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Relationship between sensory attributes and volatile compounds qualifying dry-cured hams

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ABSTRACT

This work studies the relationship between 45 volatile compounds and 17 sensory attributes (13 flavour perceptions) of dry-cured hams. Volatile compounds were quantified by SPME-GC while the sensory assessment was carried out by 13 panellists. GC-sniffing was used to determine the odour impact zones of the chromatogram. The odour thresholds of the volatile compounds and their sensory characterisation were determined by dilution analysis. Six sensory attributes (acorn odour and flavour, rancid odour, rancid taste, fat rancid and fat pungent flavours) were explained by regression equations (adjusted –$R^2 \geq 0.70$) based on ten compounds: benzaldehyde, 2-heptanone, hexanal, hexanol, limonene, 3-methylbutanal, 3-methylbutanol, 2-nonanone, octanol, pentanol. Acorn flavour attribute was successfully emulated by mixing the volatile compounds selected by the equation. Its odour was evaluated by assessors that gave a sensory description that matches with the target. All the procedures performed for the elucidation of volatile-attribute relations showed a basic agreement in their results.

KEYWORDS:

Dry-cured hams; Flavour; Volatiles; Sensory assessment; Chemometrics

INTRODUCTION

Aroma sensory attributes are descriptions of a commodity from the sensory assessors' viewpoint. Thus, each foodstuff sensory panel produces its own list of attributes that is the result of a consensus between the food sensory perceptions and their intensities after extensive training and assessment work (Deibler & Delwiche, 2004; Piggot, 1988); a key aspect of any hypothetical consensus being to avoid sensory attributes that overlap (García-González et al., 2006). This redundancy cannot be easily resolved if the terms definition is not provided with a frame of reference but it becomes readily grasped by all the assessors when chemical compounds are provided. It explains why relating aroma sensory attributes and volatile compounds sometimes represents a challenge.

Furthermore, aroma perception is not induced by a simple stimulus but it is often a complex process in which each aroma is characterized by distinct compositions of a certain number of key volatiles (Aparicio, Morales & Alonso, 1996). A good numerical relationship (e.g. $R^2 > 0.75$) between volatile compounds and sensory attributes does not automatically imply that the relative amount of a compound quantified in the food has a sensory impact on the food since only those compounds in concentrations higher than their odour threshold are odour-active (Buettner & Schieberle, 2000a; Carrapiso, Jurado, Timón & García, 2002a; Grosh, 1994; Luna, Morales & Aparicio-Ruiz, 2006b). Little research has been dedicated to this field in fat products (Buscailhon et al., 1994; Carrapiso, Ventanas & García, 2002b; Morales & Tsimidou, 2000), the statistical sensory wheel being the most available approach in the case of virgin olive oil (Aparicio-Ruiz et al., 1996). The sensory evaluation of dry cured ham, being a solid food, is even more difficult to deal with, since the strength of the aroma perception is affected by the release of volatile compounds during mastication. To explain the sensory attributes from the flavours release during eating it is necessary to assume that only those volatiles whose concentrations in the food material exceed their odour threshold can be selected (Buettner & Schieberle, 2000a) but keeping in mind that the perceived intensity of an individual volatile is almost always higher than the sum of the intensities of the volatiles that constitute the natural mixture that defines a particular odour (Laing, Panhuber, Willcox & Pittman, 1984). Several studies have been independently conducted on ham sensory attributes (Dirinck, Van Opstaele & Vandendriessche, 1997; Pastorelli et al., 2003; Ruiz, García, Muriel, Andrés & Ventanas, 2002) and on the volatiles of dry-cured hams (Andrés, Cava & Ruiz, 2002; Luna, Aparicio-Ruiz & García-González, 2006a; Ruiz, Ventanas, Cava, Andrés & García, 1999; Sánchez-Peña, Luna, García-González & Aparicio-Ruiz, 2005; Timón, Ventanas, Carrapiso, Jurado & García, 2001). Authors agree that the aroma is perhaps the most important quality parameter of hams, and it is due to the presence of many volatile compounds, most of them produced by chemical and enzymatic mechanisms during the post-mortem process (Flores, Grimm, Toldrá & Spanier, 1997); the main biochemical reactions being lipolysis and proteolysis (Toldrá, 1998). But the sensory quality depends not only of the curing process but also on factors such as the breed, age and feeding of pigs. Furthermore, ham samples are heterogeneous and, in consequence, the variability of the analytical results is related with the amounts of muscles and subcutaneous fat in every sample (García-González, Luna, Morales & Aparicio-Ruiz, 2005; Luna et al., 2006a). It is well-established that chemical changes occurring in different muscles during the ripening of hams influence the ham aroma and flavour (Ruiz, Ventanas, Cava, Timón & García, 1998). It is only recently that the contribution of the most important parts of the hams (subcutaneous fat, *biceps femoris*, *semimembranosus* and *semitendinosus* muscles) to their aroma and flavour has begun to be elucidated (Luna et al., 2006a; Monin et al., 1997; Sánchez-Peña et al., 2005). The aim of this work is to determine the relationship between 13 odour and flavour sensory attributes and 45 volatile compounds in 41 hams from diverse geographical origins, maturation times, pig feeding, etc. Mathematical procedures have been used as a filter system to reduce the set of attributes and volatiles to those with high probabilities of being related. Odour threshold and GC-sniffing/olfactometry (henceforth, GC-O) complete the filtering process prior to formulating sensory attributes with volatile compounds.

