Correlations of Sensory and Volatile Compounds of Spanish "Serrano" Dry-Cured Ham as a Function of Two Processing Times

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Spanish "Serrano" dry-cured hams were processed under traditional practices which included two different length of the ripening–drying stage dry-curing methods. Dry-cured hams typically have high production cost because of the length of the ripening–drying stage which makes the product less competitive. In order to study the generation of dry-cured ham flavor the volatile components were investigated. Sensory properties were analyzed by GC/olfactometry and descriptive sensory techniques. The relationship of the volatile components with sensory descriptors was examined by factor analysis and resulted in a solution composed of four factors defined as "pork", "cured", "pleasant", and "off-flavor". The short ripening process was characterized by aldehydes, such as hexanal and 3-methyl butanal, alcohol (1-penten-3-ol), and dimethyl disulfide, that gave an olfactory sensation of fresh-cured pork flavor. The "pleasant" aroma in the short process had already been developed and was defined by ketones, esters, pyrazines, and aromatic hydrocarbons. On the other hand, the longer ripening–drying procedure produced an increase in "pork", "cured" and "off-flavor" that masked the "pleasant" aroma.

INTRODUCTION

Meat flavor quality is attributed to several factors including age of animal, genetic composition, preslaughter diet, environmental conditions, etc. but postmortem processing is a major factor that will affect the final product quality (Spanier et al., 1990). Independent of the antemortem factors, the processing of meat will affect the concentrations of different flavor precursors such as sugars, amino acids, nucleotides, peptides, etc., which, in turn, will affect the final product flavor (Spanier et al., 1992; Spanier and Miller, 1993).

Dry-curing of hams is a traditional process in the Mediterranean region that leads to a product with unique flavor (Flores and Toldrá, 1993). Serrano dry-cured ham is produced in Spain from different cross-breeding of white pigs. These hams are characterized by low marbling, firm texture, and a typical flavor that can be more or less intense depending on the length of the drying stage (Toldrá et al., 1997a). During the drying stage, the hams are submitted to different time–temperature treatments. Depending on the final quality desired, the drying could take from 9 to 24 months. Proteins and lipids constitute the major chemical components of meat and are the main substrates of action of the muscle enzyme systems (Spanier et al., 1990; Toldrá, 1992). Throughout the drying stage, there exists a noticeable enzyme activity (Toldrá et al., 1995) that will result in the generation of flavor precursors, such as amino acids and peptides (Aristoy and Toldrá, 1995). These precursors will contribute to the generation of flavor volatiles via the Strecker degradation system and the formation of Maillard reaction products (Ventanas et al., 1992).

The high quality of the dry-cured ham depends on its unique flavor. However, the increased production cost of long-term dry-curing makes the product less competitive in the market. Several studies have attempted to reduce the processing time (Marriot et al., 1987, 1992), but the length of the ripening–drying stage is necessary in order to have complete cured color formation and dry-cured flavor development (Toldrá et al., 1997b).

Several investigations have been performed to identify and quantify the volatile compounds in French (Berdague et al., 1991; Buscailhon et al., 1993), Italian (Barbieri et al., 1992; Careri et al., 1993; Hinrichesen and Pedersen, 1995), and Spanish (Garcia et al., 1991; Lopez et al., 1992) dry-cured ham. The volatile compounds consist of a wide range of organic classes, but only few correlations have been established between these volatile compounds and the sensory characteristics, such as in the Italian (Careri et al., 1993; Hinrichsen and Pedersen, 1995) and French (Buscailhon et al., 1994) dry-cured hams, but none in the Spanish dry-cured ham. Moreover, the results obtained have been confused due to the different analytical methods used by the different groups and also due to the different origin of the dry-cured ham samples studied. For example, Careri et al. (1993) established that in Italian dry-cured hams there is a high correlation between methyl branched short esters and 3- and 4-carbon alcohols with hams that have high acceptability scores. On the other hand, in French dry-cured hams there is a significant correlation between methyl ketones and straight chain alcohols with the aroma of dry-cured ham (Buscailhon et al., 1994). Recently, Hinrichsen and Pedersen (1995) studied the microbiology of dry-cured hams, taking into consideration the influence of microorganisms on the flavor of Italian dry-cured ham.

The contribution of the volatile compounds to the final characteristics of the ham is still not clear. A large number of compounds have been identified in the aroma of dry-cured ham (Barbieri et al., 1992), but none has been described as having a unique pork-cured flavor. It is most likely that dry-cured aroma depends upon a balance of various components.

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Moreover, the wide diversity of flavor descriptors has led to controversy because different terms are used by different people to characterize the same flavor and odor. Furthermore many desirable flavor compounds have very low flavor threshold concentrations, making their identification difficult.

Due to the conflicting results obtained during the study of the volatile compounds and their sensory contribution to the flavor of dry-cured ham, the objective of this study was to identify the volatile compounds of the Spanish "Serrano" dry-cured ham that contributes to the characteristic aroma by using a combination of analytical flavor methods such as GC/olfactometry and sensorial descriptive analysis. The study of the presence and development of the dry-cured aroma was performed on two different ripening–drying stages in the process of preparation of Spanish "Serrano" dry-cured ham.

MATERIALS AND METHODS

Materials. The study consisted of a total of 20 Spanish "Serrano" dry-cured hams that were purchased from a local factory (Jamones Segorbe) in Castellón (Spain). Ten hams were submitted to a ripening process of 7 months (short process), consisting of the traditional stages of salting (12 days at 3 °C), post-salting (50 days at 4 °C), and ripening–drying (1st phase, 60 days at 12 °C; 2nd phase, 60 days at 18 °C; 3rd phase; 30 days at 25 °C). Another group of ten hams was ripened for 12 months (long process). In addition to the short process, the hams in the long process experienced a 4th phase of 150 days at 15 °C. A 750 g portion was cut from the center part of each ham, perpendicular to the bone, composed essentially of the biceps femoris, semitendinosus, and semimembranosus muscles. Samples for sensory and volatile analyses were sent within three days to the Southern Regional Research Center (U.S.D.A.) in New Orleans, LA, after appropriate documentation was filed with the Food Safety Inspection Service (F.S.I.S.) and U.S. Customs.

