A Comparative Study of Cooked Ham Volatile Compounds of Large White and Iberian Pig Breeds

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Abstract— **The Iberian pig (IB) is a breed native of some areas of the Iberian Peninsula (Spain and Portugal) with a high culinary interest due to its marbling, fat composition and antioxidant status. The aim of this work was to compare the effect of the pig breed, IB or Large White (LW), on cooked ham aroma by different extraction techniques: a) Simultaneous distillation–extraction method (SDE) and subsequent Gas Chromatography Olfactometry (GC-O) and; b)Solid Phase Microextraction (SPME) or Stir-Bar-Sorptive-Extraction (SBSE) and posterior Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Cooked ham samples (5 per breed) were prepared by injecting 20 % of a brine containing salt, nitrite, ascorbate, dextrose and tri-polyphosphates, curing for 8 days and cooked at 70 ºC to a core temperature of 68ºC. The Nasal Impact Frequency was used as the GC-O method to obtain aromagrams from both distilled LW and IB cooked hams. The analysis by SPME-GC-MS and SBSE-GC-MS showed that lipid oxidative volatiles such as aldehydes, ketones and acids were predominant in both breeds. However, some breed differences were found linked to non-lipid derived compounds that seem to be related to the dietary regimes. In addition the aromagrams showed relevant differences between the two breeds both in variety and intensity of the odour-active zones. We conclude that cooked ham aroma not came only from lipid oxidation and main differences between breeds were due to the intensity of the most odorant active volatiles.**

Keywords— **cooked ham, Iberian pig, volatile compounds.**

I. INTRODUCTION

Cooked ham is one of the most popular ready-to-eat meat products. A good aroma profile with absence of Warmed-Over Flavour (WOF) notes is an important parameter in consumers' choice. The Iberian pig (IB) is a breed reared in some areas of the Iberian Peninsula (Spain and Portugal). The IB meat has specific features such as marbling, fat composition, antioxidant status likely link to a slow growth rate and unique feeding practices that include significant amount of acorns. The effect of pig breed on meat volatiles has been previously studied in *longissimus dorsi* muscle [1] and Spanish meat products such as dry-cured loins [2] and Iberian ham [3]. Some studies have also been reported on flavour profiles of cooked ham [4], however, only limited data is available. Solid-Phase-Micro-Extraction (SPME) is commonly used for analysis of meat volatiles because is a high sensitive and rapid extraction technique [5]. Complementary to SPME the novel Stir-Bar-Sorptive-Extraction (SBSE) has a higher performance in extraction of water soluble volatiles [6]. Moreover, cooked ham flavour may be further analysed by Gas-Chromatography-Olfactometry (GC-O) nasal impact frequency technique [4], to identify the most active odour zones. The aim of this research was to identify differences between the cooked ham profiles of two different pig breeds (IB and LW pig) by combining the use of two non-invasive techniques (SPME and SBSE) and characterizing the most active odour zones by GC-O.

II. MATERIALS AND METHODS

A. Sample preparation

Pork hams were selected from 10 gilt carcasses (n=5 Large White and n=5 Iberian genetic lines) with a pH measured on the s*emimembranosus* muscle at 45 min *post mortem* of above 6.0 and at 24h *post mortem* (pH_{24}) lower than 6.2. The hams were trimmed and subcutaneous fat, connective tissue and rind were

removed. Brine was manually injected into the pork legs to increase their weight by 20% and including 0.3% pentasodium tripolyphosphate, 0.05% sodium ascorbate, 1.8% NaCl and 0.01% sodium nitrite after injection. Hams were then placed in a vacuum tumbler at 4 ºC at a pressure of 200mbar. The tumbling schedule was set for the hams to rotate a total of 2000 times at 14 rpm. Then, the hams were packaged in bags (CN330, Sealed Air, Italy) and matured at 2 ºC for 8 days. After maturation, hams were packaged and cooked in a steam oven to an internal temperature of 68 °C using an external temperature of 70 °C.

B. SPME sampling

A representative minced sample of each ham (about 2 g) was weighed in a 20 mL glass vial and capped with a Silicone-PTFE septum. The closed vial was then put in a CTC SPME AutoSampler (CTC Analytics AG, Zwingen, Switzerland). A Carboxen/PDMS/DVB (Supelco, Bellefonte, PA) 30/50 µm fiber was exposed to the headspace of the vial for 30 min at 40°C to adsorb the volatile compounds and then desorbed in the injection port of a chromatograph for 10 min at 250ºC in splitless mode.