MATERIALS AND METHODS

<u>Samples</u>:

A total of 41 hams from several geographical parts of Spain and France were used for this study. These different samples somewhat reproduce the actual variability in dry-cured ham features that the consumer can find in the market, and allow enough scope to study the influence of different sensory traits on their acceptability. Thirty were white hams from several crossbreeds – (French Landrace × Large White) × (Piétrain × Large White), (Duroc or Landrace) × (Landrace or Large white or Landrace × Large white) and Landrace × Large White crossbred sows mated with several genetic types – eight were Iberian hams – Iberian × Duroc-Jersey with a minimum of 50% Iberian pig-, and three were Gasconne and Basque hams although crossed with Large White and other genetic types. The ripening time varied from one ham to another although they can be clustered into various groups, French hams were cured for less than 12 months with the exception of the hams from Bayonne. Spanish white hams were cured for a period between 10 and 18 months while Iberian hams were cured for more than 18 months. All the hams were processed by local manufacturers using the traditional method of each geographical origin (Flores & Toldrá, 1993; Sabio, Vidal-Aragón, Bernalte & Gata, 1998). The samples were stored in vacuum plastic bags at –5°C until they were required for the sensory and chemical studies.

<u>Sensory analyses</u>:

Twenty-seven traits related to sensory characteristics of dry-cured hams (Table 1) were evaluated by the quantitative-descriptive analysis method (Stone, 1992). The traits were grouped into appearance (red colour, homogeneous red colour, subcutaneous fat, fat colour, heterogeneous fat colour, intramuscular fat), texture (crust, dry, melting, fibrous, elastic, sticky, doughty, fat greasy), odour (cured ham, rancid, acorn, mouldy, smoke), taste (salty, rancid) and flavour (raw meat, cured ham, acorn, fat rancid, fat pungent, pungent). Sensory attributes were assessed with a 9-points structured scale. The total number of assessors was 13, trained during 10 training sessions, although not all of them evaluated the whole set of samples. The minimum number of assessors per sample was ten. All the samples, slices of 1.5 mm thickness with 1 cm of subcutaneous fat, were evaluated at 20–22°C in sensory panel rooms equipped with fluorescent lighting. About 50 ml of water and 20 g of unsalted bread were provided to assessors between successive ham samples. Samples were evaluated in eight sessions. The order of the sample presentation was randomised (García-González et al., 2006).

Reagents

Four chemical compounds (2-propanone, 2-ethyl furane, 2,3-butanodione and isobutyric acid) were identified by mass-spectrometry. All the other chemical compounds, described in Table 2, were purchased from Fluka–Sigma– Aldrich (St. Louis, MO). 4-methyl-2-pentanol was used as external standard.