Volatile Analysis. Volatile compounds were isolated from the ham samples by dynamic headspace technique using a polymeric material as the adsorbing trap. Each ham sample (150 g) was cut in small pieces and ground while cooling the food processor in ice. The ground ham sample was divided in 15 g portions, vacuum sealed in oxygen impermeable bags, and kept frozen at −20 °C until analysis. The volatile compounds were extracted by putting the content of the bag in a fritted purge sampler tube (Supelco, Bellefonte, PA) and adding 10 μL of 100 ppm chlorodecane as internal standard to check the reproducibility of the purge and trap technique. The sample was maintained at 40 °C for 5 min and then flushed with nitrogen at a flow rate of 150 mL/min for 11 min. The volatile compounds were adsorbed onto 100 mg of TENAX (60/80 mesh size) in a 4 mm i.d. glass-lined tube (Supelco, Bellefonte, PA). The trap was flushed with nitrogen for 5 min to eliminate the adsorbed water that could interfere in the analysis.

Volatile compounds were thermally desorbed from the TENAX trap using a short path thermal desorbtion unit (SPTD, TD-2 Scientific Instruments Services, Inc., Ringoes, NJ) connected directly to the gas chromatograph (GC). The trap was heated at 200 °C for 2 min and the desorbed compounds were cryofocused at 0 °C at the entrance of a DB-624 capillary column (J&W Scientific; 60 m, 0.32 mm i.d.; film thickness 1.8 μm) installed on a gas chromatograph (GC 5890-HP series II, Hewlett-Packard, Palo Alto, CA), equipped with flame ionization (FID) and olfactometry (sniffer port) detectors. Helium was used as a carrier gas with a linear velocity of 24.2 cm/s. The temperature program used was as follows: 0 °C maintained for 2 min and then raised from 0 to 90 °C at 10 °C/min, then from 90 to 160 °C at 2 °C/min, and from 160 to 250 °C at 5 °C/min with a final holding time of 5 min; total run time 69 min. Injector and detector temperatures were set at 220 and 240 °C, respectively. The content of each of the volatile compounds in the ham sample was calculated from

Table 1. Spanish "Serrano" Dry-Cured Flavor Descriptors Used in the Analysis

descriptor	description
fat complex (FCX)	aromatics associated with lipid products such as animal fat
boar taint (BOR)	aromatic associated with boar meat; hormone-like (skatole)
barnyard (BYD)	aromatics associated with free fatty acids
pork (PRK)	aromatics associated with cooked pork muscle meat
sour (SOR)	taste on the tongue associated with citric acid
salty (SLT)	taste on the tongue associated with sodium ions
bitter (BTR)	taste on the tongue associated with caffeine
mouth-filling (MTH)	mouthfeel associated with monosodium glutamate (MSG)

the FID areas and expressed as area units normalized by the area of the chlorodecane internal standard. The internal standard was used to minimize the variability inherent in the purge and trap technique. Each sample was analyzed by five replicates. Kovats indices (KI) of the volatile compounds were calculated according to the method of Kovats (1965).

The sensory characteristics of the different compounds separated on the GC were measured by olfactory test by a splitter system installed at the exit of the capillary column (1:1 ratio). One side of the column was directed to an FID detector and the other side to a room where the sniffer port was located separated from the instrument, and having a positive air pressure. Four trained panelists took part in the olfactory test to develop the aromagram of the "Serrano" dry-cured ham.

The identity of the volatile compounds in the ham samples was confirmed by using a HP-5970 quadripole mass selective detector, directly coupled to a HP-5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA). Separation was performed with a J&W Scientific fused capillary column (25 m × 0.2 mm i.d.), coated with DB-624 (film thickness 1.1 μm). The carrier gas used was helium with a linear velocity of 26 cm/s. The same temperature program was used for both GC–FID and GC–MS analyses. Mass spectra were obtained by electron impact at 70 eV. Mass spectral data were acquired across the range 35−450 amu. The GC/MS interface was maintained at 260 °C.

Sensory Analysis. A descriptive sensory profile of Spanish "Serrano" dry-cured ham was obtained by sensory evaluation with a trained panel of thirteen panelists. The panel was previously trained during a period of 4 months as described by Flores et al. (1996). The panel developed a lexicon of descriptors for Spanish "Serrano" dry-cured ham including the following sensory attributes: fat complex (FCX), boar taint (BOR), barnyard (BYD), pork (PRK), sour (SOR), salty (SLT), bitter (BTR), and mouth-filling (MTH) (Table 1). These sensory attributes describing desirable and off-flavors of dry-cured ham were analyzed for this experiment. The ham samples were sliced (approximately 3 mm thickness), and 3 g rolls were prepared. Each sample was presented in a Petri dish in which 2 rolls were included. The sample was served uncooked and at room temperature. Intensities were based on a universal intensity scale established in reference to flavor intensities of several commercial food products (Meilgaard et al., 1991). Sensory evaluations were recorded via computer system using a light pen to record the descriptor intensity. For further analysis, data were analyzed with SAS (SAS Institute Inc., Cary, NC) and subjected to specific tests.

