25 ± 0.08	32.7	0.30 ± 0.09	31.2
61	c,t-2,4-Decadienal	0.30 ± 0.10	32.8	0.29 ± 0.16	57.1
62	t,t-2,4-Decadienal	0.29 ± 0.02	7.3	0.25 ± 0.20	79.2

n.q.: Not quantified (because of integration problems : no pure peak, very small peak, ...).

Because of their relatively high threshold values, the saturated alcohols detected in the headspace should be of less importance to the overall flavour. In contrast, 1-octen-3-ol, an unsaturated alcohol derived from lipid oxidation and imparting a potent mushroom odour, could be a major contributor.

Sulfur-containing compounds always have low threshold values. Despite their low concentration, dimethyl disulfide, dimethyl trisulfide and dimethyl tetrasulfide might influence overall flavour.

Although extreme care was taken during headspace enrichment of volatiles on Tenax, such as sampling in an air-conditioned room, we were not able to avoid air contamination occurring in the chromatograms (we also observed a whole series of suspected contamination compounds in several other publications dealing with dynamic headspace analyses of dry-cured hams). Probably due to the high fat content of hams, adsorption of volatile air contaminants is promoted. Taking into consideration the relatively high standard deviation and

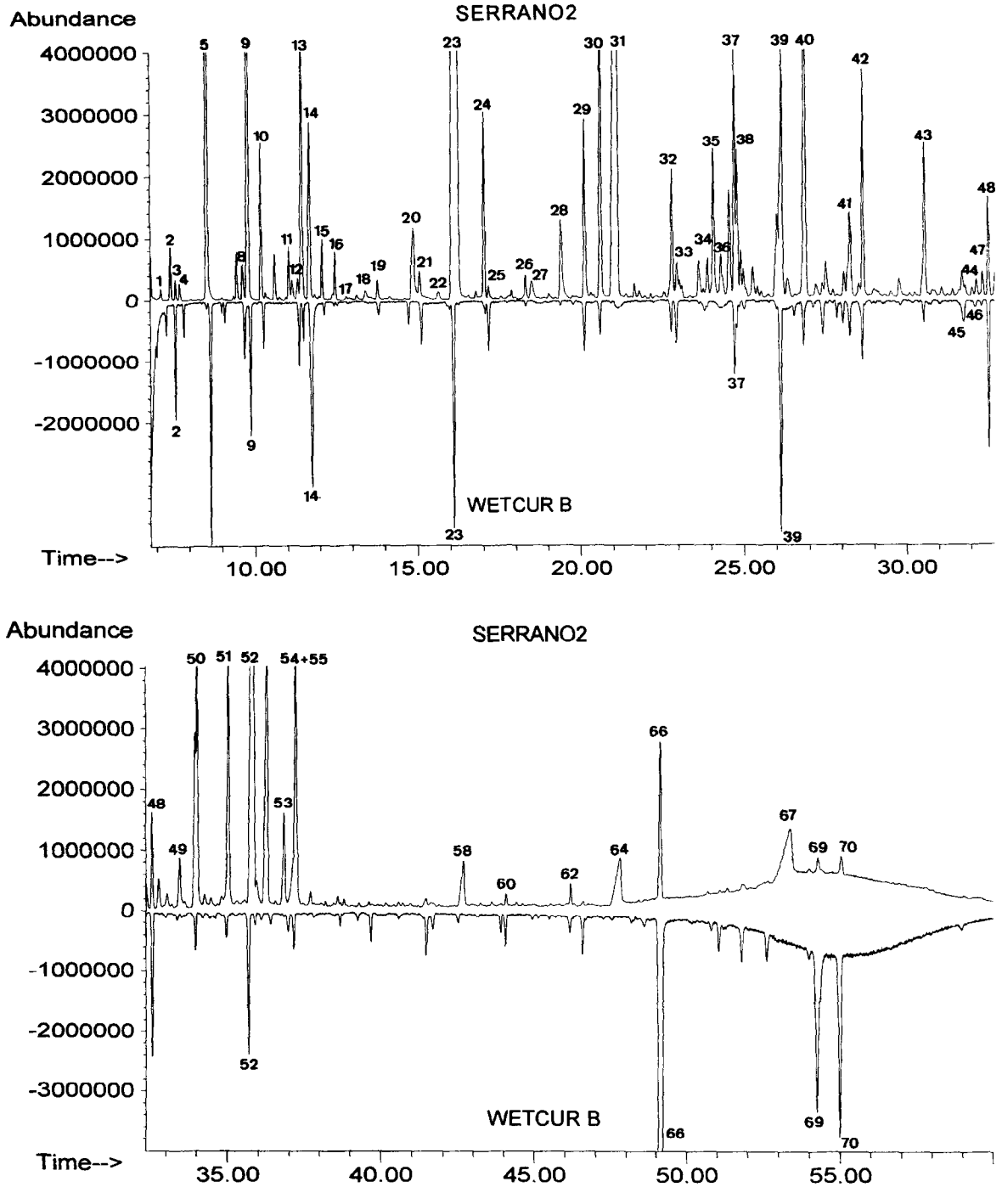

Fig. 2. GC–MS analyses of the Likens-Nickerson extracts of a dry-cured Serrano ham compared to a brine-cured ham. Peak numbering is in accordance with the numbering of Table 2.

Table 2. Semi-quantitative data of volatiles, isolated by Likens-Nickerson extractions, from dry-cured and brine-cured hams (expressed as ng g^{-1} ham)