Gas-chromatography (SPME-GC)

A sample of approximately 350 g of the part located along and behind the femur was collected from each one of the hams, composed essentially of subcutaneous fat and *biceps femoris*, *semimembranosus* and *semitendinosus* muscles. Three grams representative of the ham portion, previously minced to increase the interface between the ham and the vapour phase during the concentration step, were placed into 20 ml glass vials tightly capped with a PTFE septum and left for 10 min at 40 °C to allow equilibration of the volatiles in the headspace. The septum covering each vial was then pierced with a solid-phase microextraction (SPME) needle and a Carboxen/PDMS/DVB fiber (Supelco, Bellefonte, PA) exposed to the headspace for 180 min (Gianelli, Flores & Toldrá, 2002). When the process was completed, the fiber was inserted into the injector port of the GC for 5 min at 260 °C using the splitless mode. The temperature and time were automatically controlled by a Combipal (CTC Analytics AG, Zwingen, Switzerland) using the Workstation v.5.5.2 (Varian, Walnut Creek, CA) software. The volatile compounds were analysed using a DB-WAX column (J&W Scientific, Folsom, CA; 60 m × 0.25 mm id × 0.25 μm film thickness) installed on a Varian 3900 gas chromatograph (Varian, Walnut Creek, CA) with a flame ionization detector. The carrier gas was hydrogen.

The oven temperature was held at 40 °C for 4 min and programmed to rise 1 °C/min to a temperature of 91 °C, and then to rise 10 °C/min to a final temperature of 201 °C, where it was held for 10 min. Each sample was analysed in triplicate. The identification of volatile compounds by GC–MS was carried out on a GC8000 (Carlo Erba, Milano, Italy) gas chromatograph coupled to a MSD-800 (Fisons, Manchester, UK) mass-selective detector. Column and analytical conditions were identical to those described for gas-chromatography with the exception of the carrier gas that was helium (head pressure 15 psi). Volatile compounds were tentatively identified by the library MassLab v.1.3. (VG MassLab, Altrincham, UK). The content of each volatile compound was calculated from the FID area and expressed as area units. A solution of 4-methyl-2-pentanol (1.2 mg/kg) was used as standard in order to standardise the results of all the analyses. Thus, the amount (mg/kg) of each volatile compound was computed by relating the peak area of the volatile compound to the area of the standard and taking into account the sample weight and the response factor of each volatile. Table 2 shows the volatile compounds quantified in the samples.

Response factors

Standard solutions (Table 2) were prepared using fully deodorised edible oil as matrix. Concentrations in the range 0.1–5.0 μg/g, with the exception of 3-methylbutanol whose range was 0.5–20 mg/kg, were analysed under the conditions described above. The absolute response factors of the standard compounds were calculated as the slopes of the linear regressions obtained from the ratio of total peak area as a function of concentration. Relative response factors were obtained as the ratio of the absolute response factor of each compound to that of the internal standard (4-methyl-2-pentanol).

GC-olfactometry (GC-O)

GC-olfactometry (GC-O) was applied to assess the aroma notes corresponding to ham volatile compounds. The effluent of the GC column was split 1–10 to the detector and the sniffing port, respectively. Three assessors with a large experience of odour recognition and of sniffing virgin olive oils carried out the evaluation. Elution of each aroma compound through the sniffing port was recorded by writing the beginning and end of the entire sensation of any odorant as well as its odour properties. The final aromagram (sensory description versus t_R) is the result of merging the information from the individual analyses of the assessors.

Odour threshold of volatile compounds

Fully deodorised edible oil was the matrix for the assessment of the odour threshold values; the absence of volatile compounds in the matrix was checked by the SPME-GC procedure described above. The sensory assessment was carried out in the test room used for evaluating sensory characteristics. The same assessors who carried out the GC-O were in charge of the detection of the volatile thresholds. Three samples were presented to the assessors following the triangle test whose results were statistically analysed. 15 ml of each sample was kept in standardised glasses at 29 °C±2 °C for 15 min and then tested. The samples were diluted until none of the assessors were able to classify samples by odour intensity. The odour activity values (OAVs), or ratio of the concentration to the odour threshold (Aparicio-Ruiz & Morales, 1998; Rothe & Thomas, 1963), of the volatile compounds were calculated to determine their sensory significance. Thus, the concentration of each volatile found in the ham samples was divided by its corresponding odour threshold value previously determined as described above.

Statistical analysis

Univariate and multivariate algorithms have been used by means of Statistica (Statsoft, 2001). Correlation was used to determine the relationship between the sensory attributes while the first screening of the relationship between volatiles and sensory attributes was carried out by principal component analysis and canonical correlation. Stepwise ridge linear regression analysis (RR) was applied to explain the sensory attributes by means of volatile compounds. Later the selected compounds were checked for odour activity values higher than 1 in order to ensure that they actually contribute to the sensory attribute. The criterion for the selection of variables (volatiles) was the strictest, the minimum F-to-enter value was selected according to $F(1, n–k–1)$, n being the number of samples and k the number of selected volatiles, for a F-distribution of 0.95. Tolerance was fixed at 10^3.