Statistical Analysis. The analysis was performed on the panel average for each individual replicate ham sample after using the standard to adjust out the session effect. The analysis consisted of a t-test (Steel and Torrie, 1980) to study the differences of the data with regard to the processing time. Principal factor analysis using an oblique (Promax) rotation was performed on the sensory and chemical data, to study the generation of the dry-cured flavor, and to find the chemical compounds that contribute to the dry-cured flavor.
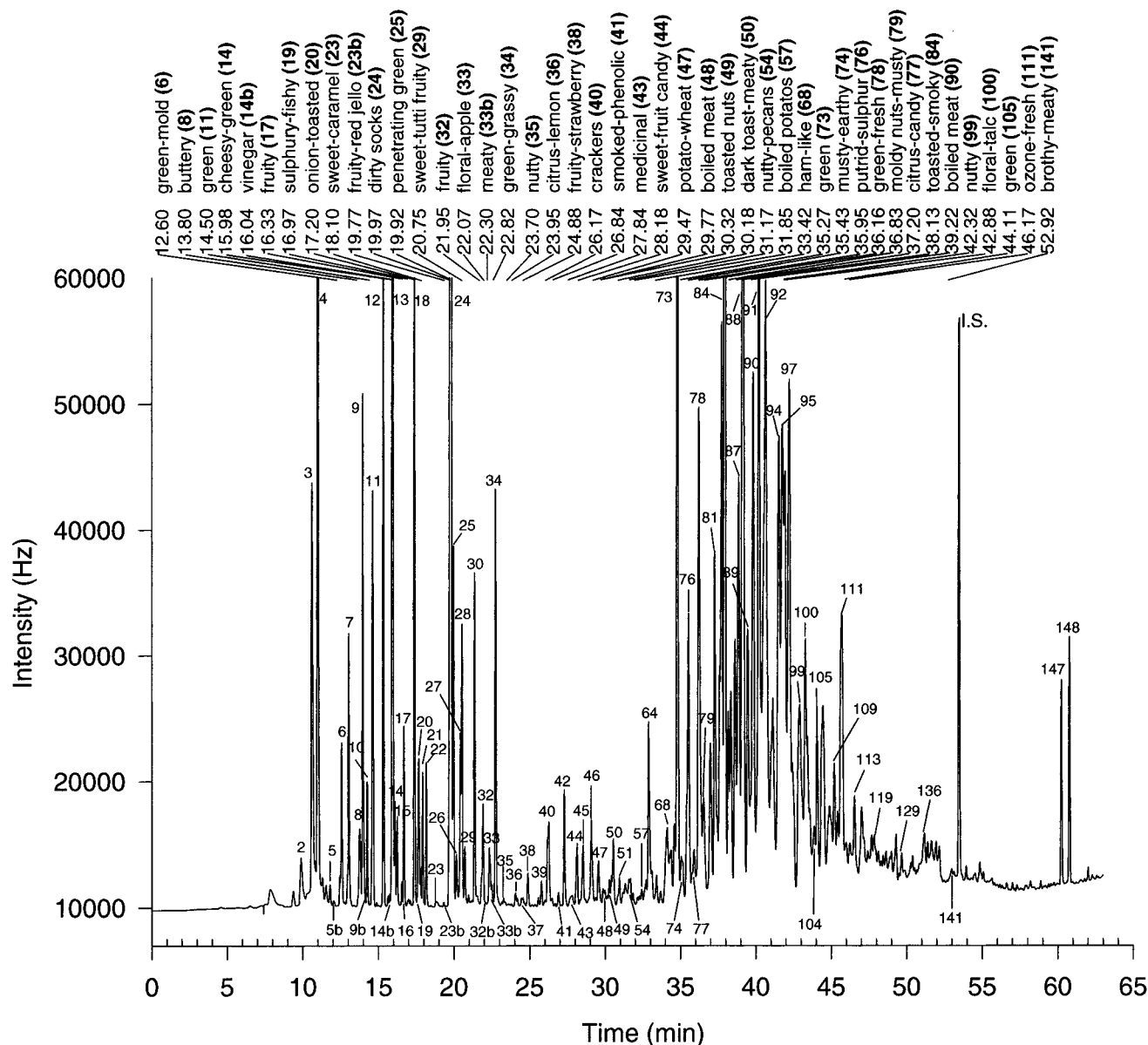
Figure 1. Gas chromatogram of Serrano dry-cured ham. The numbers represent compounds identified and listed in Table 2. The aromagram is presented on the top of the graph and is expressed by retention time (in min) at which the descriptor eluted; in parenthesis is the peak to which the descriptor corresponds.

RESULTS AND DISCUSSION

Volatile Compounds. The dynamic headspace analysis of the Spanish Serrano dry-cured ham was carried out by purge and trap concentration of the volatile compounds on TENAX. During the purge and trap a temperature of 40 °C was chosen to mimic the release of the volatile compounds on the mouth during mastication. Over 100 volatile compounds were detected (Figure 1) and 84 were identified. The volatile compounds identified, or tentatively identified, are listed in Table 2. The references where the compounds were previously reported, their Kovats index, and their average quantities at the two different ripening–drying processes (short and long) are also presented. When possible the identity of the volatile compounds was confirmed by using an authentic standard.

Among the peaks identified or tentatively identified 77 were quantified. Molecular weights of the identified compounds range from 46 (ethanol) to 184 (branched chain hydrocarbons). The identified compounds consisted of 17 alcohols, 11 aldehydes, 9 ketones, 7 aliphatic hydrocarbons, 10 esters, 4 aromatic hydrocarbons, 2

chloride compounds, 3 nitrogen compounds, 2 furans, 1 carboxylic acid, 1 sulfide compound, and 17 branched chain hydrocarbons.

The headspace volatiles of the two different processes (short and long) contained essentially the same components. The total quantity of volatiles extracted did not show a significant change. Changes affecting chemical classes cannot be considered globally because of individual variations (Table 2). Ranking of the chemical classes following their quantitative importance was as follows: branched chain hydrocarbons (47–48% of the total volatile area), alcohols (16–17%), aldehydes (13–15 %), ketones (6–7%), aliphatic hydrocarbons (6.0–6.6%), esters (3–3.5%), aromatic hydrocarbons (1.3–1.4%), furans (0.75%), chloride compounds (0.6–0.9%), nitrogen compounds (0.5%), and others (1.7–2.2%).

Branched chain hydrocarbons represented the group with the highest concentration (approximately 48% of the total area in both processes). Moreover, this group interfered in the identification of other compounds that elute in the same area between 33 and 47 min (Figure 1). Because branched chain hydrocarbons have been

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considered as non-contributors to meat flavor (Chang and Peterson, 1977; Shahidi et al., 1986), we did not focus on their identification and they were tabulated as tentatively identified.

The long processing of the dry-cured hams (12 months) contributes to lipolytic and oxidative degradation of unsaturated fatty acids which are abundant in both intramuscular and adipose tissue of swine. Aliphatic hydrocarbons are derived from oxidative decomposition of lipids (Shahidi et al., 1986). Most of the sixteen alcohols identified, linear and branched, are oxidative decomposition products of lipids. For example, 1-propanol and 1-butanol can come from miristoleic acid, 1-pentanol from linoleic acid, 1-hexanol may be formed from palmitoleic and oleic acids, and 1-octanol from oleic oxidation (Forss, 1972). 1-Penten-3-ol seems to come from linolenate oxidation and have a penetrating, grassy ethereal odor (Shahidi et al., 1986). The methyl branched alcohols are probably derived from the Strecker degradation of amino acids. 2-Propanol can be distinguished from the other alcohols, considering its very high quantities, although the concentration of 2-propanol was increased considerably in the long process (Table 2) in contrast with 2-methylpropanol, 1-butanol, 1-penten-3-ol, 2-pentanol, and 1-hexanol which were reduced in the long process.

