

Analysis of Volatiles in Meat from Iberian Pigs and Lean Pigs after Refrigeration and Cooking by Using SPME-GC-MS

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The volatile compounds generated in meat from Iberian and lean pigs after four different treatments (raw, refrigerated, cooked, and refrigerated cooked meat) were analyzed. The different treatments showed different volatile profiles. Methyl alcohols and ketones (such as 2-ethyl-hexan-1-ol, 2-methyl-butan-1-ol, 3-methyl-butan-1-ol, and 3-hydroxy-butan-2-one) were the most representative in refrigerated meat because of the degradation of carbohydrates and proteins together with the Strecker degradation pathway. Lipid-derived volatiles were the most abundant in cooked meat and refrigerated cooked meat. Meat from different pig breeds presented different volatile profiles, probably due to different enzymatic and oxidative deterioration susceptibility. Otherwise, the fat content and its compositional characteristics also played an important role in the generation of volatiles. As compared to samples from lean pigs, muscles from Iberian pigs showed a higher content of heme iron that may have promoted the generation of higher content of total lipid-derived volatiles during the refrigeration of cooked meat. Despite that, the formation of volatiles with low thresholds and related to intense rancidity perception likely to be derived from polyunsaturated fatty acids was higher in lean pork than in meat from Iberian pigs. This might be expected to lead to a more intense development of a warmed over flavor during refrigeration of cooked samples from lean pigs.

KEYWORDS: Pork; volatile compounds; refrigeration; cooked meat; fat content; fatty acid profile; heme iron; WOF

INTRODUCTION

The generation of volatile compounds in meat and meat products has been largely studied because of the role of flavor in the overall acceptability of meat and meat products (1). The solid phase microextraction (SPME) sampling, combined with gas chromatography and mass spectrometry (GC-MS), has been successfully introduced to collect aroma components in the headspace of several foods and more recently in cooked pork and other meat products (2, 3). Among several advantages, the SPME sampling offers chemical data, which can be closely related to olfactory assessment (cf. review by Pawliszyn, 4).

The fat portion of meat, and especially the phospholipid fraction, undergoes autoxidation phenomena, producing an overwhelming number of volatiles, such as acids, aliphatic aldehydes, ketones, and alcohols, and promoting the formation of some others such as nitrogen- and sulfur-containing compounds (1). Some of them are thermally derived, are odor active compounds, and have a great effect on cooked meat flavor (1). Also, the generation of volatiles has been related to meat deterioration during its refrigerated storage (5). Among the different precursors of volatiles in meat, lipids are possibly the most important (1). Thus, the lipid fraction is the most variable

component of meat, and some aspects such as its content in meat (6) and its compositional characteristics (7) are influential on the generation of volatiles, with both desirable and undesirable repercussions. The breed, feeding regime, and rearing systems can also be important factors (7, 8).

The Iberian pig is a rustic animal reared free-range in the southwest of the Iberian Peninsula. This pig offers a meat with excellent properties for the preparation of cured products (9) and meat for fresh consumption (10). The high content of fat, monounsaturated fatty acids (MUFAs), heme iron (10), and antioxidants (11) in meat from Iberian pigs have been identified as critical aspects of their higher quality as compared to meat from lean pigs. In fact, the fresh meat from lightweight Iberian pigs has higher nutritional and technological properties than meat from lean pigs (10, 12). Despite that, there is no information of the effect of this particular breed and its free-range rearing system on the formation of volatile compounds after meat refrigeration and meat cooking. Previous studies indicated that MUFAs are positively correlated and polyunsaturated fatty acids (PUFAs) are negatively correlated with pork flavor (7). Moreover, pork flavor is thought to have declined with selection strategies that reduce intramuscular fat (IMF) content (8). This paper examines the aroma profile generated after refrigeration and cooking of meat from free-range-reared Iberian pigs and

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intensively reared lean pigs in order to investigate how these breeds and treatments affect the generation of volatile compounds.