RESULTS AND DISCUSSION

Relating flavour attributes and volatile compounds represents a challenge whatever the food product studied. The concentration of a compound in a food is not necessarily a measure of its sensory impact as it depends on its sensory threshold (Carrapiso et al., 2002b; Carrapiso et al., 2002a). Only a small percentage of volatiles are odour active and the sensory characteristics of their odour can change with their concentration and possible synergy with other compounds from the matrix (Aparicio-Ruiz & Morales, 1998). In addition, overlaps between sensory attributes have been described in different fat products (Aparicio-Ruiz & Morales, 1995;

García-González et al., 2006), and there is also a potential source of variation related to the training of the assessors. Thus, we have followed a series of steps before attempting to tentatively explain the flavour attributes of the hams by means of the volatile compounds: (a) multivariate and univariate studies of the dataset of sensory attributes to determine possible mathematical overlaps between the descriptors; (b) relationship between sensory attributes and volatile compounds by principal components and regression procedures; (c) relationship between volatiles and sensory attributes analysing the muscles and subcutaneous fat of the hams independently.

Analysing the sensory dataset

Table 1 shows the sensory attributes evaluated by the assessors and their mean intensities when evaluating the two main kinds of hams from the breeding viewpoint. 17 out of 27 sensory attributes were significantly different ($p < 0.05$) when classifying the hams by their breeds (white hams vs. Iberian hams). The intensities of almost all the flavour attributes (the exception was cured ham flavour) and many of colour and odour attributes distinguished these kinds of hams. However, half of the texture attributes did not show significant differences despite the Iberian hams being cured for a longer time. Correlation analysis of the sensory attributes showed that the texture attributes were poorly correlated between them with the exception of the fibrous texture and melting attributes ($R = 0.78$), which means the perceptions are quite diverse. The highest correlations, on the contrary, were detected between the attributes related to the rancid perception. Thus, fat rancid flavour was highly correlated with rancid odour (0.89) and rancid taste (0.86), fat pungent flavour with rancid odour (0.85), and rancid odour with fat colour (0.81). The correlations between the pairs of perceptions of the same attribute (e.g. odour and flavour) were not high with the exception of rancid (0.83) and cured hams (0.72). In order to analyse the whole sensory assessment, the multivariate statistical procedure of principal components analysis (PCA) was applied to odour and flavour sensory attributes; colour and texture attributes were projected on the model (Statsoft, 2001) to avoid them disturbing the relation between volatiles and odour and flavour attributes that is the aim of this work. Figure 1 shows the sensory attributes of the first quadrant (Q1) are raw meat flavour, homogeneous red colour and doughy and sticky texture attributes these texture attributes refer to substances with a soft texture and their location is opposed to the crust attribute (A7) and partially opposed to the sectors where cured ham and rancid attributes are situated (third and fourth quadrants). The second quadrant contains the salty taste perception and texture attributes (fibrous, melting, dryness, elastic) related to the process of ham curing. These attributes also appear together in previous studies on the acceptability of dry-cured hams (Ruiz et al., 2002). The third quadrant could be qualified by the cured ham sensory perception while the fourth quadrant contains the attributes related to rancid and acorn sensory perceptions. Since the rancid attribute increases through lipid oxidation, attributes related to fat description (A3, A6, A14) were also placed in this quadrant. No disagreement seems to be detected in the locations of the sensory attributes by the first two principal components that explain 55.78% of the total variance. Factor 1 (38.24% of explained variance) can be labelled as the ham ‘‘rancid perception”, Factor 2 (17.54%) can be qualified as the ‘‘cured ham perception” while Factor 3 (12.67%) can be labelled as the ‘‘acorn and smoke” odour and Factor 4 (8.54%) explains the acorn flavour.