Many of the aldehydes are formed by oxidation of unsaturated fatty acids like hexanal that comes from linoleic acid oxidative decomposition. Aldehydes have shown to be contributors to the loss of desirable flavor in meats because of their high rate of formation during lipid oxidation and low flavor thresholds (Frankel, 1984). It is important to note the high concentration of octanal and 3-methyl butanal, although both compounds decreased in concentration during long processing. Drumm and Spanier (1991) reported that the unsaturated aldehydes undergo further oxidation to shorter chain aldehydes as happens with 2,4-decadienal and 2-undecenal that were higher in the short process than in the long process (Table 2). St. Angelo et al. (1980) suggested that 2,4-decadienal is an oxidation product of linoleic acid. On the other hand, branched aldehydes such as 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal are not usually derived from lipid oxidation but often arise from Strecker degradation of the amino acids valine, isoleucine, and leucine, respectively (Forss, 1972). The intense proteolytic activity produced during the dry-curing process results in an increased concentration of free amino acids (Toldrá et al., 1995) that serves as a pool for the Strecker reaction.

Aliphatic esters are very important constituents of foods, particularly in fruits. Most of the esters in plant material are formed by enzymatic reactions. Often precursors and enzymes are located in separate cells in the tissue and esters are formed after the rupture of the membrane (Forss et al., 1972). In meats, the intramuscular fat plays an important role in flavor retention. It acts as a solvent for the aroma volatiles formed in the muscle lean tissue and serves as a site for further reactions. The esters are formed from the interaction of free fatty acids and alcohols generated by lipid oxidation in the intramuscular tissue (Baines and Mlokiewicz, 1984; Shahidi et al., 1986). In the headspace of the Serrano dry-cured ham, ten esters were detected. These compounds have been found in greater amounts and are characteristic in the aroma of Italian dry-cured ham (Barbieri et al., 1992) but have not previously been found in the Spanish type hams (Garcia

et al., 1991; Lopez et al., 1992). It should be taken into account that nitrate is not used in Parma ham (Parolari, 1996) while it is usually added to Spanish dry-cured ham, being the most possible reason for the lower concentration of esters found in Spanish hams. In any case, microbial origin is discarded on the basis of the very low microbial counts found in both types of products (Molina and Toldrá, 1992; Baldini et al., 1992).

Seven saturated ketones were found in the Serrano dry-cured ham (Table 2). Acetone was presented with the highest concentration and represented 1.81% of the total volatile area compared to the 36−78% of the total volatile area that acetone constituted in French dry-cured ham (Buscailhon et al., 1993; Berdague et al., 1993). This important difference in the content of acetone could be due to the starting material and processing technique used. Moreover, the French dry-cured hams had been characterized for a high content in ketones in contrast with the 7% of the total volatile area found in the Italian type (Barbieri et al., 1992) that is very close to the 6−7% found in the Spanish "Serrano" ham. Ketones are also products from lipid oxidation one of them, 2-heptanone, has been found to be an oxidation product of linoleic acid (St. Angelo et al., 1980), although its mechanism of formation is not clear (Forss, 1972; Shahidi et al., 1986).

Two furans were found (Table 2) although as a chemical class they are considered to contribute insignificantly to the basic meaty taste. However, they can contribute to the overall odor of broiled and roasted meats. One of them, 2-pentylfuran, has been suggested to be an oxidation product of linoleic acid (Forss, 1972, St. Angelo et al., 1980; Baines and Mlotkiewicz, 1984).

Of the total volatile compounds in Serrano dry-cured ham only one carboxylic acid was found, acetic acid; however, it was not possible to accurately quantify (Table 2). This is remarkable compared to the large number of carboxylic acids identified in French (Berdague et al., 1991) and Italian (Barbieri et al., 1992; Hinrichesen and Petersen, 1995) hams. The origin of free fatty acids comes from the action of enzymes on triglycerides and phospholipids, in that these enzymes are active during the curing process (Motilva et al., 1993).

Two pyrazines were found in the headspace of "Serrano" dry-cured ham (Table 2). Pyrazines are products from Maillard reactions. These pyrazines are extensively generated during meat cooking (Mottram and Edwards, 1983); moreover, the proportion of pyrazine compounds increase in cooked meat as influenced by aging time, with longer aging resulting in higher values probably due to increases in sugars and free amino acids associated with aging (Maga, 1982). During the dry-cured process the temperature used is not as high as in cooking, thereby being the reason of the few pyrazines found.

Sulfide compounds are formed principally from the sulfur containing amino acids, such as methionine, cysteine, and cystine via Strecker degradations to thiols (Shahidi et al., 1986). Dimethyl disulfide is an oxidation product of methane thiol, and can react to form dimethyl trisulfide and dimethyl sulfide (Drumm and Spanier, 1991). Only dimethyl disulfide was detected, and its concentration was halved during the long process (Table 2). Two chloride compounds were detected both at lower concentration in the long process than in the short

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Table 2. Volatile Compounds Detected in the Headspace of Spanish "Serrano" Dry-Cured Ham

