Relationship among Flavor, Volatile Compounds, Chemical Changes, and Microflora in Italian-Type Dry-Cured Ham during Processing

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To improve the flavor of modern cured meat products by means of microorganisms, Parma ham can serve as a model. Therefore, 29 dry-cured Parma hams representing six different stages in the manufacturing process were examined to reveal the relationship between the formation of flavor and the occurrence of microorganisms. Quantities of volatile compounds, chemical changes, sensory profiles, and quantitative isolation of microorganisms showed that flavor formation can be divided into two phases, the first including salting, drying, and ripening and the second including only ripening and postripening. The first phase is characterized by autoxidation, while the second phase reflects secondary metabolism of microorganisms, especially amino acid catabolism. The results suggest that flavor-improving microorganisms should possess a secondary metabolism which generates methyl-branched aldehydes, secondary alcohols, methyl ketones, ethyl esters, and dimethyl trisulfide in a distinct balance.

Keywords: *Dry-cured ham; flavor; volatile compounds; microbiology; multivariate statistics*

INTRODUCTION

The consumers' consciousness of meat quality in the industrialized world has during recent years become more profound. Consumers prefer products of higher quality than is produced by modern cost-efficient methods. An important contributor to the quality of the products is the flavor, which is decisive for the continuous purchase of a product and, therefore, the success of the product (Verplaetse, 1994). Parma ham is a classic nonsmoked, dry-cured meat product of northern Italy characteristic of a distinct flavor; it may therefore serve as a model for development of new cured meat products with improved flavor. The flavor of these new meat products could be manipulated by the use of microorganisms, as it is well-known that microorganisms possess the potential of changing the flavor in fermented meat products (Berdagué et al., 1992; Hinrichsen and Andersen, 1994; Stahnke, 1994). It is also well-known that the flavor of dry-cured meat products is the result of various enzymatic and nonenzymatic degradations of the macromolecules within the tissues of the hams. However, the importance of the secondary metabolism of the microorganisms present in the meat is not known.

The content of volatile compounds is of major importance to the flavor of dry-cured meat products (Berdagué et al., 1991); consequently, a better understanding of the underlying mechanisms that lead to the formation of volatile compounds in Parma ham and further their relation to the perception of the product is needed. Several investigations have been performed to quantify and identify volatile compounds in French (Berdagué et al., 1991; Buscailhon et al., 1993), Spanish (Garcia et al., 1991), and Italian (Barbieri et al., 1992) dry-cured ham. The volatile compounds in these products are evidently composed of a wide range of various organic compounds.

Careri et al. (1993) tried to correlate a few selected volatile compounds from Parma ham to a sensory profile and found that short-chain methyl-branched oxy compounds correlated well with the "aged" flavor of the hams. This is not the case in French dry-cured ham as Buscailhon et al. (1994) could not confirm this; they found that methyl ketones and straight-chain alcohols correlated to the "cured ham" flavor.

In none of these investigations was the microbiology of the hams taken into consideration. Most microbiological investigations of dry-cured hams deal with spoilage; hence, these microorganisms are unwanted in the product. Therefore, a collaborative study to find the relationship between flavor and microorganisms was undertaken. Development of volatile compounds, chemical changes, sensory profile, and microorganisms was measured and, subsequently, sensory profiles and quantities of volatile compounds were correlated by multivariate statistics.

MATERIALS AND METHODS

Products. In total 29 Parma hams from a factory in Langhirano, Italy, were purchased directly at the manufacturer. The hams were manufactured according to a traditional processing scheme based on a 25-day dry salting period at 1–3 °C followed by a rest period of 90 days at 1–4 °C and air-drying and primary ripening at 15–20 °C for 90 days at 60–90% relative humidity. Pork fat is then smeared on cut surfaces; final ripening takes place at 17–18 °C for 160 days followed by postripening at 17–18 °C for a further 120 days as described by Parolari et al. (1994). Hams for analyses were selected at specific stages in the process: five hams prior to salting (3 days), five hams after salting (25 days), five hams after drying (125 days), five hams after the first ripening (211 days), five hams after the second ripening (fully matured product, 365 days), and three hams after postripening (485 days). Furthermore, a spoiled, fully matured ham was included in the analyses.

Sampling. Initially samples were taken for microbiological analyses (see below). Then hams were derinded, leaving 1 cm of fat on the hams. To obtain representative sampling, only the cushion part of the ham was examined. This included essentially M. biceps femoris, M. semimembranosus, and M. semitendinosus. The cushion part was sliced from the hip end: 20 slices (1 mm) for sensory analyses, 1 for gas chromatographic analyses (5 mm), and finally 1 for chemical analyses (5 mm). When this sequence was ended, it was reiterated ($n = 2$) from samples for gas chromatography.

Samples for sensory analyses were stored in sealed vacuum bags without vacuum at 2 °C for no longer than 24 h. Samples

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Table 1. Family or Genus Criteria According to *Bergey's Manual* (Sneath, 1986)

Gram	cell morphology	catalase	oxidase	motility	O/F	family/genus
+[a]	cocci	+	D[b]	−[c]	D	Micrococcaceae
+	cocci	−	−	−	F[d]	Gram-positive cocci
+	rod	+	−	−	F	*Corynebacterium* spp.
+	rod	−	−	−	F	*Lactobacillus* spp.
−	rod	+	−	−	O[e]	*Acinetobacter* spp.
−	rod	+	−	D	F	Enterobacteriaceae
−	rod	+	+	D	D	Gram-negative rods

[a] Positive reaction. [b] Variable reaction. [c] Negative reaction. [d] Fermentative reaction. [e] Oxidative reaction.

for gas chromatography were wrapped in aluminum foil and then packed in vacuum bags with vacuum at −20 °C for no longer than 2 months.

Microbiology. Samples were taken from meat and rind surface by cutting 10−15 g in a depth of 3 mm. From within the meat, samples were taken from the cutting surface perpendicular to the femur.

Samples were diluted in 0.1% peptone with added 4% NaCl and subsequently poured on brain heart infusion agar with added 4% NaCl (BHI, Merck). Samples from surfaces were incubated aerobically at 30 °C, while samples from within the meat were incubated under microaerophilic atmosphere (Anaerocult C, Merck) at 30 °C for 5 days.

Additionally, direct epifluorescent filter technique (DEFT) was applied to all samples according to the NMKL (1990) procedure to differentiate between viable and sublethal damaged microorganisms. A Bactis ScanBeam system connected to an Olympus microscope (BH-2) was used.

After incubation for 5 days, microorganisms were isolated quantitatively by selecting BHI plates with counts between 100 and 150. These plates were divided into four parts, and from one part all colonies were subcultivated. Colony morphology, Gram reaction [according to the method of Gregersen (1975)], oxidase reaction (Bactident Oxidase, Merck), and catalase reaction were tested on the subcultivated colonies on BHI with added 4% NaCl at 30 °C. Cell morphology was examined by phase contrast microscopy of a 1 day old culture in BHI bouillon with added 4% NaCl at 30 °C. The oxidative/fermentative (O/F) reaction was examined in OF basal medium (Merck) with added 1% serum. Isolates were identified according to Table 1.