No.	Volatile Compounds	Serrano 1	Serrano 2	Parma	Drycur 2 M	Drycur 4 M	Drycur 6 M	Wetcur C	Wetcur B	Wetcur D	Wetcur S
1	Dichloroethene	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.
2	Diacetyl	69.3	117.5	88.1	33.4	70.6	55.4	86.0	74.0	99.9	97.3
3	Butanal	16.1	17.6	10.7	—	—	—	—	—	—	—
4	2-Butanone	22.7	28.4	19.2	19.4	30.2	60.3	12.7	20.4	12.6	14.4
5	Chloroform	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.
6	Trimethyloxirane	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.
7	Tetrahydrofuran	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.	s.i.
8	1-Hydroxy-2-propanone	71.1	168.1	62.3	28.7	44.7	50.4	55.5	64.1	62.4	50.2
9	3-Methylbutanal	360.8	491.7	257.4	18.9	91.7	116.9	46.9	114.8	70.2	82.0
10	2-Methylbutanal	116.5	157.1	69.2	—	26.5	33.2	15.3	38.6	23.9	29.3
11	2-Pentanone	106.8	59.1	50.8	—	—	—	—	—	—	—
12	2,3-Pentanedione	55.2	42.2	24.2	20.2	33.4	24.0	29.4	57.8	24.7	17.3
13	Pentanal	270.9	320.1	281.1	76.2	115.9	76.2	14.5	31.0	18.2	7.7
14	3-Hydroxy-2-butanone	341.5	769.3	375.4	163.3	373.9	261.4	322.2	245.3	342.2	176.0
15	2-Ethylfuran	108.1	59.1	31.5	25.7	32.6	27.7	6.7	14.1	12.9	2.1
16	Heptane	31.8	41.7	36.6	7.8	12.1	12.7	—	2.2	7.0	—
17	Pyrazine	4.6	16.9	4.6	—	—	1.9	1.0	1.0	9.6	11.2
18	3-Methyl-1-butanol	38.1	18.3	n.q.	22.6	23.5	16.0	3.9	—	—	—
19	2-Pentenal	34.1	26.9	5.3	11.3	14.5	11.1	12.5	n.q.	n.q.	n.q.
20	1-Pentanol	298.7	148.2	128.1	64.5	82.1	94.2	18.1	—	—	—
21	Toluene	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
22	2-Hexanone	14.6	n.q.	n.q.	n.q.	n.q.	n.q.	—	—	—	—
23	Hexanal	1787	2243	1612	1031	1634	1256	142.2	234.5	137.1	41.5
24	Octane	137.1	136.3	161.6	23.3	23.3	48.9	6.1	16.2	17.1	—
25	Furfural	35.1	22.2	7.4	17.8	36.0	37.2	26.5	54.2	52.0	38.5
26	2-Hexenal	35.1	30.0	20.1	14.1	13.9	14.4	3.2	7.9	8.9	—
27	Furfuryl alcohol	23.4	76.6	22.6	—	13.4	12.7	n.q.	n.q.	64.3	22.6
28	1-Hexanol	136.3	117.8	105.9	—	—	51.4	—	—	—	—
29	2-Heptanone + methional	142.5	175.8	114.6	48.4	99.5	56.6	26.4	54.8	50.3	43.7
30	Heptanal	366.4	312.6	363.6	94.5	140.1	111.8	26.0	36.2	33.8	12.1