C. SBSE sampling

Aliquots of 5 mL of cooked ham juice as a result of the exudation process were collected and placed in 50 ml vials. The samples were extracted for 90 min at 1400 rpm using 10 mm x 0.5 mm PDMS phase thickness Twister stir bars (GERSTEL GmbH & Co.KG, Mülheim an der Ruhr, Germany). Afterwards the Twister stir bars were rinsed with distilled water, dried with a clean tissue and transferred to desorption tubes in a MPS Gerstel Autosampler System.

D. SDE Sampling

Simultaneous distillation–extraction method, SDE, was performed using a Lickens-Nickerson apparatus. 300 g of minced ham plus 300 g of a 20 % solution of NaCl in deionized water were distilled and extracted with 150 ml of dichloromethane for 3h. The solvent-phase extract was concentrated to 0.2 ml in a nitrogen stream at 40°C using a Turbovap system (Biotage AB, Uppsala, Sweden).

E.GC-MS

All analyses were performed with an Agilent 6890 gas chromatograph coupled to 5973N mass selective detector from Agilent. The separation of volatiles was performed using a Supelcowax 10 (30m x 0.25 mm x 0.25 µm) capillary column and helium was used as carrier gas. Retention Time Locking was used setting a limonene peak at 6.7 min. Volatile compounds were identified comparing their mass spectra with those of known compounds from library databases such as proprietary libraries, the NIST08, the Willey 275 or from data previously reported in the literature. Retention times were also compared with data from proprietary databases.

F. GC/O- FID-nasal impact frequency

GC/O-FID-nasal impact frequency was used to obtain aromagrams from both (LW and IB) distilled cooked hams. All the GCO-FID-nasal impact frequency analysis were performed using an Agilent 6890 gas chromatograph equipped with a flame ionitzation detector (FID) and a Gerstel ODP2 sniffing olfactory detection port. The sniffing panel was constituted by 8 selected and non-trained panellists. All of them carried out two sessions per each type of ham. During analysis, panellists were asked to record the length of the olfactive impression and a description of each perceived note. All panellist records were summed up and their descriptions categorized in eight olfactory classes: meat products, earthy-toasted-caramel, fatty-dairy products, fruity-floral, green-vegetable-herbaceous, sulphurous, plastic-chemical solvent-metallic, miscellaneous [7].

G. Statistical analysis

Data was analysed by means of ANOVA (SAS, 2007). Genetic breed was included in the model as main effect. Mean comparisons were carried out using the Tukey test ($p<0.05$).

III. RESULTS

A. SPME results

Table 1. Number (n) of Volatile Organic Compounds (VOCs) identified in cooked ham by SPME. Least squares means, level of significance (P) and RMSE (Root Mean Standard Error) are shown for each volatile compound of cooked ham found in Guillard et al. [4].

	LW		IB			
	n	Area signal	n	Area signal	RMSE	P
Aldehyds	16		15			
Methional		130895.4[a]		47098.4[b]	4868.8	<0.0001
Cinnamaldehyde		69707.8[a]		27385.4[b]	7882.9	<0.0001
Alcohols	13		9			
Acids	8		8			
3-Methyl butanoic acid		973253.6		945283.4	90106.4	0.6367
Ketones	8		13			
Esters	8		10			
Butanoic acid ethyl ester		1074840.4[a]		591052.0[b]	24531.4	<0.0001
Terpens	12		14			
1,8-Cineole		185853.2[a]		405849.4[b]	22329.6	<0.0001
Linalool		448888.4[a]		161148.8[b]	22593.0	<0.0001
Menthol		n.d.		n.d.		
L-carvone		n.d.		n.d.		
Thymol		119219.0[a]		73838.6[b]	8496.3	<0.0001
Eugenol		n.d.		n.d.		
Furans	2		3			
Lactones	11		9			
Nitrogen + Sulphur	6		5			
Dimethyl disulfide		n.d.		n.d.		
Allyl isothiocyanate		n.d.		n.d.		
Others	5		3			

a,b Least squares means with different superscripts within the same row, differ significantly ($p < 0.05$); n.d. not detected

B. SBSE RESULTS

Table 2. . Least squares means, level of significance (p) and RMSE (Root Mean Standard Error) for every terpen compound of cooked ham found in Guillard et al. [4].