Chemical Analyses. Sodium chloride was determined by potentiometric titration with $AgNO_3$ in an autotitrator (Radiometer, Denmark) as described by the NMKL (1986), and results are given as percent (w/w) NaCl. Nitrate and nitrite were measured spectrophotometrically at 546 nm using the diazotation reaction of sulfanilamide and subsequent coupling with *N*-(1-naphthyl)ethylenediamine (NMKL, 1982). Results are given as parts per million of KNO_3 and $NaNO_2$. For determination of fat, 3.00 g homogenized samples were extracted with 25 mL of diethyl ether for 5 min using a Sortex extractor (Tecator) with subsequent evaporation of the organic solvent, hydrolyzed with 7 M HCl for 10 min at 100 °C, washed three times with double-deionized water before final extraction with diethyl ether. Results are given as percent (w/w). Water content was determined after drying of a 3.00 g homogenized sample at 105 °C for 18 h and subsequent cooling in a desiccator. Results are given as percent water (w/w). pH was measured in a homogenate of 3.00 g of sample in 3.00 g of double-deionized water after 10 min with a combined electrode (Model GK2401C) connected to a PHM 83 Autocal pHmeter (Radiometer, Denmark). Protein content was determined in an automated system (Foss Hereaus macro N) according to the principle of Dumas (Foss Hereaus Analysensysteme GmbH, 1990). Results are given as percent (w/w). All analyses were carried out in duplicates.

Extraction of Volatile Compounds by Head Space Gas Chromatography. Thirty grams of sample was homogenized and transferred to a 500 mL conical flask with a Dreschel head joined to a trap packed with 250 mg of Tenax (Tenax TA, mesh 60−80, Chrompack). One milliliter of a 16.7 μM aqueous solution of *n*-chloroheptane in a small cup was placed in the flask as internal standard. The flask was sealed with parafilm and equilibrated for 30 min at 50 °C. Volatile compounds were purged onto the Tenax trap with ultrapure nitrogen at a flow rate of 60 mL min^{-1}. Volatile compounds were thermally desorbed from the Tenax trap (250 °C; 30 min) in a Perkin-Elmer ATD400 automatic thermal desorption system and retrapped on a Tenax-packed cold trap maintained at −30 °C. Injection into the GC column was by thermal desorption of the trap at 300 °C for 2 min with a split of 1:24. For GC analysis a Perkin-Elmer 8120 gas chromatograph connected to a Varian Star chromatography work station (ver. 4.0) was used. A flame ionization detector (FID) held at 250 °C was used. Chromatographic separations were performed with a DB-1701 capillary column (J&W Scientific; 30 m × 0.25 mm i.d., film thickness 1.0 μm) using He as carrier gas (linear flow 29.17 cm s^{-1}). The column temperature was held at 35 °C for 10 min and then raised from 35 to 150 °C at 3 °C min^{-1}, held at 150 °C for 5 min, and then ramped from 150 to 250 °C at 30 °C min^{-1} with a final holding time of 5 min. Kovats indices (KI) of the volatile compounds were computed according to the method of Kovats (1965). Gas chromatographic peak areas were calibrated to an *n*-dodecane standard curve and expressed as nanogram dodecane equivalents. Samples were analyzed with five replicates.

Head Space Gas Chromatography−Mass Spectrometry. Extraction of volatile compounds and chromatographic separations were carried out as described above; however, a Spantech thermal desorber (TD4) connected to a Varian gas chromatograph equipped with a Finnigan MAT ITS40 ion trap was used. The linear flow was 27.78 cm s^{-1}. Mass spectra were obtained with electron impact ionization with auto ion control (background mass 45, peak threshold 1, mass defect 0) in the range 15−250 amu.

Sensory Analyses. Before evaluation, the samples were equilibrated at ambient temperature for 1 h. The nine-member panel was trained according to the quantitative descriptive analysis method as described by Stone and Sidel (1993). Before analysis, the panel developed a profile with the descriptors *nutty*, *cheesy*, *meaty*, *salt*, *stale*, and *fatty* on the basis of test samples. The panel then evaluated all samples except samples taken before salting with an unstructured intensity scale ranging from 0 to 15 with anchor points at 1 and 14.

Statistical Analyses. Results from chemical and sensory analyses were examined by analysis of variance using SAS statistical software (SAS, 1988). When significant *F* values were obtained, the method of least-squares means was used to determine significant differences between means. Results from gas chromatography were examined by principal component analysis (PCA) and subsequently soft independent modeling of class analogy (SIMCA) in the Unscrambler (CAMO A/S, 1994). Results from chromatography and sensory analyses were compared by projection on latent structures (PLS). For detailed information on the applied multivariate techniques, see the extensive review by Aishima and Nakai (1991).

RESULTS AND DISCUSSION

Microbiology. Changes in the number of colony-forming units (CFU) from the microbiological analysis on BHI agar and cell counts from DEFT analyses during processing are shown in Figure 1.

At the beginning of processing there were 10^7 CFU g^{-1} on the surface of the meat. The number increased 1 log-unit after 25 days of storage and remained at 10^8 CFU g^{-1} for the rest of the storage period. Plate counts on BHI agar and DEFT matched within acceptable limits.

On the rind surface there were initially 10^8 CFU g^{-1} on BHI agar and 10^7 CFU g^{-1} at DEFT analysis. On BHI agar the number of cells decreased gradually during the storage period, ending at 10^3 CFU g^{-1} after 485 days. DEFT analysis showed an unchanged number of microorganisms at 10^7 CFU g^{-1} the first 125 days, after which time it decreased to 10^5 CFU g^{-1} after 485 days, slightly higher than the corresponding BHI count.

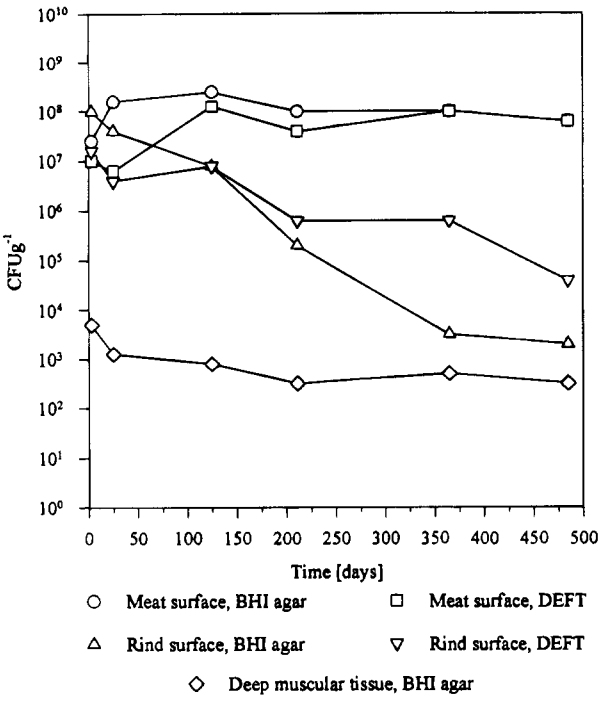

Figure 1. Number of colony-forming units from the microbiological analyses as measured on BHI agar and by DEFT on meat and rind surfaces and in deep muscular tissue.