MATERIAL AND METHODS

Sampling. Pig meat from two different origins was considered. *M. Longissimus dorsi* from free-range reared Iberian pigs commonly produced in the southwest of Spain and belonging to Iberian pig pure breed selection schemes ($n = 5$) was obtained from the carcasses after being slaughtered at 85–90 kg live weight and an age of 9 months. Iberian pigs were fed on grass and a concentrate feed based on cereals. *M. Longissimus dorsi* from an industrial genotype of lean pigs, Large White-Landrace \times Large White ($n = 5$), was obtained after they were slaughtered at 90–95 kg live weight and an age of 6 months. Lean pigs were intensively reared and fed with a concentrate feed. The day after slaughter, all of the treatments (refrigeration and cooking) were performed after which the samples were vacuum-packaged and kept frozen ($-85\text{ }^{\circ}\text{C}$) until required (3 weeks).

Lipid Extraction from Meat. Lipids were extracted from 5 g of meat samples with chloroform/methanol (2:1) according to the method described by Bligh and Dyer (13).

Lipid Extract Fractionation. Total lipid extracts were fractionated into neutral and polar lipids on aminopropyl cartridges, following the procedure described by Kaluzny et al. (14).

Fatty Acid Profile Analysis. After fractionation, triacylglycerol and phospholipid fatty acid methyl esters (FAMES) were prepared by transesterification using methanol in the presence of sulfuric acid (5% of sulfuric acid in methanol) following the method of López-Bote et al. (15). FAMES were analyzed using a Hewlett-Packard, model HP-5890A, gas chromatograph, equipped with a flame ionization detector. The derivatives were separated on a semicapillary column (Hewlett-Packard FFAP-TPA fused-silica column, 30 mm long, 0.53 mm internal diameter, and 1.0 μm film thickness). The injector and the detector temperature were held at 230 $^{\circ}\text{C}$. The column oven temperature was maintained at 220 $^{\circ}\text{C}$. The flow rate of the carrier gas (N_2) was set at 1.8 mL/min. Identification of FAMES was based on retention times of reference compounds (Sigma). Fatty acid composition was expressed as percent of total FAMES.

Heme Iron Content. The concentration of heme pigments was assayed from the total content of heme according to Hornsey (16). Hematin and heme iron contents were calculated as follows

$$\text{hematin (mg/kg)} (a) = \text{abs (640 nm)} \times 680$$

$$\text{heme iron (mg/kg)} = (a \times 8.82)/100$$

Refrigeration And Cooking Procedures. Four different treatments were given to the meat samples from both Iberian and lean pigs the day after slaughter.

Raw Meat. Raw meat was prepared by freeing *M. Longissimus dorsi* from visible fat and packaging chops (1 cm thickness) of this muscle in a vacuum.

Refrigeration of Raw Meat. The day after slaughter, chops (1 cm thickness) from *M. Longissimus dorsi* were placed on Styrofoam meat trays and overwrapped in PVC oxygen permeable films and stored at $+4\text{ }^{\circ}\text{C}$ for 10 days under fluorescent light.

Cooking of Raw Meat. Chops of *M. Longissimus dorsi* (1 cm thickness) were placed in plastic bags and cooked in a hot water bath to an internal temperature of 80 $^{\circ}\text{C}$ for 10 min. The internal temperature in loin chops was determined using a thermocouple placed in the core of the chop. They were then rapidly chilled to 15–20 $^{\circ}\text{C}$ with a cold water shower for 5 min and dried on the surface with a paper towel.

Refrigeration of Cooked Meat. The samples were cooked using the aforementioned method after which they were allowed to cool and placed on Styrofoam meat trays and overwrapped in PVC oxygen permeable films and stored at $+4\text{ }^{\circ}\text{C}$ for 10 days under fluorescent light.

After each of the treatments, meat samples (raw, refrigerated, cooked, and refrigerated cooked) were vacuum-packaged and kept frozen ($-85\text{ }^{\circ}\text{C}$) until analyzed (3 weeks) in the darkness.