31	2-Butoxyethanol	p.c.	p.c.	p.c.	p.c.	p.c.	p.c.	p.c.	p.c.	p.c.	p.c.
32	2-Heptenal	172.4	134.2	141.1	51.4	70.3	75.2	20.0	30.9	40.3	17.1
33	Benzaldehyde	46.8	46.4	57.9	24.8	35.7	24.3	50.7	48.8	45.6	48.6
34	2,3-Octanedione	n.q.	62.1	n.q.	48.2	81.2	55.7	3.1	10.2	10.3	—
35	1-Octen-3-ol	198.6	154.2	177.7	109.8	147.5	123.0	16.2	7.9	10.0	—
36	2-Octanone	61.9	50.3	29.1	n.q.	n.q.	n.q.	—	—	—	—
37	2-Pentylfuran	496.3	239.9	243.4	185.2	340.1	252.2	22.2	65.2	31.8	9.1
38	Octanal	218.1	190.1	356.1	60.5	83.6	64.9	15.3	21.1	23.3	12.1
39	Phenylacetaldehyde	499.0	530.3	309.1	27.6	138.7	182.6	102.8	313.1	467.9	263.3
40	2-Octenal	632.7	550.4	391.0	138.5	145.5	186.3	34.5	45.5	38.0	4.9
41	1,2,4-Trithiolane	76.6	41.5	67.0	n.q.	n.q.	29.1	51.8	49.2	73.1	52.4
42	Nonanal	373.0	266.7	461.8	159.3	228.3	174.5	46.5	61.0	66.8	8.9
43	2-Nonenal	160.1	161.3	140.5	51.3	76.7	64.1	15.3	23.3	27.6	7.7
44	Decanal	29.5	25.8	28.7	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
45	Alfa-terpineol	—	—	—	—	—	—	41.4	15.5	n.q.	n.q.
46	Beta-pinene	—	—	—	—	—	—	14.8	7.5	4.6	4.7
47	2,4-Nonadienal	41.9	31.7	22.6	12.9	18.2	17.0	19.8	4.1	3.9	—
48	Dodecane	—	i.s.	—	i.s.	i.s.	i.s.	—	i.s.	i.s.	i.s.
49	Gamma-octalactone	40.0	46.3	28.5	—	95.0	6.5	—	—	—	—
50	2-Decenal	223.7	335.6	354.6	88.6	125.9	123.6	14.6	43.6	50.9	7.3
51	c,t-2,4-Decadienal	207.5	228.6	104.2	60.6	86.0	119.8	16.7	52.1	47.7	—
52	t,t-2,4-Decadienal	1021	1132	534.4	245.4	394.9	569.4	66.9	193.5	279.1	24.5
53	Gamma-nonalactone	57.9	78.5	85.2	—	135.2	9.0	3.0	—	—	—
54	Decanoic acid	111.9	115.7	54.3	—	48.8	34.3	—	38.4	108.0	59.1
55	2-Undecanal	192.3	303.8	273.0	71.9	97.6	103.9	10.9	42.5	54.0	—
56	Ethyl decanoate	—	—	19.1	—	—	—	—	—	—	—
57	Tetradecane	i.s.	—	i.s.	—	—	—	i.s.	—	—	—
58	Dodecanoic acid	75.6	64.8	41.8	33.6	48.8	94.2	12.2	23.0	87.0	35.7
59	Ethyl dodecanoate	—	—	3.5	—	—	—	—	—	—	—
60	Tetradecanal	14.7	34.1	23.3	15.4	—	—	22.0	27.2	—	18.5
61	Gamma-dodecalactone	7.3	n.q.	—	—	21.0	—	—	—	—	—
62	2-Pentadecanone	—	41.3	—	31.2	49.8	35.5	—	35.2	48.1	47.0
63	Pentadecanal	18.4	47.3	45.9	9.1	30.0	16.3	29.2	40.1	—	16.6