This difference between plate counts and DEFT can be explained by the physiological state of the microorganisms. In the DEFT procedure nonviable cells are included. These cells will not be able to grow on BHI agar, and therefore a lower plate count is achieved compared to DEFT. This indicates that microorganisms on the rind surface become stressed during the storage period, resulting in lack of growth on BHI agar.

Within the meat the number of microorganisms was 10^4 CFU g^{-1} on BHI agar after 3 days. The number decreased to 10^3 CFU g^{-1} and remained constant for the rest of the storage period (Figure 1). It was not possible to count the microorganisms in the deep muscular tissue of the hams by DEFT analysis, because the number of microorganisms was below the detection limit of the DEFT analysis (10^5 CFU g^{-1}).

The quantitative distributions of microorganisms on the meat and rind surfaces and within the meat are shown in Figure 2. The percentage distribution is calculated from the total number of microorganisms isolated at the same time and from the same sampling point.

After 3 days, the meat surface was covered with 49% Gram-negative rods, which were catalase and oxidase positive but not further identified (Figure 2a). Besides, there was 29% Enterobacteriaceae, 5% Micrococcaceae, 5% *Corynebacterium* spp., 4% yeasts, and 12% unidentified.

Micrococcaceae gradually became dominating, and after 125 days, these species comprised 95% of the total microflora on the meat surface. After 211 days, Micrococcaceae was still dominating (98%). However, on the ripened product (365 days) Gram-positive, catalase and oxidase negative cocci were dominating (75%). Micrococcaceae made up 17% and unidentified, yeasts, and *Lactobacillus* spp. the last 8%. After postripening for an additional 120 days (485 days in all), the distribution of microorganisms remained unchanged. The occurrence of unidentified Gram-positive bacteria in dry-cured ham has also been observed by Molina et al.

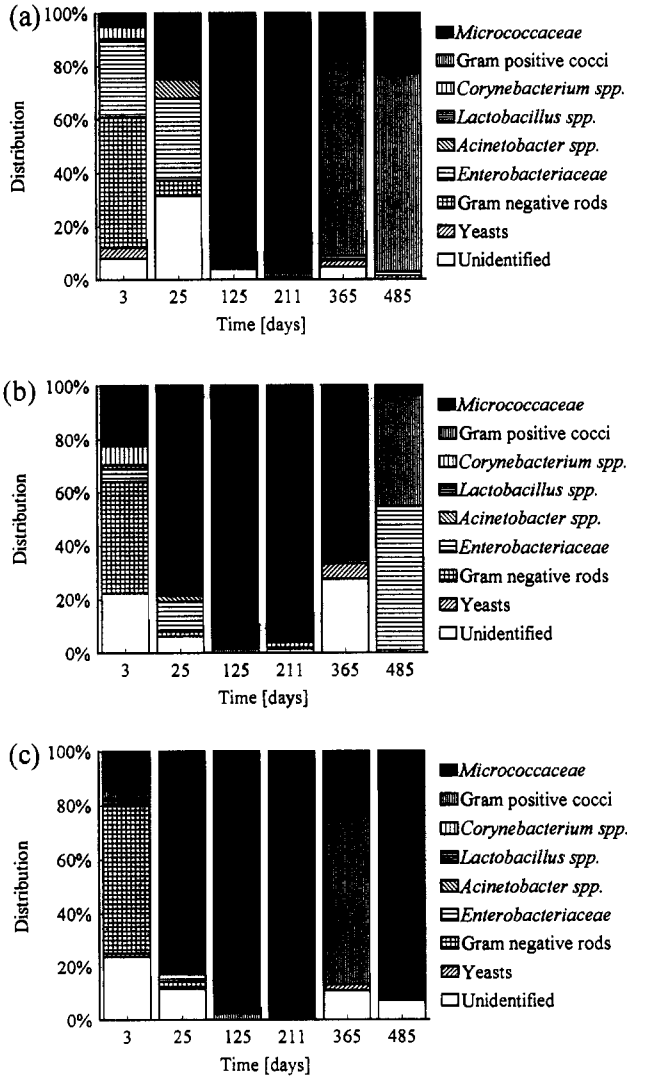

Figure 2. Quantitative distribution of microorganisms on meat surface (a) and rind surface (b) and within the meat (c). Distributions are based on colony-forming units per gram.

(1989a,b), who found 26% atypic streptobacteria in Spanish Serrano hams.

Initially in the processing Gram-negative rods dominated the rind surface (Figure 2b). Micrococcaceae made up 22%, *Corynebacterium* spp. 7%, Enterobacteriaceae 5%, and unidentified 22%. Again, Micrococcaceae gradually became dominating and was almost 100% dominating after 125 days. On the matured product (365 days) Micrococcaceae was still dominating (63%). Yeasts made up 6% and unidentified 28%.

After postripening 120 days later (485 days in all), the microflora changed completely on the rind surface. Now Enterobacteriaceae constituted 55%, Gram-positive cocci 42%, and Micrococcaceae 4%.

Carrascosa et al. (1992) found that Enterobacteriaceae did not survive in postripened ham; however, we found high numbers of Enterobacteriaceae late in the processing, which was surprising. According to Baldini and Raczynski (1979) *Enterobacter* spp. are inactivated during the first weeks of processing due to the combination of low temperature, progressive dehydration, and increase of sodium chloride. The reason for our findings may be that the hams were contaminated immediately before sampling. Another possibility is that the isolated bacteria are categorized incorrectly. The DEFT counts indicated that microorganisms on the rind surface were stressed. This may lead to loss of characteristics in the strains and, subsequently, erroneous classification.

Table 2. Chemical Analyses ($n = 4$)[a]

time (days)	NaCl (%)	nitrate (ppm)	nitrite (ppm)	pH	fat (%)	free water (%)	protein (%)
3	0f	0c	0.0d	5.87bc	17.5ab	63.4a	18.6c
25	1.9e	9.6a	2.6a	5.72c	16.7b	60.7ab	19.6bc
125	2.8d	9.4a	2.1ab	5.93b	17.5ab	57.8b	21.2ab
211	3.6c	6.8a	1.0c	5.97ab	17.7ab	55.7b	21.4ab
365	4.4a	7.6a	1.3c	5.96ab	20.3ab	50.4c	23.0a
485	4.2ab	7.8a	1.5bc	5.88bc	22.1ab	48.8c	23.0a
spoiled	3.8b	3.0bc	2.0ab	6.12a	25.1a	48.5c	21.3ab

[a] Values followed by different letters in each column are significantly different ($P < 0.05$).

Within the meat Gram-negative rods dominated (55%) after 3 days (Figure 2c). Unidentified made up 24% and Micrococcaceae 14% of the total microflora.

Micrococcaceae dominated (83%) after 25 days and was still dominating after 211 days. After 365 days, it was still Gram-positive cocci that dominated, but the distribution was 65% Gram-positive cocci and 22% Micrococcaceae; 120 days later (485 days in all), Micrococcaceae dominated (93%) again.