Analysing the volatile dataset

Table 2 shows the chemical compounds quantified in the hams as well as their mean concentrations in Iberian and non-Iberian hams from several pig breeds and feeding systems and maturation time. The information from the volatiles concentrations shows that twenty compounds (coded as 5-6, 13, 17–18, 21, 24–27, 29–31, 34, 36–38, 40–42) distinguished non-Iberian from Iberian hams ($p < 0.05$), applying the Brown-Forsythe test (Brown & Forsythe, 1974) in agreement with previous studies (Sánchez-Peña et al., 2005). The seventh column of Table 2 shows the odour thresholds of volatiles assessed by the panellists. Comparing the mean concentrations of the volatiles and their odour thresholds, 25 out of 45 volatiles (coded as 3, 6, 12, 15, 17, 21–28, 30–38, 40–42) can contribute to dry-cured aroma because their odour threshold is lower than their mean concentration. Sixteen of these volatiles were aldehydes or alcohols. Although alcohols have been considered unimportant due to their relatively higher threshold compared with other carbonyl compounds (e.g. aldehydes), their flavour becomes stronger as their carbon chain increases (Shahidi, Rubin & D’Souza, 1986) and, in this case, the alcohols varied from C4 to C8. In order to know the individual contribution of the volatiles to odour and flavour sensory attributes, a representative group of all the ham samples was analysed by trained assessors using GC-O technique (Flores et al., 1997; Morales, Luna & Aparicio-Ruiz, 2005). However, sometimes the retention times of two sequential compounds in the chromatogram are so close that the assessor perceives them as a single odour. In this case, the individual sensory characterization of the volatiles was taken from the information reported by the assessors in their odour threshold evaluation at the most similar concentration detected in the evaluated hams. According to the information reported by the assessors (Table 2), the most frequent sensory attributes were fruity, green, sweet, pungent/astringent, woody, spicy, lemon, and some undesirable attributes (fishy, iron, rancid, unpleasant). No peak was qualified by the characteristic of cured-ham odour meaning that the global flavour perception of dry-cured ham is produced by the action of several volatile compounds which interact producing the final flavour. Analysing the main series of volatile compounds with OAV≥1, the alcohols (butanol, 3-methylbutanol, pentanol, hexanol, 2-heptanol, 1-octen-3-ol, octanol) contribute with greenish, woody, fruity and fatty sensory notes among others. C6-C9 aldehydes (hexanal, heptanal, E-2-heptenal, octanal, nonanal, E-2-nonenal) contribute with green, fatty, rancid flavours while 3-methylbutanal is characterised by fruity, acorn-like and cheesy sensory attributes. Ketones are responsible for the flavour notes floral and spicy while the rest of chemical series contributes a varied set of sensory attributes. This information about the odour threshold and sensory characteristic of volatiles also revealed which volatile compounds, distinguishing Iberian vs. non-Iberian hams, contribute to their respective aromas; they are coded as 6, 17, 21, 24–26, 27, 30–31, 34, 36–38, 40–42. It is noticeable that fruity, woody, spicy and green fatty sensory descriptors were associated with major volatiles in both kinds of hams although their concentration was usually higher in Iberian hams. Comparing these volatiles with those identified by Carrapiso et al. (2002b), both studies agree that three odour active volatiles (3-methylbutanal, hexanal, E-2-octenal) were quantified in higher concentrations in Iberian hams while two other odour active volatiles (octen-3- one, 1-octen-3-ol) were in higher concentrations in non-Iberian hams; Flores et al. (1997) also detected high concentrations of 3-methylbutanal and hexanal in Iberian hams. The present work, however, shows that other eleven odour active volatiles (butanol, limonene, 3-methylbutanol, 2-pentyl furane, octanal, hexanol, nonanal, E-2-octenal, benzaldehyde, E-2-nonenal, octanol) were present in higher concentrations in Iberian hams. Furthermore, two compounds (2-octanone and E,E-2,4 decadienal) were detected in higher concentrations in non-Iberian hams although E,E-2,4 decadienal does not seem to contribute to the ham aroma. Table 2 also shows that nine volatile compounds (octane, α-pinene, dimethyl disulfide, 2-heptanone, heptanal, pentanol, E-2-heptanal, 2-heptanol, 2- nonanone) are also odour-active though their concentrations are similar in Iberian and non-Iberian hams. It is remarkable that none of C7 volatiles distinguishes both kinds of hams ($p > 0.05$). A study centred on the geographical origin of the white hams revealed that the concentration of 2-heptanone was higher in Spanish white hams while French white hams showed the highest concentration of pentanol.