Table 2. *Cont'd.*

64	Tetradecanoic acid	218.3	51.2	48.0	159.6	832.7	621.2	44.5	31.4	72.0	—
65	Ethyl tetradecanoate	—	—	10.8	—	—	—	—	—	—	—
66	Hexadecanal	347.3	1280	1675	271.8	1149	799.9	1081	1500	450.4	1115
67	Hexadecanoic acid	383.9	—	127.3	663.3	1187	1570	246.3	—	—	36.0
68	Ethyl hexadecanoate	—	—	154.4	—	—	—	—	—	—	—
69	9-Octadecenal	101.3	201.7	173.0	279.8	159.2	173.3	203.3	279.8	94.5	146.0
70	Octadecanal	66.7	197.9	164.8	—	114.3	114.3	70.9	215.3	74.4	138.9
	Sum	10719	12240	10199	4521	8853	8071	3131	4293	3256	2719

s.i.: Solvent impurity.
n.q.: Not quantified (because of integration problems: no pure peak, very small peak, ...).
p.c.: Plastic contaminant.
i.s.: Internal standard.

coefficients of variation in our experiments (Table 1), we also concluded that dynamic headspace analyses suffered from lack of reproducibility to be used for comparing different types of ham by semi-quantitative GC–MS profiling. Although dynamic headspace was recognized to be very valuable for isolation of volatiles from products like beverages, fruits and vegetables (Dirinck *et al.*, 1984; Dirinck & De Winne, 1994), in the case of fatty and inhomogeneous materials, in our ham experiments it was very difficult to standardize the release of volatiles after disintegration of the product. For both reasons described before, we concentrated our efforts on the development and the use of a 'total volatile analysis' method (SDE, Likens-Nickerson extraction) for comparing semi-quantitative GC–MS patterns of the different ham types.

GC–MS profiling of Likens-Nickerson extracts

In Fig. 2, a typical GC–MS profile of the Likens-Nickerson extracts of a dry-cured ham (Serrano 2) is compared to the profile of a brine-cured ham (Wetcur B). Peak numbering is in accordance with the numbers in Table 2, which presents the semi-quantitative data (mean of triplicate analyses, dodecane as internal standard) of all hams in this study. Comparison of the dry-cured ham in Fig. 2 with the dynamic headspace analysis in Fig. 1 illustrated that aroma patterns were largely influenced by the isolation procedure. However, from a qualitative point of view, and despite the heat processing during SDE-extraction, a lot of similar components could be detected in the chromatograms of both isolation procedures. Saturated and unsaturated aldehydes were also major volatiles in Likens-Nickerson chromatograms: one, a whole series of aldehydes formed by autoxidation of unsaturated fatty acids (e.g. pentanal, 2-pentenal, hexanal, 2-hexenal, heptanal, 2-heptenal, octanal, 2-octenal, nonanal, 2-nonenal, 2,4-nonadienal, decanal, 2-decenal, 2-undecenal and the isomeric 2,4-decadienals); two, branched and aromatic aldehydes formed by oxidative deamination-decarboxylation (Strecker degradation) of amino acids and related to proteolysis (2-methylpropanal, 2- and 3-methylbutanal, and phenylacetaldehyde). Besides these high volatile aldehydes, several high molecular weight aldehydes (e.g. tetradecanal, pentadecanal, hexadecanal, octadecanal) were also identified in the SDE-extracts. These higher aldehydes also occurred in raw meat (Dirinck & De Winne, 1996). They could act as precursors for the volatile alkanals and alkenals, but because of lower volatility their direct contribution to ham aroma should be of less importance.

A whole series of methylketones (e.g. 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, 2-pentadecanone) occurred in the chromatograms. Methylketones are also lipid degradation products and play a major role in the flavour of cheeses (De Frutos *et al.*, 1991). Polyfunctional ketones, such as diacetyl and acetoïne, could be important as contributors to buttery notes. Important concentrations of two non-carbonyl oxidation products, 2-pentylfuran and 1-octen-3-ol (mushroom), were also found in the SDE GC–MS profiles of dry-cured hams.

Further evidence of the major role of fat degradation in flavour formation was provided by the occurrence of the γ-lactones, which are products of dehydratation and cyclisation of the γ-hydroxyacids. Lactones are very potent aroma compounds with fatty, creamy, fruity and coconut-like odours.

In our opinion, the SDE approach resulted in a more complete picture of the different volatiles contributing to ham flavour, and artefact formation as a result of the heat treatment during isolation was limited. Sulfur-containing constituents, which have the reputation to be reactive and easily transformed, probably formed an exception to this statement. Dimethyl disulfide, dimethyl trisulfide and dimethyl tetrasulfide could not be detected in the SDE-extracts and probably the identified 1,2,4-trithiolane was not a genuine component of ham but was formed as a result of the isolation procedure.