peak no.[a]	compound	RI[b]	KI[c]	short[d] (7 mon)	long (12 mon)	R[e]	P[f]
	Alcohols						
2	ethanol	a	522	0.832 ± 0.036	0.760 ± 0.032	3, 6	ns
4	2-propanol	a	555	1.546 ± 0.133	3.658 ± 0.356	3	***
7	1-propanol	a	613	0.592 ± 0.048	0.523 ± 0.052	3, 4	ns
10	2-butanol	a	647	0.541 ± 0.031	0.532 ± 0.045	3, 4	ns
12	2-methylpropanol	a	677	1.030 ± 0.084	0.483 ± 0.030	4, 6	***
13	2-methyl-3-buten-2-ol	c	689	0.094 ± 0.003	0.089 ± 0.004	4, 2	ns
18	1-butanol	a	712	0.392 ± 0.019	0.278 ± 0.014	4, 6	***
20	1-penten-3-ol	a	728	1.542 ± 0.150	0.618 ± 0.040	3, 4, 6	***
22	2-pentanol	a	740	0.839 ± 0.071	0.461 ± 0.023	3, 4	***
25	3-methyl-1-butanol	a	786	1.032 ± 0.105	1.074 ± 0.103	1–6	ns
26	2-methyl-1-butanol	a	790	0.417 ± 0.023	0.415 ± 0.035	1–3, 5, 6	ns
32	3-methyl-2-butenol	a	824	nq[g]	nq		
42	1-hexanol	a	915	0.774 ± 0.052	0.448 ± 0.020	1–5	***
47	3-methyl-2-hexanol	b	947	0.230 ± 0.008	0.250 ± 0.032		ns
87	2,2-dimethyloctanol	c	1077	1.166 ± 0.053	1.204 ± 0.047		ns
97	1-octanol	a	1122	2.147 ± 0.086	1.973 ± 0.084	1, 4, 5	ns
	Aldehydes						
5b	2-methylpropanal	a	594	nq	nq	3, 6	
14	3-methylbutanal	a	694	3.378 ± 0.364	2.323 ± 0.257	2–6	*
16	2-methylbutanal	a	702	0.439 ± 0.031	0.659 ± 0.116	2–4	ns
21	pentanal	a	734	0.534 ± 0.033	0.531 ± 0.055	1–3, 4, 6	ns
34	hexanal	a	838	0.913 ± 0.054	0.655 ± 0.032	1–6	***
46	heptanal	a	942	0.776 ± 0.036	0.660 ± 0.030	1–4	*
78	octanal	a	1049	3.228 ± 0.173	2.654 ± 0.152	1, 3–6	*
105	nonanal	a	1151	0.773 ± 0.027	0.731 ± 0.032	1–3, 5, 6	ns
136	decanal	a		0.443 ± 0.015	0.408 ± 0.016	1–6	ns
147	2,4-decadienal (*E,Z*)	c		0.511 ± 0.018	0.438 ± 0.019	2	**
148	2-undecenal	c		0.603 ± 0.024	0.504 ± 0.021		**
	Ketones						
3	2-propanone	a	542	1.027 ± 0.074	1.338 ± 0.093	3, 4, 6	*
8	2,3-butanedione	a	633	0.444 ± 0.019	0.374 ± 0.015	2, 3, 6	**
9	2-butanone	a	638	1.043 ± 0.056	1.059 ± 0.063	2–6	ns
19	2-pentanone	a	726	nq	nq	1, 3, 4, 6	
23	3-hydroxy-2-butanone	a	780	nq	nq	2–6	
33	2-hexanone	a	831	0.644 ± 0.023	0.539 ± 0.023	6	**
45	2-heptanone	a	934	0.662 ± 0.033	0.531 ± 0.027	2, 3, 5, 6	**
77	6-methyl-5-heptan-2-one	a	1044	0.644 ± 0.023	0.646 ± 0.035	4, 6	ns
104	5-ethyl-3-methyl-5-hepten-2-one	b	1148	0.584 ± 0.031	0.656 ± 0.035		ns
	Aliphatic Hydrocarbons						
6	hexane	a	600	0.828 ± 0.087	0.712 ± 0.030	6	ns
15	heptane	a	700	0.587 ± 0.125	1.534 ± 0.216	3, 4, 6	***
28	octane	a	800	0.493 ± 0.025	0.485 ± 0.022	3, 4, 6	ns
30	1-octene	b	814	1.214 ± 0.110	0.526 ± 0.021	4, 6	***
40	nonane	a	900	0.746 ± 0.104	0.613 ± 0.073	1, 3, 5, 6	ns
64	decane	a	1000	0.756 ± 0.125	0.484 ± 0.040	2, 5, 6	*
119	dodecane	a	1200	0.543 ± 0.017	0.491 ± 0.018	2, 5	*
	Esters						
5	methyl acetate	a	569	0.199 ± 0.009	0.188 ± 0.011	4	ns
9b	ethyl acetate	a	641	nq	nq	3, 4, 6	
17	methyl 2-methylpropanoate	b	709	0.142 ± 0.005	0.124 ± 0.010	4	ns
23	methyl butanoate	a	745	0.380 ± 0.022	0.275 ± 0.019	4	***
32	ethyl butanoate	a	823	0.524 ± 0.022	0.435 ± 0.016	3, 4	**
37	ethyl 1-methylbutanoate	c	868	0.124 ± 0.004	0.111 ± 0.005		ns
38	ethyl 2-methylbutanoate	a	875	0.278 ± 0.010	0.316 ± 0.014	2–4,6	*
39	ethyl 3-methylbutanoate	c	892	0.151 ± 0.010	0.119 ± 0.007	3,4	**
48	methyl hexanoate	a	954	0.581 ± 0.022	0.547 ± 0.025	4	ns
51	hexyl propanoate	b	964	0.337 ± 0.024	0.300 ± 0.020		ns
	Aromatic Hydrocarbons						
27	toluene	a	797	0.410 ± 0.017	0.360 ± 0.019	3–6	ns
41	*p*- or *m*-xylene	a	910	0.198 ± 0.007	0.170 ± 0.007	1, 4–6	**
43	styrene	a	923	0.102 ± 0.004	0.095 ± 0.006	1, 4, 6	ns
44	*o*-xylene	a	928	0.385 ± 0.018	0.348 ± 0.022	1, 3–6	ns
	Furans						
19	2,5-dimethylfuran	a	721	0.063 ± 0.002	0.057 ± 0.003	1	ns
68	2-pentylfuran	a	1014	0.512 ± 0.019	0.496 ± 0.024	4	ns
	Chloride Compounds						
11	chloroform	a	657	0.573 ± 0.040	0.369 ± 0.016	2, 5, 6	***
36	2,2-dichloroethanol	b	861	0.101 ± 0.004	0.084 ± 0.004	6	**
	Nitrogen Compounds						
33	1*H*-pyrrole	a	835	nq	nq	4	
35	methylpyrazine	a	847	0.093 ± 0.003	0.082 ± 0.004	4	*
49	2,6-dimethylpyrazine	a	955	0.282 ± 0.014	0.280 ± 0.019	4–6	ns