The dominance of Micrococcaceae in dry-cured ham has frequently been reported in Parma ham (Giolitti et al., 1971; Baldini and Raczynski, 1979) and in Spanish ham (Molina and Toldrá, 1992; Carrascosa et al., 1992; Cornejo et al., 1993). However, the present results suggest several subsuccessions of microbial populations on and within the dry-cured ham according to the alternating distribution of Gram-positive bacteria throughout the process. The actual impact of the microorganisms in the product is therefore extremely complicated as each population may participate with a distinct contribution.

Microbiological analysis of the spoiled ham did not show any difference compared to the postripened hams at 485 days (results not shown). However, during cutting of the ham it appeared that the spoilage occurred in soft spots in the deep muscular tissue. If these spots were not represented in the microbiological analysis, there was no difference. Specific isolation of bacteria from the soft spot revealed the presence of a monoculture of a Gram-negative, swarming rod.

Chemical Analyses. Results from chemical analyses are shown in Table 2. Statistical analysis of variance showed that all measured parameters changed significantly during storage ($P < 0.001$). Most changes can be explained by the loss of water, which leads to an increase in the concentration of water-soluble substances. Nonetheless, changes in nitrate and nitrite concentrations and pH are very small and may be coincidental. NaCl, nitrate, and nitrite display the same diffusion rates within lean meat. Therefore, an increase in the concentrations of nitrate and nitrite would be expected as observed by Huerta et al. (1988) in Spanish dry-cured ham. The absence of this increase in Parma ham may be explained by the presence of nitrate-reducing microorganisms within the meat; such microorganisms have been observed in other cured meat products (Eddy and Kitchell, 1961; Andersen and Hinrichsen, 1995). The pH of the spoiled ham is slightly increased ($P < 0.001$) compared to that of the fully ripened hams. This indicates excessive proteolysis in this ham and has been reported to be a major problem in Parma ham production (Parolari et al., 1994).

Volatile Compounds. A total of 84 volatile compounds were quantified from the Parma hams, and of these 73 were identified. Of the identified compounds 21 were aldehydes, 14 alcohols, 10 esters, 5 aliphatic hydrocarbons, 5 aromatic hydrocarbons, 5 carboxylic acids, and 4 sulfur compounds. The identifications and amounts of volatile compounds are listed in Table 3. Identified compounds have all previously been reported in Spanish Iberian ham (Garcia et al., 1991) and French dry-cured ham (Berdagué et al., 1991; Buscailhon et al., 1993). In Parma ham Barbieri et al. (1992) detected more than 100 volatile compounds. The reason for this high number compared to the present results is the use of different methodologies. Of reliably identified compounds (a and b identifications, Table 3) Barbieri et al. (1992) did not detect acetaldehyde, propanal, 2-methylpropanal, nonanal, methanol, ethanol, 2-methylbutanol, ethyl pentanoate, propyl acetate, diacetyl, heptan-2-one, nonan-2-one, nonane, and 3-methylbutanoic acid.

Results from principal component analysis of the volatile compounds are shown as score and loading plots in Figure 3 and included the spoiled ham. The first two principal components describe 88% of the total variance in data. The score plot (Figure 3a) illustrates that the spoiled ham (31) is different from the rest of the hams. Also, ham 28 is different and like the spoiled ham.

The reason for this difference is illustrated in the loading plot (Figure 3b) and is due to increased concentrations of the volatile compounds ethanol (KI 585), 3-methylbutanal (KI 734), 2-methylpropanal (KI 629), and 2-methylbutanal (KI 739). It seems that too much of these compounds in the hams indicates spoilage.

If hams 31 and 28 are excluded from the statistical analysis and, further, the outlying volatile compounds methanol (KI 530) and ethanol (KI 585) are omitted, the new loading and score plots are shown in Figure 4.

The first two principal components in Figure 4 describe 79% of the total variance in data. From the score plot in Figure 4a it appears that there are at least four groupings: group I, fresh and nonsalted hams (1–5); group II, hams after second salting and first drying (6–15); group III, hams after first ripening (16–20); and group IV, hams after second ripening (21–25) and postripened hams (26, 27).

Comparing the score plot with the loading plot (Figure 4a,b) illuminates the reason for these groupings. Group I (fresh hams) is characterized by having a low amount of volatile compounds, although it seems that toluene (KI 817) is characteristic for this group. Toluene may be a contaminant from the packing material used during transportation, as toluene is frequently found in plastic packagings (Hinrichsen et al., 1994). For group II (slightly older hams) there are still no characteristic volatile compounds except acetone (KI 603).

Group III, however, is characterized by very high contents of hexanal (KI 886) and pentanal (KI 781). Also, compounds such as *n*-pentanol (KI 883), acetic acid (KI 791), and heptanal (KI 990) are characteristic for this group. These compounds indicate that the reaction taking place at this stage in the processing is mainly autoxidation of lipids, as these compounds are secondary end products from autoxidation (Belitz and Grosch, 1986).

The last group, IV, is characterized by high contents of 3-methylbutanal (KI 734), 2-methylbutanal (KI 739), pentan-2-one (KI 777), heptane (KI 700), 2-methylpropanal (KI 629), butan-2-one (KI 686), and pentan-2-ol (KI 801), indicating that reactions taking place at this step are different and more complex than autoxidation.

Testing the observed groupings by SIMCA revealed that all groupings were significant ($P < 0.05$). SIMCA makes it possible to describe how the groupings differ with respect to the content of volatile compounds. In

Table 3. Identification and Quantification of Volatile Compounds in Parma Ham [Quantities Are Expressed as Nanogram Dodecane Equivalents (n = 50); for the Spoiled Ham n = 5]