Relationship between sensory attributes and volatile compounds

Independently of the results of GC-O analysis of the volatiles and their odour threshold values, assessed by trained panellists, a mathematical procedure was applied to explain the relationship between volatiles and sensory attributes. The objective was to elucidate possible disagreements between the information reported by assessors and the mathematical results, prior to formulating regression equations that might explain sensory attributes by means of volatile compounds. PCA was the statistical procedure selected as it pointed out differences and similarities between the sensory attributes evaluated by assessors (Figure 1). The same procedure has also been used to point out the relationships between volatile compounds and sensory attributes (Buscailhon et al., 1994). The concentration values of the volatile compounds were projected onto the plot built with the sensory information given by the sensory panel. The position of volatiles and sensory attributes determines their information content; the vicinity of a volatile to an attribute indicates a good correlation between them, from a mathematical viewpoint, but also its sensory qualification according to the sensory attributes surrounding it. The assessors, in qualifying a volatile by sniffing, actually search the sensory attributes that better explain the perceptions in their brains. The projection process follows a similar process because each volatile is placed in the PCA plot near the set of sensory attributes that better qualify it. Furthermore, the relevance of the volatiles contributing to the aroma depends on their position in the circle (Aparicio-Ruiz et al., 1996). The distance of a volatile to the circle centre points out how much it contributes to the aroma (Figure 1). Thus, the volatiles near to the centre of the circle contribute less than those placed near to the perimeter of the circle; hence, we can see the circle in terms of probability. Thus, the most noteworthy volatile compounds, in terms of basic contribution to ham flavour matrix, are the following: hexanal, 3-methylbutanal, limonene, hexanol, octanol and E-2-nonenal (Figure 1). Fig. 1 shows the volatile compounds projected onto a PCA plot of sensory attributes. The volatiles projected were those with average concentrations (mg/kg) higher than their odour thresholds (mg/kg) since they are the only volatiles that can contribute to ham aroma. A few disagreements were found in the volatiles-sensory descriptors correlation when compared with the GC-O results. These disagreements might be occur for several reasons. Firstly, the assessors were free to qualify the ham samples with their own semantic sensory notes (free-choice sensory terms), while the flavour and odour attributes of the sensory assessment were already pre-established (Table 1). Furthermore, this is a solid foodstuff and its volatile compounds are also released during mastication and perceived via retronasal. The information reported by GC-O should be understood as a tentative explanation since the volatiles released during mastication might change not only resulting in higher amounts but also in variation of the flavour profile (Buettner