	LW	IB		
	Area signal	Area signal	RMSE	P
Cinnamaldehyde	54045.0[b]	77233.0[a]	7760.5	0.0015
1,8-Cineole	203614.2[a]	115547.2[b]	43209.3	0.0122
Linalool	82504.4[b]	133992.0[a]	18353.7	0.0022
Menthol	331332.8[a]	39364.2[b]	71392.6	0.0002
L-carvone	n.d.	n.d.		
Thymol	n.d.	n.d.		
Eugenol	n.d.	n.d.		

a,b Least squares means with different superscripts within a row, differ significantly ($p < 0.05$); n.d. not detected.

C. Analysis of odour active compounds

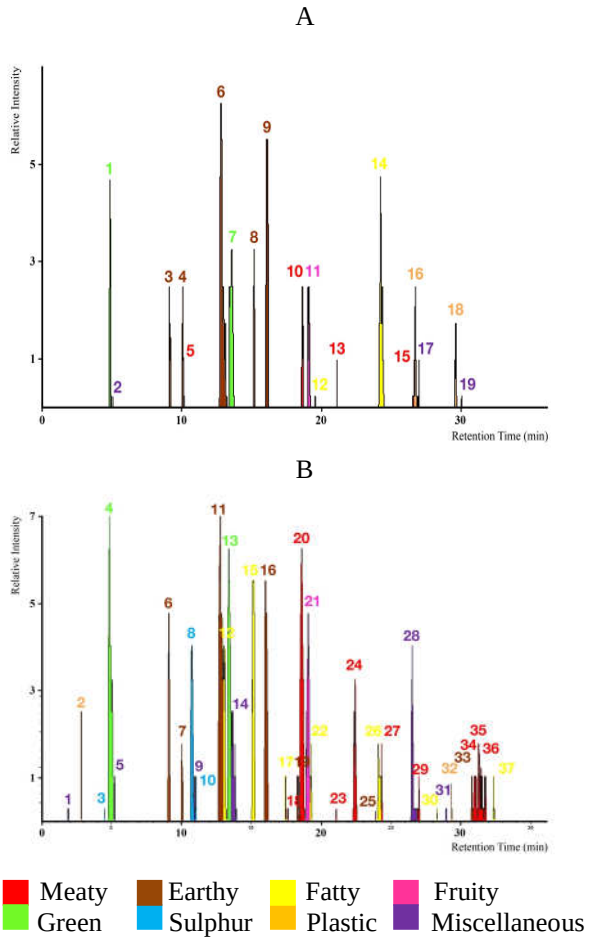

Figure 1. Aromagrams for the LW (A) and the IB (B) samples obtained by GC-O-FID/nasal impact frequency of 16 individual panellists per each breed.

IV. DISCUSSION

The same number of compounds (89 per breed) were identified by SPME analysis (Table 1) in both breeds. The aldehyde family was the largest group identified in both LW and IB, with 16 and 15 volatiles accounting for 34% and 38% of the total

chromatographic area (CA) respectively Aldehydes, acids, ketones and alcohols derived from lipid oxidation [2] [8] were predominant in both breeds, representing almost 75 % of the CA (72.3 % in LW and 76.4% in IB).

Particular emphasis was given to the volatile compounds of cooked ham found by Guillard and co-workers [4] that were identified and quantified for both breeds by SPME (Table 1). In addition, the terpen compounds were also analyzed by SBSE (Table 2), encouraged by the robust response observed by others using this extraction technique [6]. The results showed significant differences ($P<0.05$) between the two breeds for six compounds by SPME and four by SBSE. Most of these compounds were terpens and they are thought to be related to the diet. Terpens seems to derive from classical IB diets and tend to accumulate in IB fat stores [2] .

On the other hand, clear differences in the number of odour-active zones were found between the two breeds. In LW 19 odour clusters were defined whereas in IB the number was almost doubled to 37, indicating a higher complexity and richness of the latter. The main odour active zones in LW were also identified in the IB aromagram but they differed significantly in their odour intensity. Meat products and earthy-toasted-caramel odour classes accounted for almost half of the total odour active zones (47% % in LW and 44% in IB) while lipid oxidation related odour families (fatty, fruity and green) were 26% and 27% respectively.

V.CONCLUSIONS

Volatile compounds derived from lipid oxidation have a predominant role in cooked ham aroma being the most identified in both breeds. However, significant differences are reported in some non-lipid derived volatile compounds that seem related to differences in the dietary regimes of the two breeds. In addition there are more odour active zones in both aromagrams not related to lipid oxidation that affect the overall aroma. Based on intensity and variety of odour-active zones the IB cooked ham has a richer profile than the LW what explains its world renowned flavour uniqueness. Further work will be carried out to relate the volatile differences to the odour active zones.

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