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Table 2 (Continued)

peak no.[a]	compound	RI[b]	KI[c]	short[d] (7 mon)	long (12 mon)	R[e]	P[f]
	Miscellaneous						
14	acetic acid	a	681	nq	nq	1, 3, 4	
24	dimethyl disulfide	a	782	2.916 ± 0.253	1.490 ± 0.119	3, 4, 6	***
50	2-butoxyethanol	a	960	0.537 ± 0.026	0.601 ± 0.041	2, 4, 6	ns
	Branched Hydrocarbons						
73	(MW 156)	c	1028	3.864 ± 0.241	3.244 ± 0.213	1, 2, 6	ns
76	(MW 156)	c	1040	0.392 ± 0.065	0.525 ± 0.067	1, 2, 6	ns
79	(MW 170)	c	1055	1.555 ± 0.160	1.944 ± 0.181	1, 2, 6	ns
81	(MW 170)	c	1061	1.698 ± 0.080	1.363 ± 0.070	1, 2, 6	**
84	(MW 170)	c	1068	2.511 ± 0.148	1.989 ± 0.131	1, 2, 6	*
88	(MW 170)	c	1080	1.402 ± 0.054	1.201 ± 0.057	1, 2, 6	*
89	(MW 170)	c	1084	2.163 ± 0.094	1.945 ± 0.083	1, 2, 6	ns
90	(MW 170)	c	1088	4.876 ± 0.249	4.562 ± 0.215	1, 2, 6	ns
91	(MW 170)	c	1093	1.739 ± 0.137	1.928 ± 0.183	1, 2, 6	ns
92	(MW 170)	c	1099	2.950 ± 0.126	2.712 ± 0.114	1, 2, 6	ns
94	(MW 184)	c	1111	4.772 ± 0.242	4.823 ± 0.243	1, 2, 6	ns
95	(MW 184)	c	1116	2.032 ± 0.095	2.098 ± 0.110	1, 2, 6	ns
99	(MW 170)	c	1128	2.733 ± 0.116	2.666 ± 0.114	1, 2, 6	ns
100	(MW 170)	c	1135	1.198 ± 0.104	1.514 ± 0.115	1, 2, 6	*
109	(MW 170)	c	1169	0.813 ± 0.026	0.756 ± 0.028	1, 2, 6	ns
111	(MW 170)	c	1177	1.931 ± 0.066	1.779 ± 0.067	1, 2, 6	ns
113	(MW 170)	c	1182	0.638 ± 0.021	0.602 ± 0.023	1, 2, 6	ns
	Unknown Compounds						
29	unknown		802	0.444 ± 0.027	0.432 ± 0.032		ns
54	unknown		973	0.304 ± 0.023	0.249 ± 0.013		*
57	unknown		981	0.416 ± 0.013	0.380 ± 0.019		ns
74	unknown		1035	1.285 ± 0.221	1.856 ± 0.258		ns
141	unknown			0.674 ± 0.024	0.639 ± 0.025		ns

[a] Number of peak as in Figure 1. [b] RI, reliability of identification: a, mass spectrum and retention time identical with an authentic sample; b, mass spectrum and Kovats index from GC−MS and GC−FID in agreement; c, tentative identification by mass spectrum. [c] KI, Kovats index calculated for DB-624 capillary column (J&W Scientific; 60 m, 0.32 mm i.d.; film thickness 1.8 μm) installed on a gas chromatograph equipped with flame ionization (FID), chromatographic conditions detailed in Materials and Methods. [d] Results expressed as means of ten samples ± sem of the area of GC−FID peak normalized by the area of the internal standard. [e] Already reported in (1) Lopez et al. (1992), (2) Garcia et al. (1991), (3) Hinrichesen and Petersen (1995), (4) Barbieri et al. (1992), (5) Berdague et al. (1991), and (6) Buscailhon et al. (1993). [f] P: ns, nonsignificant; *, significant at $P < 0.05$; **, significant at $P < 0.01$; ***, significant at $P < 0.001$.[g] nq, nonquantified.

process (Table 2). Their origin probably arises from pesticide residues ingested by the pigs (Buscailhon et al., 1993).

In summary, the drying-ripening processes were differentiated by 3-methylbutanal, octanal, and dimethyl disulfide being the levels higher in the short (7 months) than in the long (12 months) process. Moreover, the short process was also characterized by high contents of 2-methylpropanol, 1-penten-3-ol, and 1-octene, whereas the long process was characterized by high contents of 2-propanol, 2-propanone, and heptane.

Olfactory Test. The impact of an odor component on the total aroma depends on a number of factors such as odor threshold, concentration in the material measured, solubility in water or fat, and temperature. During the olfactory test 44 odor descriptors were repeatedly reported by the four trained panelists (Figure 1). Many of the compounds responsible for these odors were identified, except odors corresponding to peaks 29, 54, 57, 74, and 141 due to their low concentrations or because they elute in the area between 33 and 47 min where the branched chain hydrocarbons also eluted thereby interfering in the identification of other compounds. The high flavor thresholds of hydrocarbons make minimal contribution to desirable and undesirable flavors (Min et al., 1979; Chang and Petersen, 1977), although some unsaturated hydrocarbons are moderately potent odorants (Forss, 1972). On the other hand, the aromatic hydrocarbons have completely different characteristics such as *o*-xylene and *p*-xylene that give sweet and fruity like odors, respectively (Shahidi et al., 1986). In the Serrano dry-cured ham, *m*- or *p*-xylene

gave an aroma described as smoked-phenolic while *o*-xylene gave a sweet-fruit candy flavor (Figure 1).

The flavor of alcohols was considered unimportant due to their relatively higher threshold compared to other carbonyl compounds (Drumm and Spanier, 1991). It has been shown that the straight chain primary alcohols (C2, C3, 1-alkanols, and 2-alkanols) are relatively flavorless, but as the carbon chain increases, the flavor becomes stronger (Forss, 1972; Shahidi et al., 1986) giving greenish, woody, and fatty floral notes. In the Serrano dry-cured ham only three alcohols were related to specific aromas. 1-Penten-3-ol gave an onion-toasted aroma, 3-methyl-1-butanol gave a penetrating green aroma, while 3-methyl-2-hexanol was defined as a potato-wheat aroma (Figure 1).