& Schieberle, 2000b). Thus, the selection procedure, based on the volatile odour threshold, does not guarantee that all the volatiles contributing are selected but, on the contrary, all the volatiles selected contribute to aroma. Secondly, the disagreements might also be explained by the fact that sensory properties of the volatiles can change with concentration and that new sensory properties can be achieved if other compounds are present because of synergism, suppression and enhancement. The resulting plot (Figure 1) shows that almost all the volatiles are in Q4 quadrant. These compounds are aldehydes, excepting E-2-heptenal, and alcohols, excepting 3-methylbutanol and 1-octen-3-ol. A study of dry-cured hams (Dirinck et al., 1997; Sabio et al., 1998) also showed these compounds cluster together. From a sensory viewpoint, this quadrant has three circular segments that are characterised by the pungent, fatty-rancid and acorn sensory attributes. The location of aldehydes and alcohols in Q4 quadrant agrees with the sniffing results (Table 2) and previous studies about the odour and flavour compounds from lipids (Flores et al., 1997; Forss, 1972). Thus, E-2-octenal and pentanol are near the pungent perception while hexanal, octanol and E-2-nonenal are near the rancid perceptions and 3-methylbutanal, benzaldehyde and 2-heptanone are in the vicinity of acorn attributes. Heptanal, octanal and nonanal that were mainly characterised with a rancid odour by sniffing are not as near the rancid attributes as other volatiles are. The main disagreement is, however, hexanol which is characterised as ‘‘green” but is near the rancid attributes. This disagreement was also detected studying the relationship between attributes and volatiles of virgin olive oils (Aparicio-Ruiz et al., 1996). Excepting 3-methylbutanol and limonene that are inside Q3 quadrant, which can be qualified by the sensory perception to ‘‘cured ham”, the rest of projected volatiles are inside or in the vicinity of Q1 whose only sensory attribute is ‘‘raw meat” flavour. These compounds contribute the sensory perceptions of fruity, sweet, floral and spicy, the three first being sensory attributes of the raw meat (Reineccius, 1994). The main disagreement is the location of 1-octen-3-ol in this quadrant as it is characterised with ‘‘mushroom and earthy” sensory attributes and should be located near mouldy odour. An analysis of its concentration in the hams shows the maximum corresponds to Bayonne and Aosta hams. Their concentrations are double those in Iberian hams and 50% higher than Spanish white hams. The next step was to formulate the sensory perceptions related with odour, flavour and taste by means of a regression equation combining the information on volatile compounds. In order to diminish the selection of explicative variables (volatiles) by chance, the regression procedure (SLRA, stepwise linear regression analysis) was carried out under the strictest conditions (Ridge Regression, k = 0.01; F-to-Enter = 8.00; p-level 0.60) (Statsoft, 2001). Under these conditions, six attributes were explained by the selected volatile compounds: acorn odour and flavour, rancid odour and taste, fat rancid flavour, and fat pungent flavour, as described in Table 3. All these attributes are explained by only 9 volatile compounds – benzaldehyde, 2-heptanone, 3-methylbutanal, 3-methylbutanol, hexanal, hexanol, octanol, 2-nonanone, limonene – some of them being contributors to more than one sensory attribute. Some of these volatiles (V6, V17, V22) have been reported as potent odorants with clear contributions to dry-cured ham aroma (Carrapiso et al., 2002b). Acorn odour is explained, from a mathematical viewpoint, by the presence of benzaldehyde (V40), 3-methylbutanal (V6) and 2-heptanone (V22) which are characteristic compounds of dry-cured hams (Sánchez-Peña et al., 2005) and are responsible for woody, almond and acorn sensory perceptions as their odour thresholds are low enough. All these volatiles are located in the vicinity of acorn odour attribute (Figure 1). The mathematical explanaion of acorn flavour attribute needed the combined information of more compounds – 3-methylbutanal (V6), hexanol (V34), 2-nonanone (V35) and 3-methylbutanol (V25) – probably due to the higher number of volatile compounds that are released during mastication. Three of these compounds are relatively near this sensory attribute (Figure 1) while 3-methylbutanol is far enough away from the attribute location although it was characterised with acorn sensory note in the GC-O. Rancid taste is explained by the presence of two alcohols (V34, hexanol, and V42, octanol) and 3-methylbutanal while rancid odour is also explained by two alcohols (V28, pentanol, and V34, hexanol) and an aldehyde (V17 hexanal). The double contribution of 3-methylbutanal, to acorn and rancid attributes, had already been reported by other authors (Hinrichsen & Pedersen, 1995; Buscailhon et al., 1994). The contribution of hexanol to the fatty-rancid sensory attribute has also been described in virgin olive oils (Aparicio-Ruiz et al., 1996; Morales et al., 2005). Fat pungent flavour is explained mathematically by octanol (V42) and limonene (V24) that mixed with 3-methylbutanal (V6) explains the fat rancid flavour attribute. The main disagreement is the fact that neither E-2-octenal nor pentanol were selected by the mathematical procedure to explain the attribute fat pungent flavour despite being nearer the attribute location in the PCA plot (Figure 1) than octanol and limonene. Although the relationship between sensory attributes and volatiles does not show great disagreements, the fact that ham is not an homogeneous product and the percentages of the fat and muscle in the samples could have influenced the results was the determining factor in deciding to repeat the study with the volatiles quantified in the subcutaneous fat and *biceps femoris*, *semitendinosus* and *semimembranosus* muscles. We have used the information