compound	Kovats index	reliability[a]	3 days mean	3 days SD	25 days mean	25 days SD	125 days mean	125 days SD	211 days mean	211 days SD	365 days mean	365 days SD	485 days mean	485 days SD	spoiled mean	spoiled SD	SIMCA[b]	PLS[c]
aldehydes																		
acetaldehyde	2,06	a	19	10	26	10	55	45	43	15	67	29	109	102	329	118	I–II	–
propanal	601	b	620	341	1439	529	2035	1417	3016	1331	3146	790	2958	729	1662	303	II–III–IV	–
2-methylpropanal	637	b	3	9	5	3	12	6	45	13	120	51	696	902	4759	1466	II–III	+
2-methyl-2-propenal	713	c	3	2	3	2	5	3	6	2	7	2	8	3	14	5	I–II	–
3-methylbutanal	736	a	0	1	5	3	27	18	126	45	379	213	1357	1463	14370	3520	II–III–IV	+
2-methylbutanal	742	b	20	9	9	6	34	21	83	32	183	88	704	829	4129	1199	II–III–IV	+
pentanal	782	b	7	5	11	19	13	16	15	20	101	231	36	33	41	29	I–II	+
2-methyl-2-butenal	838	b	0	0	0	0	0	1	2	2	1	3	1	2	6	3	ns[d]	+
hexanal	887	b	161	128	1202	649	2220	1402	4158	1483	3414	1183	2504	1091	1236	461	I–II–III–IV	–
2,4-octadienal	934	c	3	14	1	3	1	2	1	2	2	2	2	3	7	5	ns	–
hexenal	945	b	1	4	0	0	0	0	0	1	0	1	0	1	1	2	ns	–
2-hexenal	963	c	0	1	2	2	3	3	5	4	3	3	3	3	0	1	ns	–
heptanal	988	b	1	2	6	3	11	6	22	11	42	30	39	15	42	27	I–II	–
2,4-nonadienal	1032	c	0	1	0	0	0	0	0	0	0	0	1	2	3	4	I–II	–
2-heptenal	1071	c	2	2	16	5	22	15	44	9	34	13	33	13	14	7	I–II–III	–
benzaldehyde	1086	c	1	2	2	2	4	5	11	7	16	6	14	4	9	5	I–II	+
octanal	1090	b	7	4	7	2	7	3	13	3	16	3	17	4	17	6	I–II	+
2-octenal	1174	c	5	8	1	2	5	20	0	2	2	4	4	4	3	3	ns	–
benzenacetaldehyde	1183	c	3	6	8	7	13	9	24	12	15	9	15	13	3	3	ns	–
nonanal	1192	b	74	29	90	21	107	35	182	35	168	34	152	40	101	24	ns	–
decanal	1296	b	17	11	10	3	10	4	14	4	16	4	17	5	11	5	I–II	–
alcohols																		
methanol	530	a	15	7	153	47	344	175	771	437	929	562	1288	914	1382	632	I–II	–
ethanol	590	a	2193	1110	2450	2836	1555	379	1054	261	5682	4627	7886	4928	12769	20696	ns	+
2-propanol	610	a	877	409	1105	1455	642	193	436	213	487	135	498	140	333	58	ns	–
n-propanol	671	a	4	3	7	4	16	9	26	10	72	36	209	212	1256	572	I–II–III–IV	+
butan-2-ol	708	a	4	2	2	2	4	2	6	2	10	4	14	4	19	4	III–IV	+
1-ethylcyclopropanol	784	c	9	9	130	94	317	238	727	234	627	287	578	228	297	131	ns	–
1-penten-3-ol	787	b	0	1	2	11	3	3	5	4	7	3	11	4	8	3	ns	–
pentan-2-ol	806	b	1	2	1	2	3	3	4	2	8	4	8	3	39	18	I–II–III	+
3-penten-1-ol	845	c	10	8	7	7	18	8	10	7	9	6	7	5	3	2	ns	–
3-methylbutanol	850	a	85	70	32	28	108	100	116	68	165	93	115	102	992	385	I–II	–
2-methylbutanol	855	a	18	31	9	16	29	42	27	34	30	31	25	23	242	70	ns	–
n-pentanol	882	a	28	12	131	55	207	91	348	116	352	115	254	119	149	63	I–II	–
n-hexanol	979	b	1	2	0	1	0	1	1	2	2	3	2	2	3	3	ns	–
1-octen-3-ol	1080	a	1	2	3	5	3	6	14	10	8	8	11	7	8	7	ns	+
ester compounds																		
ethyl acetate	678	a	1	2	9	7	18	12	39	9	42	14	77	116	193	408	I–II	–
ethyl propionate	774	b	0	0	0	2	0	1	1	2	2	2	3	2	41	82	ns	+
ethyl butanoate	863	b	2	3	1	3	1	2	3	3	13	13	21	10	102	29	I–II	+
ethyl 2-methylbutanoate	904	a	0	1	0	0	0	0	0	1	1	2	1	2	14	5	ns	–
ethyl 3-methylbutanoate	911	a	5	10	0	0	1	1	1	2	3	4	3	3	21	5	ns	+
ethyl pentanoate	961	b	0	0	1	1	0	0	2	4	2	3	1	2	8	2	ns	–
propyl acetate	994	c	0	1	0	0	0	0	0	0	3	3	4	3	4	2	I–II	–
ethyl hexanoate	1060	b	0	0	0	1	2	12	1	2	10	9	18	10	68	11	ns	–
ethyl heptanoate	1159	b	0	0	0	0	0	1	2	1	1	2	1	1	0	0	ns	–
ethyl octanoate	1258	b	0	1	0	1	0	2	0	0	0	1	3	2	17	3	III–IV	–
ketones																		
acetone	607	a	131	269	227	695	31	114	66	94	84	63	140	130	184	114	ns	–
butan-2-one	681	c	4	4	5	4	6	6	4	3	38	37	51	30	614	311	I–II, III–IV	+
diacetyl	691	a	49	19	45	20	80	34	131	41	240	106	283	100	255	67	I–II, III–IV	+
pentan-2-one	777	b	7	3	15	11	39	27	74	39	305	201	236	132	381	163	II–III	+
pentane-2,3-dione	794	b-c	2	5	23	10	56	26	96	29	115	36	95	32	94	29	ns	–
heptan-2-one	982	b	5	4	18	12	43	25	60	26	89	51	55	23	58	29	ns	+
4-octen-3-one	1067	c	0	1	3	2	6	3	13	4	11	4	11	5	4	3	ns	–
butyrolactone	1128	c	2	3	0	0	1	1	1	1	1	2	1	2	2	2	ns	–
nonan-2-one	1186	b	0	1	3	2	3	2	2	3	5	7	7	2	16	6	ns	–
aliphatic hydrocarbons																		
heptane	701	a	2	3	10	5	35	24	132	103	268	299	422	492	570	595	I–II–III	–
octane	796	a	13	6	16	7	38	22	121	83	221	225	300	298	417	513	I–II	+
nonene	900	c	0	0	0	0	1	2	1	2	2	3	1	1	0	0	I–II	–
nonane	900	a	1	3	0	1	0	1	3	3	2	2	2	2	4	1	I–II	–
decene	1001	c	9	14	1	2	3	2	3	3	4	4	7	6	7	4	ns	–
aromatic hydrocarbons																		
benzene	719	a	20	20	12	15	32	13	20	13	25	17	22	11	45	19	I–II	–
toluene	819	a	282	522	28	39	29	31	28	28	32	31	41	44	37	19	I–II	–
dimethylbenzene	920	c	10	18	1	2	3	4	2	3	2	4	1	2	4	4	ns	–
aromatic compound	958	d	1	1	0	0	1	2	1	1	1	1	1	1	2	2	ns	–
aromatic compound	1044	d	0	0	3	2	5	4	10	7	11	7	14	8	12	12	ns	–
carboxylic acids																		
acetic acid	791	a	1	2	0	0	0	2	0	0	2	5	0	0	4	5	ns	–
propanoic acid	888	a	8	7	7	7	12	6	9	7	7	6	4	4	1	1	ns	–
2-methylpropanoic acid	935	c	0	0	0	0	0	0	0	1	0	1	1	1	8	2	II–III	–
3-methylbutanoic acid	1019	a	1	4	1	3	0	1	0	1	0	1	3	4	17	14	ns	–
pentanoic acid	1058	c	0	1	0	0	1	4	1	2	2	3	2	2	5	5	ns	–