C3 and C4 aldehydes have sharp and irritating flavors; intermediate (C5−C9) have green, oily, fatty, tallowy flavors, and the higher (C10−C12) have citrus, orange peel flavors (Forss, 1972). In the headspace of the Serrano dry-cured ham four aldehydes were identified as responsible of four aroma descriptors such as 3-methyl butanal (cheesy-green, aromatics associated with the smell of cheese and green cut grass), hexanal (green-grassy, aromatics associated with the smell of green cut grass), octanal (green-fresh, aromatics associated with fresh cut green) and nonanal (green). Unsaturated ketones are responsible for characteristic flavor notes in animal and vegetable fats (Forss, 1972). 3-Hydroxy-2-butanone gives a buttery note to cooked meat (Shahidi et al., 1986), but in the Serrano dry-cured ham it was identified as responsible for a fruit-red Jell-o aroma (aromatics associated with the smell of com-
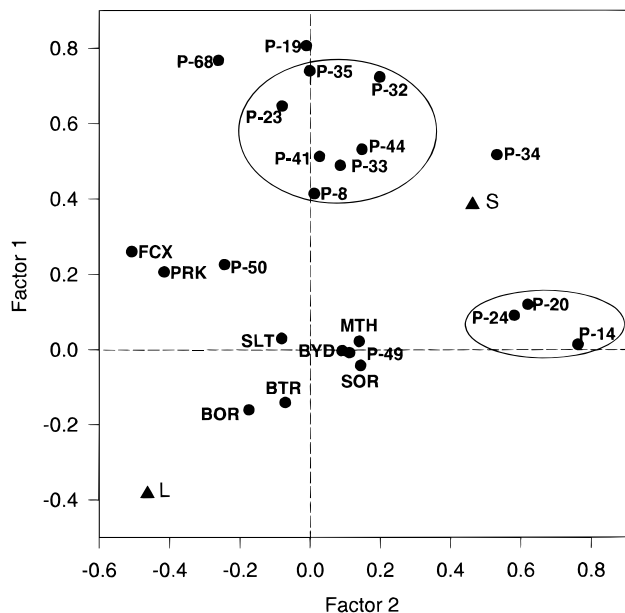
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Figure 2. Principal factor loadings of the attributes (●) onto factor 1 and factor 2 and the average factor scores of the treatments (▲) for factor 1 and factor 2. The attribute represented by the P-number is the volatile compound number as seen in Figure 1 and Table 2. The three-letter codes of the attributes represent the sensory descriptors defined in Table 1.

Figure 3. Principal factor loadings of the attributes (●) onto factor 2 and factor 4 and the average factor scores of the treatments (▲) for factor 2 and factor 4. The attribute represented by P-number is the volatile compound number as seen in Figure 1 and Table 2. The three-letter codes of the attributes represent the sensory descriptors defined in Table 1.

mercial red-strawberry Jell-o) and 2,3-butanedione was responsible for the buttery note. Also, two other ketones were responsible for two different aromas: 2-hexanone (floral-apple) and 6-methyl-5-heptan-2-one (citrus-candy, aromatics associated with the smell of candies with citrus aromas) (Figure 1).

Baines and Mlokiewicz (1984) reported that esters from C1−C10 acids tend to impart a fruity sweet note to pork meat whereas esters derived from long chain fatty acids give a more fatty flavor character as found in beef. In the headspace of Serrano dry-cured ham, we found principally esters from C1−C10 acids that gave fruity (methyl 2-methylpropanoate, ethyl butanoate, ethyl 2-methylbutanoate) and sweet-caramel (methyl butanoate) aromas. Only the methyl hexanoate was responsible for the boiled meat aroma (Figure 1).

Fatty acids, particularly acetic and propionic, are sometimes present in sufficient amounts to make the food acidic. These conditions will modify the flavor contribution of alkaline compounds such as pyrazines and amines. We detected only acetic acid at a level too low to be quantified but high enough to give a vinegar odor to the ham sample. Sulfur compounds are among the important contributors to meat flavor because of their low flavor threshold (Chang and Petersen, 1977; Drumm and Spanier, 1991). In this study we only detected dimethyl disulfide, which gave an unpleasant aroma defined as dirty socks. On the other hand, some of the meaty aromas detected were 2-pentylfuran (ham-like), 1*H*-pyrrole (meaty), and 2-butoxyethanol (dark toast-meaty) (Figure 1).

Many pyrazines have been recognized as the volatiles contributing to roasted aromas of cooked foods being responsible for aromas such as, nutty, green, earthy, potato-like, etc. (Maga, 1982). The processing of Serrano dry-cured ham does not include a cooking step which would result in the generation of pyrazines, although two of them were identified and gave nutty (methylpyrazine) and toasted nut (2,6-dimethylpyrazines) aromas (Figure 1).
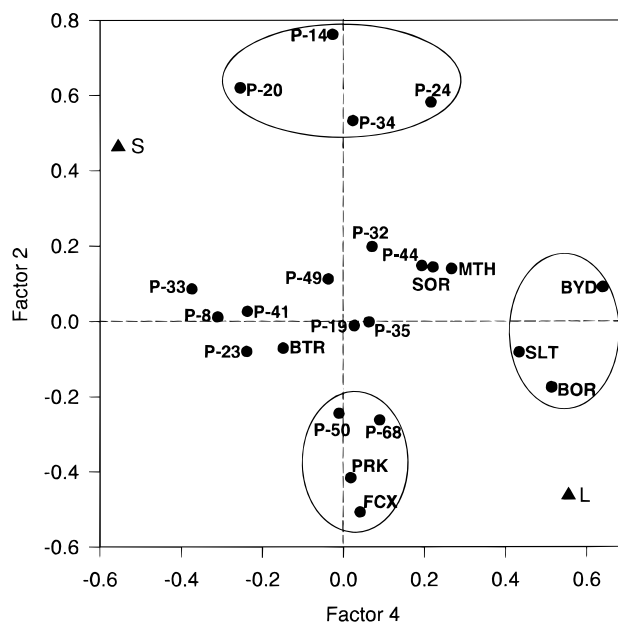
Sensory Analysis and Its Relationship with Instrumental Data. The sensory analysis of the ham samples resulted in significant differences observed between the two processes in four of the eight attributes studied. Boar taint, barnyard, sour, and salty were higher in the long (12 months) than in the short (7 months) process. In order to study which of the volatile compounds contributed to the generation of the dry-cured flavor, the data were analyzed using the statistical method of factor analysis. The impractical amount of replication required to obtain a statistically stable factor-analytic solution, precluded the examination of all of the volatile compounds. Hence, we selected those volatile compounds that were responsible for specific aromas in the olfactory test to see their relation with the sensory descriptors.