Analysis Of Volatiles From Raw, Refrigerated, Cooked, and Cooked and Refrigerated Meat Samples. The SPME fiber, coated with divinylbenzene-carboxen-poly(dimethylsiloxane) (DVB/CAR/PDMS) 50/30 μm , was purchased from Supelco Co. (Canada). This coating phase was chosen because of the high reproducibility presented and the lower coefficients of variance obtained as compared to CAR/PDMS fiber (17). The SPME fiber was preconditioned prior analysis at 220 $^{\circ}\text{C}$ during 45 min. The headspace sampling technique was used as follows: 5 g of meat was homogenized with 15 mL of water, and aliquots of 2 mL were placed in 2.5 mL vials. The fiber was exposed to the headspace of the solution while the sample equilibrated during 30 min immersed in water at 37 $^{\circ}\text{C}$. On the basis of the literature data, the sampling method was selected because in those conditions most of the analytes might have reached the equilibrium. Analyses were performed on a HP5890GC series II gas chromatograph (Hewlett-Packard) coupled to a mass selective detector Agilent model 5973. Volatiles were separated using a 5% phenyl-95% dimethyl polysiloxane column (30m \times 0.25 mm i.d., 1.0 mm film thickness; Restek). The carrier gas was helium at 18.5 psi, resulting in a flow of 1.6 mL min^{-1} at 40 $^{\circ}\text{C}$. The SPME fiber was desorbed and maintained in the injection port at 220 $^{\circ}\text{C}$ during the whole chromatography run. The injector port was in the splitless mode. The temperature program was isothermal for 10 min at 40 $^{\circ}\text{C}$ and then raised at the rate of 7 $^{\circ}\text{C} \text{ min}^{-1}$ to 250 $^{\circ}\text{C}$ and held for 5 min. *n*-Alkanes (Sigma R-8769) were run under the same conditions to calculate the Kovats index (KI) values for the compounds. The GC-MS transfer line temperature was 270 $^{\circ}\text{C}$. The mass spectrometer operated in the electron impact mode with an electron energy of 70 eV and a multiplier voltage of 1650 V and collected data at a rate of 1 scan s^{-1} over a range of m/z 40–300. Compounds were tentatively identified by comparing their mass spectra with those contained in the NIST/EPA/NIH library and by comparison of KI with those reviewed by Kondjoyan and Berdagué (18) and some others from the literature. The identifications of some volatile compounds were only performed by using mass spectrometry data because the retention index was unavailable.

Data Analysis. The effect of meat origin (meat from Iberian or lean pigs) on fat content, fatty acid profiles of neutral and polar fractions of IMF, and heme iron content was analyzed using a student's test for independent variables. Chromatographic areas of all identified peaks were used as variables. To determine the effect of the meat origin and the four different treatments ($n = 5$ in each group) on the generation of volatiles, an analysis of variance (ANOVA) following the generalized linear model for a four (treatments) \times two (meat origin) with the interaction was used (19). HSD Tukey's tests were used when ANOVA found significance differences between treatments. Significance was defined at $p < 0.05$.

RESULTS AND DISCUSSION

Fat Content and Fatty Acid Composition Of Raw Meat. There was a clear effect of the meat origin on fat content of *M. Longissimus dorsi* as it was significantly higher ($p < 0.05$) in Iberian pig muscles (3.7%) than in those from lean pigs (2.0%). These results agree with those previously obtained by other authors in Iberian pigs slaughtered at 90–110 kg and lean pigs slaughtered around 100 kg (20) and by ourselves (10). These differences could be caused by the high lipid synthesis capacity of the Iberian pig breed although the slaughtering age (higher for the Iberian pigs; 9 vs 6 months) could have been the influence (20). The higher content of IMF in meat from rustic pigs (as Iberian pigs) is regarded as one of the essential aspects in their higher meat quality as compared to meat from lean pigs (21). Regardless to the effects of IMF on eating quality traits, fat content can also be influential in the production of volatile compounds so that low fat content meats could be related to loss in flavor development (8).