Table 3 (continued)

compound	Kovats index	reliability	3 days mean	3 days SD	25 days mean	25 days SD	125 days mean	125 days SD	211 days mean	211 days SD	365 days mean	365 days SD	485 days mean	485 days SD	spoiled mean	spoiled SD	SIMCA[b]	PLS[c]
sulfur compounds																		
dimethyl disulfide	809	a	0	0	1	2	7	8	33	24	64	34	59	19	36	9	I–II–III	–
methyl ethyldisulfide	897	c	0	0	0	0	2	3	4	4	3	3	1	2	0	0	ns	–
dimethyl trisulfide	1046	c	0	1	0	1	2	3	7	3	12	4	14	4	16	4	II–III	+
methyl *n*-pentyl disulfide	1207	c	1	1	0	0	0	2	1	2	0	1	0	0	2	3	ns	–
unknowns																		
unknown 1 (MP[e] = 55, 28, 29)	835	d	0	0	0	0	0	0	0	1	2	3	2	2	1	2	ns	–
unknown 2 (MP = 28, 32, 41)	1005	d	0	1	0	0	0	1	0	1	0	1	1	2	2	4	ns	–
unknown 3 (MP = 43, 28, 71)	1075	d	4	2	8	3	13	8	27	12	22	10	22	12	8	3	I–II	–
unknown 4 (MP = 43, 28, 58)	1083	d	8	3	42	13	60	27	72	21	58	16	45	13	37	10	I–II	–
unknown 5 (MP = 28, 32, 41)	1099	d	17	6	42	13	65	33	160	35	141	44	133	45	56	24	I–II	–
unknown 6 (MP = 28. 32, 57)	1131	d	0	0	0	1	1	2	4	1	3	2	2	2	1	1	ns	–
unknown 7 (MP = 28, 32, 67)	1135	d	0	0	0	1	0	1	0	1	1	1	0	1	0	0	I–II	+
unknown 8 (MP = 28, 32, 94)	1212	d	0	0	0	0	0	0	1	2	3	3	2	2	2	3	ns	+
unknown 9 (MP = 28, 73, 32)	1231	d	24	28	4	3	6	4	8	5	9	8	14	9	11	9	ns	+
unknown 10 (MP = 28, 73, 32)	1279	d	1	2	5	2	6	3	14	4	10	3	10	4	5	2	ns	–
unknown 11 (MP = 28, 32, 117)	47,20	d	0	1	0	1	0	2	2	3	1	1	0	1	8	4	ns	–

[a] Reliability of identification is indicated by: a, mass spectrum and Kovats index identical to authentic compound; b, mass spectrum and Kovats index in agreement with corresponding litterature data; c, mass spectrum consistent with spectrum found in the NIST library 1990 version Finnigan MAT; d, tentative identification by mass spectrum or unidentified. [b] Significant differences ($P < 0.05$) between groupings (I–IV) found by principal component analysis tested by soft independent modeling of class analogy. [c] Volatile compounds correlating to nutty/cheesy/salt (loadings >0.1 at either the first or second principal component) as found by projection on latent structures. [d] ns, nonsignificant. [e] Major peaks in mass spectrum in decreasing order.

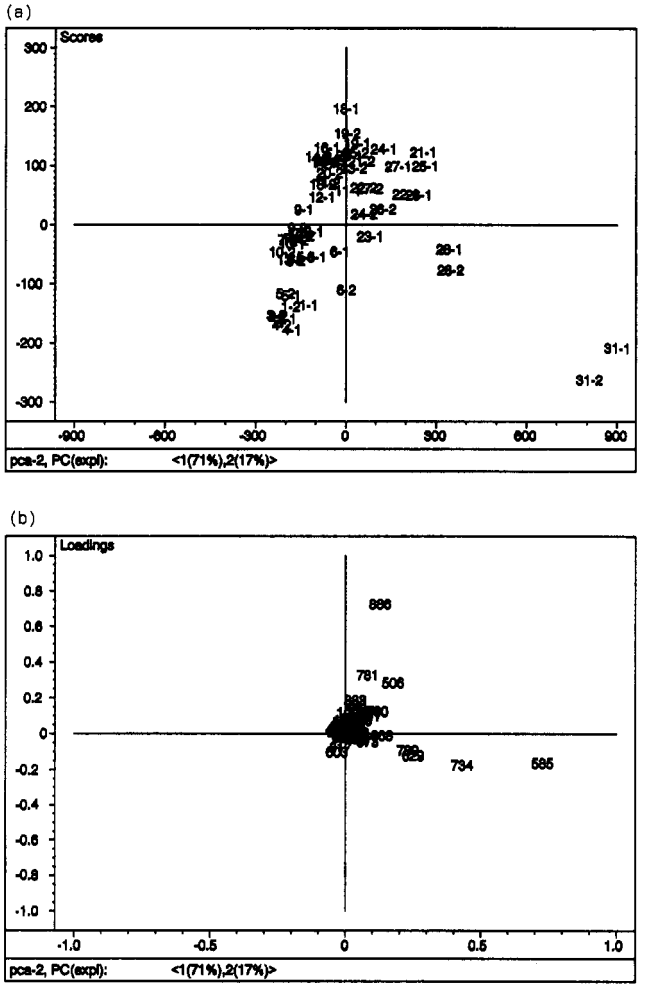

Figure 3. Score plot (a) and loading plot (b) after principal component analysis of volatile compounds in all hams. In (a) the first number designates hams at different storage times: 1–5 are after 3 days; 6–10, 25 days; 11–15, 125 days; 16–20, 211 days; 21–25, 365 days; and 26–28, 485 days. Ham 31 is spoiled. The second number designates replicates. In (b) numbers assign Kovats indices.