The multivariate factor solution consisted of four factors. Factor 1 was described as "pleasant aroma" because the response variables of peaks 8 (2,3-butanedione, buttery), 23 (methyl butanoate, sweet-caramel), 32 (ethyl butanoate, fruity), 33 (2-hexanone, floral-apple), 35 (methylpyrazine, nutty), 41 (*m*- or *p*-xylene, smoked-phenolic), and 44 (*o*-xylene, fruit-candy) exhibited the strongest correlation with the factor (Figure 2). On the other hand, factor 2 can be labeled as "pork meat" because it is defined by the sensory descriptors fat complex (FCX) and pork (PRK); furthermore it has a negative correlation with the peaks 14 (3-methylbutanal, cheesy-green), 20 (1-penten-3-ol, onion-toasted), 24 (dimethyl disulfide, dirty socks), and 34 (hexanal, green-grassy) (Figure 3). A third factor was named "cured flavor" because mouth-filling (MTH), sour (SOR), and bitter (BTR) exhibit a high correlation with the factor 3 (Figure 4). The fourth factor named "off-flavor" because it was defined by salty (SLT) and, more importantly, by barnyard (BYD) and boar taint (BOR) (Figure 3). As seen in Table 3, factors 1 and 2 were negatively correlated with factor 4 meaning that the "pleasant" and "pork" flavors are negatively correlated

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Table 3. Inter-Factor Correlations

	F. 1 (pleasant aroma)	F. 2 (pork flavor)	F. 3 (cured flavor)	F. 4 (off-flavor)
F. 1 (pleasant aroma)	1.00000	0.19612	0.14837	−0.26819
F. 2 (pork flavor)	0.19612	1.00000	0.00252	−0.31457
F. 3 (cured flavor)	0.14837	0.00252	1.00000	0.04898
F. 4 (off-flavor)	−0.26819	−0.31457	0.04898	1.00000
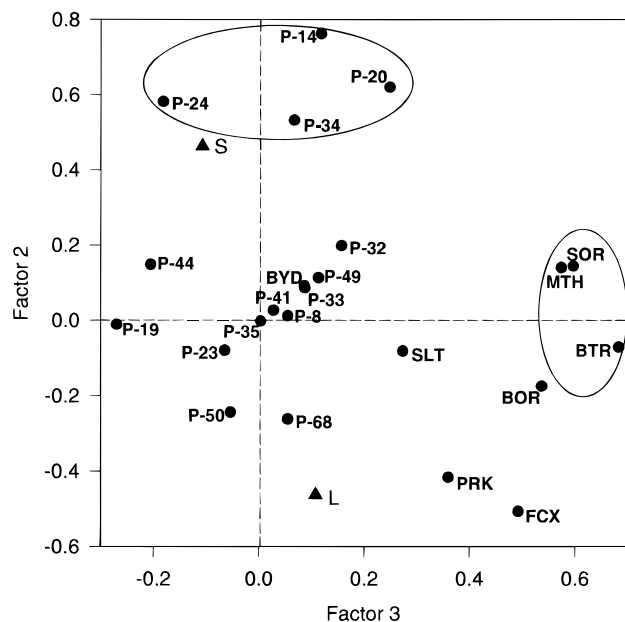
Figure 4. Principal factor loadings of the attributes (●) onto factor 2 and factor 3 and the average factor scores of the treatments (▲) for factor 2 and factor 3. The attribute represented by P-number is the volatile compound number as seen in Figure 1 and Table 2. The three-letter codes of the attributes represent the sensory descriptors defined in Table 1.

with the "off-flavor". Meanwhile, factor 3 ("cured flavor") does not have any strong correlation with the other factors.

The average factor score of the long treatment (L in Figures 2−4) clustered in the quadrant where factors 1 and 2 were negative and factors 3 and 4 were positive. On the other hand, the short average factor score (S in Figures 2−4) was onto the quadrant where factors 1 and 2 were positive and 3 and 4 were negative. From the relationship of the factors with the treatment, we explain the generation of the dry-cured flavor where the length of the drying stage produced an increase in "pork", "cured", and "off-flavors", but masked the "pleasant" aroma. On the other hand, the relationship of the volatile compounds with the treatment can be described in the short process (7 months) where some pleasant compounds such as 8 (2,3-butanedione, buttery), 23 (methyl butanoate, sweet-caramel), 32 (ethyl butanoate, fruity), 33 (2-hexanone, floral-apple), 35 (methylpyrazine, nutty), 41 (*m-* or *p*-xylene, smoked-phenolic), and 44 (*o*-xylene, fruit-candy) showed a strong correlation with the short process; furthermore, some compounds 14 (3-methylbutanal, cheesy-green), 20 (1-penten-3-ol, onion-toasted), 24 (dimethyl disulfide, dirty socks), and 34 (hexanal, green-grassy) were related with the short process and gave a character of fresh-cured pork flavor (Figure 3). Meanwhile, in the long process there is a slight correlation with compounds 50 (2-butoxy ethanol, dark toast-meaty) and 68 (2-pentylfuran, ham-like), which gave the strongest pork flavor.

These results agree in part with Careri et al. (1993) who found that esters, aromatic hydrocarbons and cyclic nitrogen compounds affect the aged odor of Italian type

dry-cured ham positively, although we did not find any positive contribution of 3- and 4-carbon alcohols to the aged aroma as they described. From the olfactory test, we did not detect any contribution of these alcohols to the aroma of the Serrano dry-cured ham. When we compared our results with those found in French type dry-cured ham (Buscailhon et al., 1994), we detected some similarities about the relation of several ketones with the pleasant aroma of the dry-cured ham and the relation of some aldehydes with the aroma of fresh-cured pork, but we did not find any contribution of 1-butanol to the aroma of Serrano dry-cured ham. On the other hand, the volatile compounds detected on Spanish Iberian dry-cured ham were principally aldehydes, ketones, hydrocarbons, and alcohols (Garcia et al., 1991; Lopez et al., 1992) but their contributions to the dry-cured ham flavor were not studied. All the differences in volatile composition and sensorial relations between the Italian, French, and Spanish dry-cured hams could be due to the different manufacturing techniques employed and also to the antemortem factors, especially the raw material, that can influence the final sensorial quality of the product.

In summary, the volatile components of the headspace of Serrano dry-cured ham contributed, both individually and in combination, to the distinctive aroma properties of the product. Ketones, esters, aromatic hydrocarbons, and pyrazines were essentially the volatile compounds that correlated with the pleasant aroma of the hams, while hexanal, 3-methylbutanal, 1-penten-3-ol, and dimethyl disulfide were related with the short ripening−drying stage. On the other hand, in the long process an increase in the pork flavor shows a low correlation with 2-butoxyethanol and 2-pentylfuran. These volatile compounds appeared to be mainly formed by lipid oxidation except for the sulfide compounds, methyl branched aldehydes, and pyrazines which were generated by Strecker degradation of amino acids.

Finally, it is important to continue these studies to identify those unknown compounds responsible for specific aromas in Serrano dry-cured ham and relate their contribution to the development of the characteristic dry-cured ham flavor.

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