Besides the qualitative feature of providing a more complete volatile composition, the major advantage of the SDE isolation procedure was experienced when determining semi-quantitative data. In our experiments, obtaining quantitative data by dynamic headspace analyses was often hindered by problems as a result of blocking of the cooling trap or leakage of the system. Even for successful dynamic headspace analyses, the mean coefficient of variation (triplicate determinations of all components) was 36% with a range of 7 to 95%.

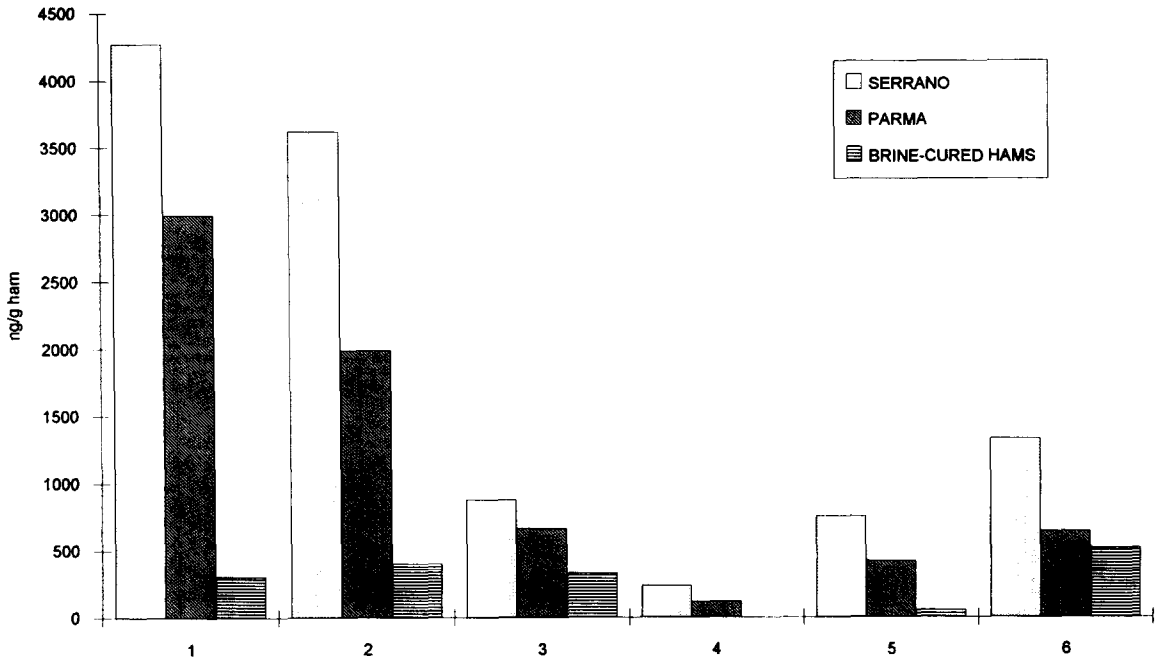

Fig. 3. Sum of different chemical classes of volatiles in Serrano, Parma and brine-cured hams: 1 = sum of alkanals (C_5–C_{10}). 2 = sum of alkenals (C_5–C_{11}) and polyunsaturated aldehydes (2,4-nonadienal and the isomeric 2,4-decadienals). 3 = sum of methylketones (C_5, C_6, C_7, C_8), acetoine; 2,3-pentanedione and 2,3-octanedione. 4 = sum of γ-lactones (C_8, C_9, C_{12}). 5 = sum of 2-pentylfuran and 1-octen-3-ol. 6 = sum of 2-methylbutanal, 3-methylbutanal and phenylacetaldehyde.

For reasons of clarity, the standard deviations and coefficients of variation were not included in Table 2. However, in the case of SDE, extracts reproducibility was consistently better and the mean coefficient of variation for all compounds in Table 2 was 20.1%. Probably due to chromatographic reasons, the highest values were obtained for the fatty acids (65%) but, for well separated peaks, values of about 5 to 10% could easily be obtained.