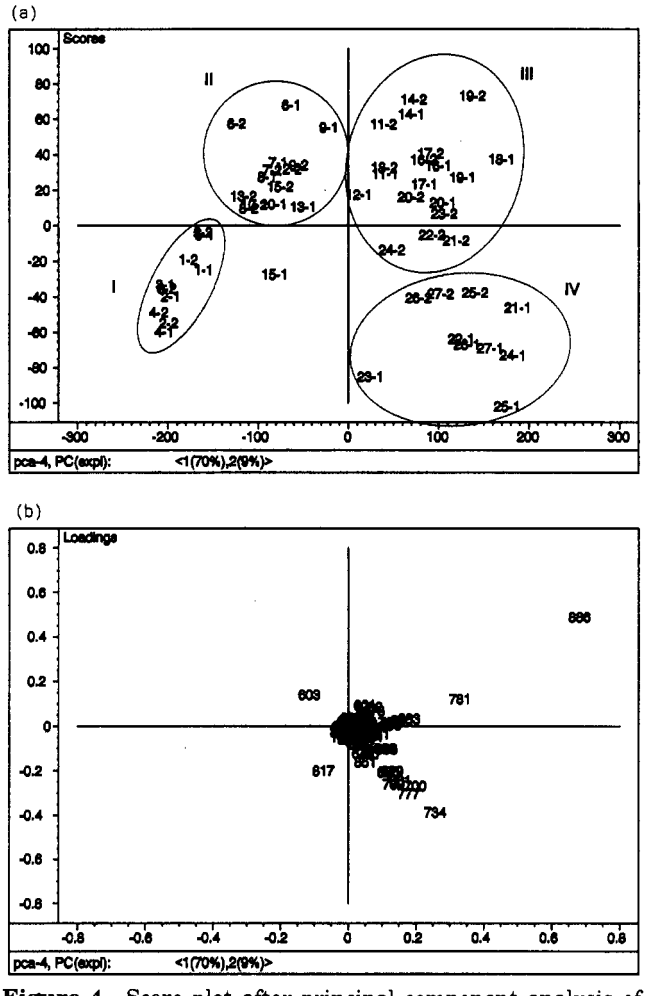

Figure 4. Score plot after principal component analysis of volatile compounds, when spoiled ham (31) and ham 28 are removed (a), and loading plot after principal component analysis of volatile compounds in hams (b). Ethanol (KI 590) and methanol (KI 530) were omitted in both analyses. In (b) numbers assign Kovats indices.

Table 3 the significant changes are indicated for each volatile compound.

From group I to group II it was confirmed that at this stage it is primarily secondary autoxidation products which are formed (Table 3). Furthermore, a number of unidentified volatile compounds, of which some may be terpenoids, seem to be formed at this stage. This is in accordance with the fact that after the first drying

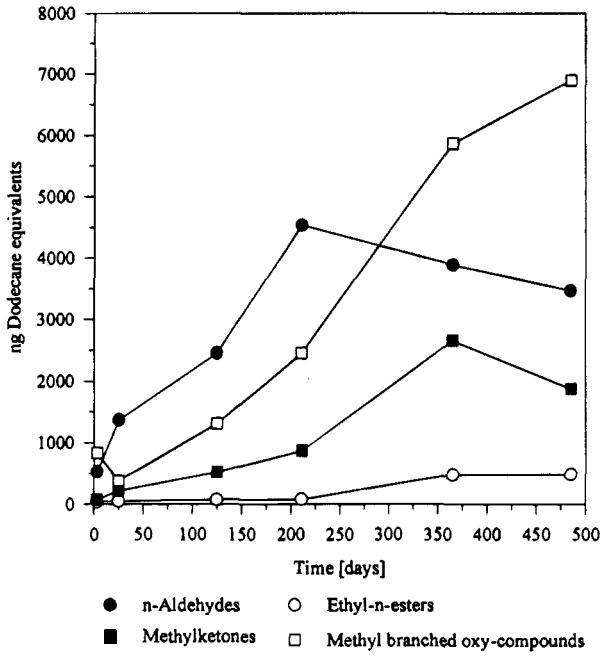

Figure 5. Development of chemical groups of volatile compounds during storage.

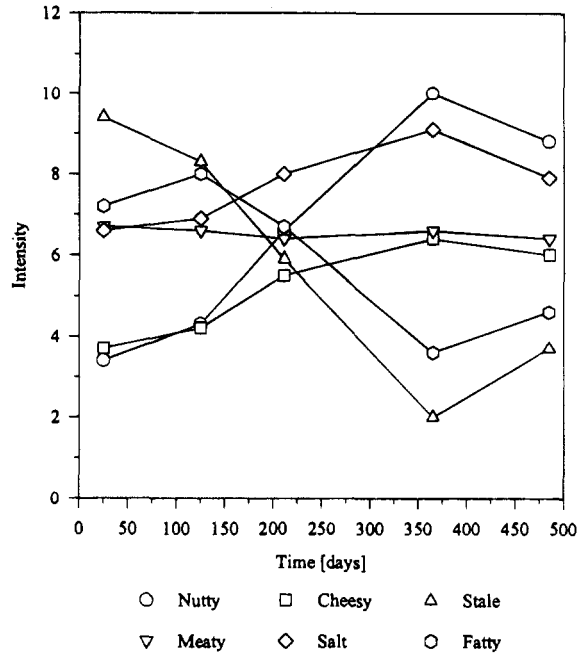

Figure 6. Development of sensory profiles during storage.

period, cut surfaces of the hams are smeared with pork fat containing pepper. As there are a high number of terpenoids in pepper (Belitz and Grosch, 1986), this may be the reason for the appearance of assumed terpenoids in group II.

From group II to III there are still formed secondary autoxidation products, but the methyl-branched aldehydes and carboxylic acids are also formed.

From group III to IV (postripening) again it is secondary autoxidation products and methyl-branched compounds, which are important. This is not in accordance with results from PCA, by which secondary autoxidation products only were characteristic for group III. The reason for this contradiction is given in Figure 5.

In Figure 5 the development of four chemical groups of volatiles is depicted. For *n*-aldehydes, which mainly represent secondary autoxidation products, there is an optimum after 211 days of storage, after which time the amount of *n*-aldehydes decreases. The observed change from group III to IV by SIMCA is actually a decrease of secondary autoxidation products rather than an increase. The observed decrease of secondary autoxidation products is confirmed in French dry-cured ham by Buscailhon et al. (1993) and has also been reported by Ventañas et al. (1992), who ascribe the decrease of aldehydes to reactions with free amino acids in the meat by Maillard reactions.

Figure 5 also shows the development of methyl-branched oxy compounds. This group is essentially composed of 3- and 2-methylbutanal. A sigmoidal increase of these compounds can be observed, with the fastest increase in the first and second ripening periods (211–365 days). These products become dominating in the final product as also described by the principal component analysis in Figure 4.

Ethyl *n*-esters show a small increase during the ripening period (Figure 5), and although this increase may seem insignificant, it is well-known that many esters possess very intense, fruity odors (Belitz and Grosch, 1986). Methyl ketones increase, as do the methyl-branched oxy compounds, during the ripening periods. However, during postripening the contents of these compounds decrease.

In summary, the development of volatile compounds in Parma ham during processing can be divided into two phases. The first phase includes salting, first drying and first ripening (211 days), in which autoxidation occurs, and the second phase includes the second ripening and postripening, in which other, more complex, reactions take place.

Sensory Analyses. Results from sensory analyses are shown in Figure 6. Although an unstructured intensity scale was used, there were significant effects among panelists ($P < 0.001$). Therefore, outlying panelists were excluded in the statistical analysis. No more than two panelists were excluded for each descriptor. Analysis of variance showed significant effects from all flavor descriptors ($P < 0.001$) except *meaty* ($P > 0.05$). *Nutty*, *cheesy*, and *salt* all showed a sigmoidal increase during storage, being most intense after second ripening (365 days). After postripening, there was a small decrease in these descriptors. It seems that these flavor descriptors contribute positively to the overall flavor of the hams. The reason for the increase in *salt* is that throughout the process there is a loss of water (Table 2). In this way the concentration of NaCl increases and thereby gives rise to more *salt* taste. Furthermore, there is a release of glutamic acid during processing, which may contribute to the *salt* flavor by umami taste (Maga, 1994).