Notable differences between meats with different origins were detected in the analysis of the fatty acid profile of neutral and polar lipid fractions (Table 1). In neutral lipids, *M. Longissimus*

Table 1. Fatty Acid Composition (Means \pm Standard Deviation) of Neutral and Polar Lipids of Raw *M. Longissimus dorsi* from Lean and Iberian Pigs^a

| | neutral lipids | | | polar lipids | | |
|---------------|------------------|------------------|-------|------------------|------------------|-------|
| | lean (n = 5) | Iberian (n = 5) | p | lean (n = 5) | Iberian (n = 5) | p |
| C12:0 | 0.08 \pm 0.09 | 0.12 \pm 0.02 | 0.420 | 0.21 \pm 0.16 | 0.14 \pm 0.08 | 0.450 |
| C14:0 | 0.98 \pm 0.07 | 1.63 \pm 0.18 | 0.000 | 0.21 \pm 0.09 | 0.68 \pm 0.32 | 0.010 |
| C16:0 | 22.59 \pm 0.71 | 26.82 \pm 1.07 | 0.000 | 19.92 \pm 0.58 | 23.39 \pm 1.64 | 0.002 |
| C17:0 | 0.23 \pm 0.01 | 0.27 \pm 0.03 | 0.040 | 0.53 \pm 0.12 | 0.54 \pm 0.09 | 0.850 |
| C18:0 | 13.85 \pm 1.34 | 12.73 \pm 0.76 | 0.140 | 8.24 \pm 0.98 | 9.18 \pm 0.65 | 0.110 |
| C20:0 | 0.38 \pm 0.07 | 0.23 \pm 0.01 | 0.002 | 0.48 \pm 0.14 | 0.21 \pm 0.12 | 0.010 |
| Σ SFA | 38.12 \pm 1.94 | 41.82 \pm 1.92 | 0.010 | 29.59 \pm 0.86 | 34.16 \pm 2.08 | 0.002 |
| C16:1 | 2.85 \pm 0.31 | 4.19 \pm 0.28 | 0.000 | 1.08 \pm 0.26 | 1.76 \pm 0.68 | 0.060 |
| C17:1 | 0.21 \pm 0.01 | 0.26 \pm 0.03 | 0.010 | 0.33 \pm 0.05 | 0.35 \pm 0.09 | 0.570 |
| C18:1 | 46.51 \pm 2.11 | 45.45 \pm 1.07 | 0.310 | 16.15 \pm 0.97 | 25.95 \pm 4.69 | 0.002 |
| Σ MUFA | 49.59 \pm 2.35 | 49.92 \pm 0.97 | 0.770 | 17.56 \pm 0.86 | 28.09 \pm 2.08 | 0.002 |
| C18:2 | 10.92 \pm 4.22 | 6.51 \pm 1.42 | 0.050 | 34.27 \pm 2.99 | 26.53 \pm 4.48 | 0.010 |
| C18:3 | 0.73 \pm 0.24 | 0.43 \pm 0.06 | 0.020 | 2.94 \pm 1.55 | 0.82 \pm 0.10 | 0.010 |
| C20:2 | 0.11 \pm 0.07 | 0.17 \pm 0.06 | 0.230 | 1.44 \pm 0.27 | 1.01 \pm 0.28 | 0.040 |
| C20:4 | 0.52 \pm 0.48 | 1.13 \pm 0.58 | 0.110 | 14.21 \pm 1.86 | 9.36 \pm 2.41 | 0.008 |
| Σ PUFA | 12.30 \pm 4.26 | 8.25 \pm 2.11 | 0.090 | 52.86 \pm 1.38 | 37.74 \pm 7.12 | 0.002 |

^a Results are expressed as means (n = 5) in percent of methyl esters from total analyzed SFA, MUFA, and PUFA.

dorsi from Iberian pigs presented a higher proportion of saturated fatty acids (SFA) ($p < 0.05$) and lower of PUFAs ($p > 0.05$) (41.82 vs 38.12% and 8.25 vs 12.30%, respectively). These results are in agreement with previous data reported in *M. Longissimus dorsi* from Iberian pigs slaughtered at low weights (10, 20) and lean pigs (20).

Fatty acid composition influences the generation of volatiles since PUFAs are extremely sensitive to oxidative deterioration, leading to lipid oxidation and off-flavors generation (22). From this point of view, the fatty acid profile of polar lipids is even more interesting because of the role played by phospholipids in lipid oxidation and flavor formation (22). In this lipid fraction, the differences between groups were even higher. *M. Longissimus dorsi* from Iberian pigs exhibited significantly higher percentages of SFAs (34.16 vs 29.59%) and MUFAs (28.09 vs 17.56%) and lower percentages of PUFAs (37.74 vs 52.86%) than the muscles from lean pigs.