Comparison of the GC–MS profiles in Fig. 2 and careful examination of the data in Table 2 showed that southern European dry-cured hams (Serrano and Parma) had a much more intensive volatile pattern (sum of volatiles: 10–12 ppm) compared to brine-cured products (sum of volatiles: 2.6–4.2 ppm). As can be seen from Fig. 3, the most differentiating compounds were related to fat oxidation: alkanals (C_5 to C_{10}), alkenals and polyunsaturated aldehydes (C_5 to C_{11} alkenals; 2,4-nonadienal and the isomeric 2,4-decadienals), methylketones (C_5, C_7, C_8), γ-lactones (C_8, C_9, C_{12}), and non-carbonyl oxidation products (2-pentylfuran and 1-octen-3-ol). Furthermore, the much more important concentration of the amino acid degradation products, 3- and 2-methylbutanal, in dry-cured products should not be neglected. Phenylacetaldehyde, which also should be related to amino acid degradation, was less differentiating between dry-cured and brine-cured hams.

The quantitative data of the experimental dry-cured hams (Drycur) as a function of ripening time showed an important increase of the fat degradation and amino

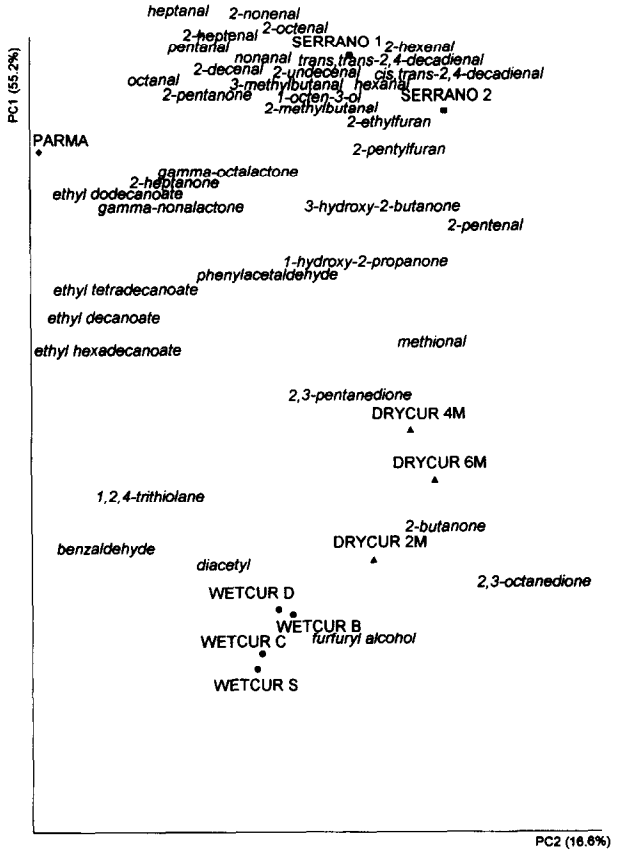

Fig. 4. Principal component analysis (plot of the two first principal components) of the volatile composition of dry-cured (2 × Serrano, Parma and 3 experimental) hams and brine-cured hams.

acid degradation compounds from two to four months of ripening. However, the GC–MS analyses showed a more intensive pattern after four months of ripening compared to six months. This was probably due to variation between individual hams.

In order to visualize the information presented in the complex data matrix in Table 2, a principal component analysis was performed.

Principal component analysis

Results of the principal component analysis are shown in Fig. 4. A plot of the two first principal components explained 71% of the total variance and permitted mapping of the different hams into a two-dimensional space. Observation of the relationships between the hams revealed some meaningful clusters and showed a clear distinction between dry-cured and brine-cured hams. Among the dry-cured hams the two Serrano hams were closely related and somewhat differentiated from the Parma ham. The different wet-cured hams (commercial production and the hams produced from pork from Belgian, Dutch and Spanish origin) formed a cluster and were strongly related. One should add that these hams were produced at the same company. The experimental dry-cured hams ripened for 2, 4 and 6 months were located between the southern European dry-cured and the brine-cured hams. Also, from PCA analysis, it was shown that the Drycur 4 M ham was closer to the dry-cured products compared to the Drycur 6 M, indicating the variability among different hams.

The relation between the hams (objects) and the aroma compounds (variables) was visualized by plotting the variables in the same plane. Inspection of the aroma components in the two-dimensional space showed that the highest concentration of fat oxidation products (alkanals, alkenals, 2-pentylfuran, 1-octen-3-ol) were found in the Serrano hams. The Parma ham was characterised by esters and by a high level of γ-nonalactone. These observations were in accordance with the use of higher temperatures during ripening of Serrano hams, resulting in a more pronounced oxidation.

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