Stale and *fatty* decreased during storage, but after the second ripening (360 days), they showed a slight increase. These flavor characteristics obviously contribute less to the overall flavor of the hams and dissappear during processing.

Volatile Compounds and Sensory Analyses. The relationship between development of volatile compounds and flavor characteristics was investigated by PLS. Before analysis, data were autoscaled to get data into the same range. Results from this analysis are shown in Figure 7 as a plot of actual intensities of the flavor attributes versus the predicted values.

The model had very high correlation coefficients for all flavor descriptors compared to earlier experiments (Figure 7). Only *meaty* was not described by the model, and this can be explained by the fact that there was no change in this flavor descriptor during storage. For

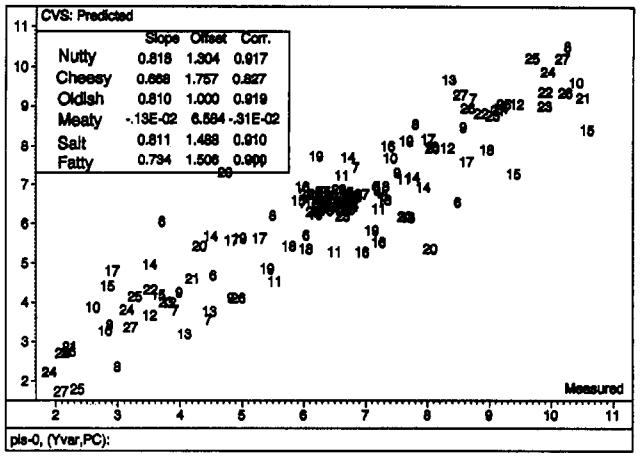

Figure 7. Observed intensities of flavor attributes versus predicted values based on a partial least-squares model with two principal components to describe the relationship between volatile compounds and sensory profiles. Correlation coefficients are listed in the plot.

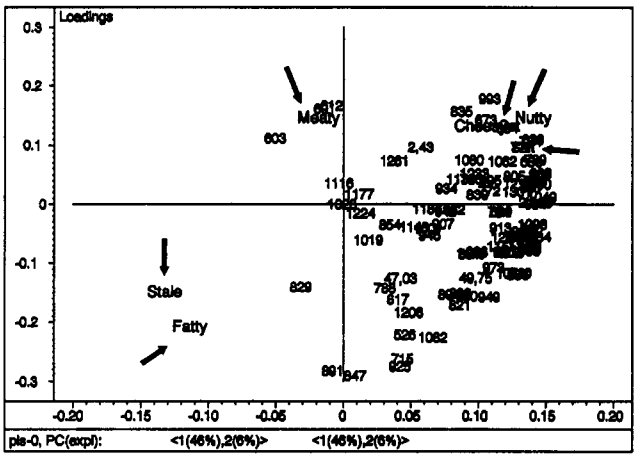

Figure 8. Loading plot after partial least-squares modeling of volatile compounds and sensory profiles. Flavor descriptors are accentuated with arrows. Numbers designate Kovats indices of volatile compounds.

nutty the correlation coefficient was 0.917, for *cheesy* 0.827, for *stale* 0.919, for *salt* 0.910, and for *fatty* 0.900.

To explore which compounds actually correlate with the flavor descriptors, the loadings plot from PLS analysis is shown in Figure 8. Volatile compounds correlated with *nutty*, *cheesy*, and *salt* are located in the first quadrant, whereas *stale* and *fatty* are located in the third quadrant, which means that volatile compounds correlated with *nutty*, *cheesy*, and *salt* are negatively correlated to *stale* and *fatty*. In Table 3 the volatile compounds correlating with the three flavor descriptors (loadings > 0.1 at either the first or second principal component) are marked.

Methyl-branched aldehydes, secondary alcohols, methyl ketones, ethyl esters, and dimethyl trisulfide are essentially the volatile compounds correlating with *nutty*, *cheesy*, and *salt* (Table 3). Methyl-branched aldehydes, ethyl esters, and dimethyl trisulfide have very intense odors (Stahnke, 1994; Hinrichsen and Andersen, 1994), indicating that these compounds do have an impact on the flavor of the product, although the specific effect cannot be estimated. These results are confirmed by Careri et al. (1993), who found that short-chain methyl-branched oxy compounds correlated well with the "aged" flavor of the hams. Buscailhon et al. (1994) could not confirm this in French dry-cured ham. These authors found that methyl ketones and straight-chain alcohols correlated with the "cured ham" flavor.

To understand the formation of flavor in Parma ham, the origins of the correlating volatile compounds are important. The methyl-branched aldehydes may be formed by microorganisms, as microorganisms are able to metabolize L-leucine to 3-methylbutanal (Hinrichsen and Andersen, 1994), L-isoleucine to 2-methylbutanal, and L-valine to 2-methylpropanal. Ventañas et al. (1992) speculate that methyl-branched aldehydes also can be formed by Strecker degradation in the presence of diketones when there is low water activity. These reactions do, nevertheless, usually take place at high temperatures or under high hydrostatic pressure (Belitz and Grosch, 1986), which is not relevant for the present conditions. Also, it is well-known that carbonyls and secondary alcohols can be formed by β-oxidation of free fatty acids and subsequent hydrolysis by a thiohydrolase to a corresponding β-keto acid of certain microorganisms. The β-keto acids are either decarboxylized to a resulting methyl ketone or further reduced to the corresponding secondary alcohol (Gottschalk, 1985). Ethyl esters are formed enzymatically on the basis of ethanol and carboxylic acids. The ability of microorganisms to form ethyl esters is well-known (Gottschalk, 1985); furthermore, Stahnke (1994) demonstrated the importance of microorganisms on the appearance of ethyl esters in fermented sausages. Finally, the presence of dimethyl trisulfide is due to the microbial metabolism of peptides or amino acids (Gottschalk, 1985).

It is difficult to state the origin of the volatile compounds conclusively, as a controlled experiment with a "sterile" Parma ham in comparison with the commercial product would be necessary. Succinctly, microorganisms can be involved in the formation of most of the compounds correlated to *nutty*, *cheesy*, and *salt*, reflecting amino acid and fatty acid degradation and esterolytic activities.

Conclusions. Microorganisms are important for the development of flavor in Parma ham, as all of the correlating volatile compounds can be generated by secondary metabolism of microorganisms, especially amino acid catabolism. That these microorganisms do have an active metabolism is supported by the observed nitrate reductase activity. In addition, autoxidation seems of less importance, because secondary autoxidation products do not correlate with the flavor development. The role of specific microorganisms during the development of flavor in Parma ham remains unclear. In the process there is evidence for several successions of different populations of microorganisms, most of which belong to Micrococcaceae. However, it is not possible to state the relative importance of each population of these microorganisms or the relative importance of microbial enzymes compared to endogenous processes in the tissues.

In conclusion, the flavor of modern meat products may be improved by means of microorganisms using Parma ham as a model. Such microorganisms should possess a secondary metabolism that generates methyl-branched aldehydes, secondary alcohols, methyl ketones, ethyl esters, and dimethyl trisulfide in distinct balance.

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