Analysis Of Volatiles. Table 2 summarizes GC-MS data obtained from volatile compounds analysis of raw, refrigerated, cooked, and refrigerated cooked meat samples. From the total volatile compounds detected in the extracts, 57 of them are shown and categorized into 11 classes. All of them were tentatively identified (good match of MS and coincidence of KI).

Analysis Of Volatiles In Raw Refrigerated Meat. In refrigerated meat, 36 volatiles were detected, being the most abundant: alcohols (2-ethyl-hexan-1-ol, butane-2,3-diol, 2-methyl-butan-1-ol, and 3-methyl-butan-1-ol), aldehydes (nonanal, hexanal), ketones (3-hydroxy-butan-2-one), and acids (acetic acid). Some of these compounds increased significantly after 10 days under refrigerated storage and were not detected in the samples in which other treatments (cooking or cooking and refrigeration) were performed. Changes in volatile compounds during refrigerated storage can indicate chemical, enzymatic, and microbial deterioration in meat (5), while desirable meat flavor is achieved by cooking (1). In general, raw meat refrigeration caused a small increase in oxidation-derived aldehydes and a large increase in methyl alcohols and ketones generated from branched chain amino acids and pyruvate catabolism. Therefore, the degradation of proteins and carbohydrates by enzymatic activity seemed to be the main cause of volatile generation during refrigeration. For example, butane-2,3-dione and butane-2,3-diol are generated from pyruvate catabolism while 2-methyl-butan-1-ol and 3-meth-

yl-butan-1-ol come from leucine or isoleucine metabolism via Strecker degradation and dimethyl-sulfide results from methionine degradation (23).

In previous work, we observed that enzymatic activity was the most important cause of meat deterioration during refrigerated storage, while oxidation phenomena had a secondary role (24). Similar results were found by other authors in refrigerated poultry (5) and refrigerated pork (25). Increases in the amount of 3-hydroxy-butan-2-one, which has been reported to be a meat aging indicator (1), were also significant.

Some large differences were found between groups, but these were not statistically different in all cases due to high standard deviations commonly found in volatile compound analysis techniques. The relatively small size of sample (n = 5) could also be a cause of the lack of differences. Refrigerated samples from Iberian pigs showed a higher content of 2-methyl-butan-1-ol and 3-methyl-butan-1-ol but not to a significant extent ($p > 0.05$). However, the content of 2-ethyl-hexan-1-ol (the most abundant volatile compound detected in the refrigerated samples) was 13-fold times higher in refrigerated *M. Longissimus dorsi* from lean pigs than in *M. Longissimus dorsi* from Iberian pigs (232.19 vs 17.33 AU; $p < 0.05$). The origin of this alcohol is controversial since some authors defended that it is generated from amino acid catabolism (26) and some others reported that this compound is derived from lipid oxidation (27). On the basis of our data, 2-ethyl-hexan-1-ol is unlikely to be originated from lipid oxidation because the amount of this compound in cooked and refrigerated cooked meat (where large quantities of oxidation products are expected to be found) is lower than in refrigerated meat. Although other possibilities could be possible, the degradation of proteins by muscle and/or microbial proteolytic enzymes into free amino acids and their consequent degradation into aldehydes and homologous alcohols through Strecker degradation is a probable way of generation, according to Stahnke et al., 2002. If so, results suggest a higher enzymatic degradation of proteins in muscles from lean pigs. A previous work in which we detected higher enzymatic products in refrigerated meat from lean pigs than in Iberian ones (24) strongly agrees with the present results. This may be related to the findings of Rossell and Toldrá (28) and ourselves (29) who described a higher residual activity of cathepsins in muscles from lean pigs as compared with Iberian pig muscles.

With regard to lipid-derived volatiles, the amount of saturated aldehydes such as pentanal, hexanal, octanal, and nonanal did not change significantly after the refrigeration period. However, differences between groups were detected. Muscles from Iberian pigs showed higher contents of oleic-derived aldehydes but not to a significant extent, such as octanal (3.68 vs 2.29 AU), nonanal (16.97 vs 13.33 AU), and undec-2-enal (2.69 vs 2.24 AU) ($p > 0.05$). On the contrary, muscles from lean pigs exhibited higher contents of heptan-2-one (5.60 vs 2.42 AU) and octan-2-one (0.76 vs 0.57 AU) ($p < 0.05$).

This is in agreement with the fatty acid composition of the muscles since polar lipids from muscles from Iberian pigs showed a significantly higher proportion of oleic acid and total of MUFAs than those from lean pigs ($p < 0.05$). On the contrary, the latter presented a significantly higher proportion of PUFAs ($p < 0.05$) (Table 1).

Analysis Of Volatiles In Cooked Meat. The volatile flavor compounds of cooked meat can be divided into two groups, those formed from lipid oxidation and those that originate from Maillard reactions (1), the first of these being the most abundant when the cooking temperature is under 100 °C (8). Lipid-derived volatiles such as acids (nonanoic acid), ketones (octane-2,3-dione), aldehydes (pentanal, hexanal, heptanal, octanal, nonanal, decanal, hept-(E)-2-enal, dec-(E)-2-enal, deca-(E,Z)-2,4-dienal, and undec-(E)-2-enal), furans (2-pentyl-furan), and alcohols (hexan-1-ol, oct-1-en-3-ol, and octan-1-ol) were the most abundant compounds in cooked meat. Hexanal was the dominant aldehyde in cooked meat from both groups. Most of the straight chain aldehydes are derived from the oxidation of unsaturated fatty acids (7). Alcohols are also derived mainly from oxidative decomposition of fat (27). The presence of hydrocarbons in cooked meat is quite small (only decane was detected), and their contribution to meat aroma is not considered important (30). Furans (as 2-pentylfuran) are oxidation products from linoleic and other *n*-6 fatty acids (31). Nitrogen-containing volatile flavor compounds originate from the breakdown of proteins, free amino acids, and nucleic acids whereas sulfur-containing volatile flavor compounds are derived from sulfur-containing amino acids (1). In the present work, the relative content of nitrogen- and sulfur-containing compounds in cooked meat is small, because of the temperature reached during the cooking process used in the present study. The thermal inactivation of hydrolytic enzymes during cooking could explain that the main compounds described in refrigerated meat and associated to proteolytic activities were not detected in cooked meat. Thus, in contrast to what happens during the refrigerated storage of raw meat, during meat cooking, the generation of volatiles occurs quickly and provides a different volatile profile related to the desirable flavor of cooked pork. Thus, the higher total amount of volatiles in cooked samples as compared to that of refrigerated meat should also be caused by the cooking loss that was not taken into account in the headspace sampling.

Cooked meat from lean pigs showed a higher number of volatile compounds. These samples tended to present a higher content of certain compounds closely related to lipid oxidation, such as hept-(E)-2-enal (5.57 vs 3.30 AU; $p < 0.05$); pent-(E)-2-enal (1.47 vs 0.58 AU; $p < 0.05$); oct-(E)-2-enal (8.15 vs 6.73 AU; $p > 0.05$); deca-(E,Z)-2,4-dienal (15.46 vs 3.40 AU; $p > 0.05$), and hexan-1-ol (15.22 vs 1.57 AU; $p > 0.05$). The content of 2-ethyl-hexan-1-ol was also larger in lean pigs' muscles, and there were some lipid-derived compounds that were only detected in those samples (i.e., methyl-ketones, heptan-2-one, and octan-2-one). These results agree with those found by Michaels and Istasse (2) who reported a higher amount

of volatiles from cooked low fat meats in comparison with meats with a high fat content. This fact implies that the higher IMF content in samples from Iberian pigs could influence the release of volatiles in those samples as long as fat could act as a reservoir of volatiles. Thus, it is accepted that the total amount of volatiles generated during cooking and the specific aromatic profile do not depend only on the fat content but also on the fatty acid composition and the balance between prooxidant and antioxidant factors in meat. The selection for high lean growth has reduced IMF, and this implies an increase in relative amount of phospholipid and concentration of PUFAs (8), which are very prone to thermal degradation. In this sense, and in agreement with the present results, a higher oxidative deterioration has been described in meat from lean pigs in comparison to meat from Iberian pigs (12). A high proportion of MUFAs and the probable presence of antioxidants in muscles from Iberian pigs as a result of a free-range rearing system (11) were reported as influential factors.

Concerning the sensory assessment of flavor in relation to the volatile profile, it must be emphasized that aldehydes are probably the most interesting of the lipid-derived volatiles, since they have low odor threshold values and may contribute to the flavor of the cooked pork samples (31). Thus, differences between groups in the volatiles profile may be related to compositional differences in their lipids. The higher proportion of linoleic acid and total of PUFAs from the polar lipids in samples from lean pigs could explain the higher content of volatile compounds derived from those fatty acids (31) in cooked samples from those pigs such as hept-(E)-2-enal ($p < 0.05$), oct-(E)-2-enal ($p > 0.05$), pent-(E)-2-enal ($p < 0.05$), deca-(E,Z)-2,4-dienal ($p > 0.05$), and hexan-1-ol ($p > 0.05$). Aromatic notes of these compounds have been described as intensely grasslike and related to rancidity (32). On the other hand, muscles from Iberian pigs showed a higher content, but not to a significant extent, of heptanal (14.17 vs 10.52 AU; $p > 0.05$), nonanal (52.92 vs 46.89 AU; $p > 0.05$), decanal (4.90 vs 3.46 AU; $p > 0.05$), and octan-1-ol (7.31 vs 6.77 AU; $p > 0.05$) with origin from oleic acid (33) and associated with pleasant notes, described as floral and sweet, in cooked meat and meat products (34). In comparison to muscles from lean pigs, muscles from Iberian pigs presented significantly higher proportions of oleic acid and total MUFAs in polar lipids. The positive correlation between pork flavor and MUFAs (8) could be related to the compounds derived from its thermal decomposition. The high content of oleic acid-derived aldehydes in meat products of Iberian pigs has been related to essential quality traits (11).

Analysis Of Volatiles In Refrigerated Cooked Meat. Among the different treatments given to meat samples, the combination of cooking and refrigerated storage was the one in which more numbers of volatiles were detected. Several volatile compounds significantly increased during the refrigerated storage of the cooked meat, namely, octane-2,3-dione, octan-2-one, hexanal, octadecanal, pentan-1-ol, heptan-1-ol, oct-1-en-3-ol, octan-1-ol, and methyl-benzene. There were also some volatile compounds that were only detected in the headspace of refrigerated cooked meat: hexanoic acid, oct-3-en-2-one, undecan-2,3-dione, hepta-(E,E)-2,4-dienal, *trans*-undec-(E)-4-enal, nona-2,4-dienal, nonan-2-ol, and pent-(E)-2-ene, most of them being related to strong lipid degradation due to the development of high oxidation phenomena. The refrigerated storage of precooked meat for a short period of time results in the development of a characteristic off-flavor caused by catalytic peroxidation of unsaturated fatty acids (35). The term warmed over flavor (WOF) describes the rapid onset of rancidity in

cooked meat during refrigerated storage. The effect of the lipid fraction on these off-flavors is very complex. Moreover, most of the lipid-derived volatiles detected in these samples could be related to the perception of off-flavors in meat since previous works have described similar results in refrigerated cooked meat. Byrne et al. (36) reported significant variations during refrigerated storage for hexanal, hept-(*E*)-2-enal, oct-1-en-3-ol, octane-2,3-dione, and alka-2,4-dienals. Other studies have also shown such volatile compounds from lipid oxidation to increase with warming over of different meats (32, 37). Thus, mild cooking of meats, to temperatures of 70–80 °C, as performed in this experiment, leads to the disruption of muscle membrane structure and facilitates the interaction of lipid oxidation catalysts with unsaturated fatty acids, resulting in the generation of free radicals and the propagation of WOF (38). At higher temperatures (above 100 °C), WOF development has been reported to be inhibited (39).

The volatile compound profiles shown by the cooked samples from both types of meat were quite similar, but several quantitative differences were detected. After refrigeration, cooked samples from Iberian pigs showed increases for lipid-derived volatiles possibly related to rancidity and WOF, such as octane-2,3-dione, hexanal, and oct-1-en-3-ol, significantly higher (Δ octane-2,3-dione = +106.90 AU; Δ hexanal = +338.49 AU; Δ oct-1-en-3-ol = +75.83 AU) than those shown by samples from lean pigs (Δ octane-2,3-dione = +24.61 AU; Δ hexanal = +199.67 AU; Δ oct-1-en-3-ol = +27.20 AU). After the refrigerated storage, the content of hexanal (589.69 vs 424.62 AU; $p > 0.05$), octane-2,3-dione (130.62 vs 42.63 AU; $p < 0.05$), oct-1-en-3-ol (95.37 vs 45.38 AU; $p < 0.05$), and some other volatiles were also higher in samples from Iberian pigs. Heme iron is thought to be the most important muscle prooxidant, because it is able to favor lipid oxidation and WOF development in cooked meat during chilled storage (38). This catalyst effect is even higher after meat cooking due to the denaturation of myoglobin followed by the iron release from the heme complex and the release and degradation of the heme molecule (40). As compared to muscles from lean pigs, muscles from Iberian pigs presented a significantly ($p < 0.05$) higher content of heme iron: 7.03 ± 1.40 vs 4.04 ± 0.78 mg/kg, respectively. It has been suggested that genetic and livestock production factors could affect the concentration of heme iron in muscles explaining in part the results obtained (10). In relation to sensory perception of WOF, there is no clear information about the specific molecules implicated in the undesirable perception, although it is thought that the equilibrium between unpleasant rancid aroma notes from PUFA-derived volatiles and meaty aroma notes plays an important role (22). During refrigeration, the evolution of oleic acid-derived volatiles such as octanal, nonanal, and octan-1-ol, which aromatic notes have been described as desirable in cooked meat and other meat products (34), is completely different between types of meat. The content of octanal and nonanal decreased in samples from lean pigs while large increases were found for meat from Iberian pigs (Table 2). The increase of octan-1-ol during refrigeration was significantly higher in samples from Iberian pigs (Δ octan-1-ol = +36 AU) than in those from lean pigs (Δ octan-1-ol = +16 AU) ($p < 0.05$). The concentration of these compounds at day 10 of refrigerated storage was also higher, but not to a significant extent, in samples from Iberian pigs (octanal: 18.97 vs 12.74 AU; $p > 0.05$; nonanal: 90.00 vs 45.80 AU; $p > 0.05$; octan-1-ol: 43.41 vs 22.77 AU; $p > 0.05$). In fact, during refrigeration, the increase in the concentration of hexanal, as compared to that of nonanal (ratio Δ hexanal/ Δ nonanal), was

higher in cooked samples from lean pigs (184.8 vs 9.1; $p < 0.05$), and the ratio between the amount of hexanal and the amount of nonanal (ratio hexanal/nonanal) after the refrigeration period also resulted higher in cooked samples from lean pigs (9.3 vs 6.5; $p > 0.05$). Moreover, refrigerated cooked samples from lean pigs presented after refrigeration a higher content of linoleic acid-derived volatiles such as deca-(*E,E*)-2,4-dienal (4.80 vs 2.74 AU; $p > 0.05$) and deca-(*E,Z*)-2,4-dienal (5.11 vs 2.14 AU; $p > 0.05$) with very low odor threshold values (0.2 ppb) as compared to those for the aforementioned hexanal (threshold value: 58 ppb) and nonanal (threshold value: 13 ppb) (41). These data indicate that samples from lean pigs tended to present a higher content of volatile compounds such as 2,4-alkadienals (33), probably related to WOF perception.

ACKNOWLEDGMENT

We are grateful to AECERIBER and Miss Elena Diéguez and Mr. Pedro Cañuelo for providing us the Iberian pigs. We also thank Miss Inmaculada Linares and Miss Ana Galaz for their excellent technical assistance.

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Received for review December 13, 2002. Revised manuscript received March 10, 2003. Accepted March 25, 2003. The authors acknowledge the MAPYA and the Junta de Extremadura for granting the present research included in the project “Establecimiento de parámetros predictores de la calidad de carne fresca de cerdo Ibérico y alentejano destinada a la elaboración de productos cárnicos curados y al consumo en fresco” (IPR99D001).

JF026218H