of the volatile compounds that were already analysed in each one of the cited muscles and the subcutaneous fat of these ham samples in previous studies (Luna et al., 2006b; Sánchez-Peña et al., 2005). First of all, the statistical procedure of canonical correlation was applied to determine the variance of the set of sensory attributes that might be explained by the set of volatile compounds of each one of the parts of the hams independently. The volatile compounds that presented an odour threshold higher than the maximum concentration in the samples were previously removed from the initial set of volatiles. The information collected showed that all the variance (100%) of odour and flavour sensory attributes was explained by the volatile compounds in a percentage that varied between 75.33% (*biceps femoris*) and 81.99% (*semitendinosus* muscle), and the redundancy of the information reported by the sensory attributes was 65.52% while the redundancy of the volatile compounds oscillated between 45.89% (*biceps femoris*) and 57.37% (subcutaneous fat). This information means, from a mathematical viewpoint, that the volatile compounds explain similar information of the sensory attributes independently of the part of ham from which they are produced although *semitendinosus* muscle and subcutaneous fat seem to be slightly more relevant. Table 4 shows the correlation detected between the sensory attributes and the volatile compounds identified and quantified in the four parts of the hams together with the maximum correlation (R-coefficient) found; the minimum having being fixed at 0.60 ($p < 0.05$). No correlation higher than 0.60 ($p < 0.05$) was found between the texture and colour attributes while the highest correlations were found with the odour, taste and flavour attributes as expected, the latter being the best explained because volatile compounds are mostly released during the mastication. Table 4 shows that 7 volatiles (3-methylbutanal, hexanal, limonene, 3-methylbutanol, hexanol, 2-nonanone and benzaldehyde) contribute to ham aroma independently of the muscle analysed. In addition, limonene, 2-nonanone and hexanal from the subcutaneous fat also contribute to aroma. The presence of limonene in the hams has been associated with the pig's diet (Buscailhon, Berdagué & Monin, 1993; Sabio et al., 1998). This may explain the highest values of limonene in the Iberian hams (Iberian pigs are fed with acorns) and hence its contribution to fat rancid and fat pungent flavour that are characteristic perceptions of the Iberian hams. 3-Methylbutanol was the most abundant alcohol, and its concentration may be due to the activity of the microorganisms on its precursor 3-methylbutanal (Muriel, Antequera, Petrón, Andrés & Ruiz, 2004) produced by Strecker degradation of amino acids during the proteolysis. This may explain why 3-methylbutanol is nearer the ‘‘cured ham” perception, which is mostly related to ham processing, than acorn sensory attributes that are qualified by 3- methylbutanal (Hinrichsen & Pedersen, 1995). In fact, 3- methylbutanal represents more than 8%, 3-methyl butanol mean 2.2%, and 2-nonanone and hexan-1-ol means approx. 0.50% of the acorn total volatile compounds (Aparicio-Ruiz, 2007). 3-methylbutanal also seems to contribute to the rancid perception when it is produced from the *semitendinosus* muscle and to acorn flavour when it comes from the *biceps femoris muscle*. These differences seem to have mathematical support only, since no plausible chemical explanation has been found yet. In general, the results of Table 4 agree with the information shown in Table 3. Once the sensory attributes were formulated with volatile compounds (Table 3), a sensory trial was carried out to check the usefulness of the equations by mixing the selected volatiles according to their coefficients. The acorn attribute was selected since it is a marker of the Iberian ham aroma though the sensory attributes with higher regression coefficients (Table 3) corresponded to rancid perception. However, rancid perception in dry-cured hams has a high number of aromatic nuances (Ruiz et al., 2002) (Table 1) that depend on the kind of ham and processing conditions. In fact, a certain level of compounds with rancid notes is needed to achieve the typical rancid flavour, but an excess in such aromatic notes leads to an overall unpleasant flavour (Andrés, Cava, Ventanas, Muriel & Ruiz, 2004). The sensory attribute was emulated by mixing the standards (3-methyl-butanal, hexanol, 3-methyl-butanol, 2-nonanone) at their mean concentrations in the Iberian hams (Table 3) multiplied by the coefficients of the mathematical equation. A fully deodorised olive oil was used as matrix of the solution. The solution was subjected to sensory analysis by the assessors who carried out the GC-O and odour threshold evaluation. Acorn, nutty, dry-meat, cheese and hay-like were the sensory notes described by the assessors to qualify the solution. This study shows the importance of the mathematical tools to elucidate the role of volatile compounds in the sensory assessment of dry-cured hams. The high complexity of the dry cured ham aroma and the heterogeneity of this solid food made necessary the use of several approaches to corroborate the different results. The agreement between the several procedures carried out proved that the most relevant attributes of dry cured ham (e.g. acorn flavour) can be explained by a limited number of volatile compounds. The chemical knowledge of these attributes may help to establish the basis for a harmonized procedure of sensory assessment of dry cured hams (García-González et al., 2006). Further studies are being carried out for a better understanding of the role of the muscles and the subcutaneous fat in the aroma of dry-cured hams.

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FIGURE CAPTIONS

Figure 1. Plot of principal component analysis of the sensory attributes and volatile compounds. Note: Codes in Tables 1 and 2.

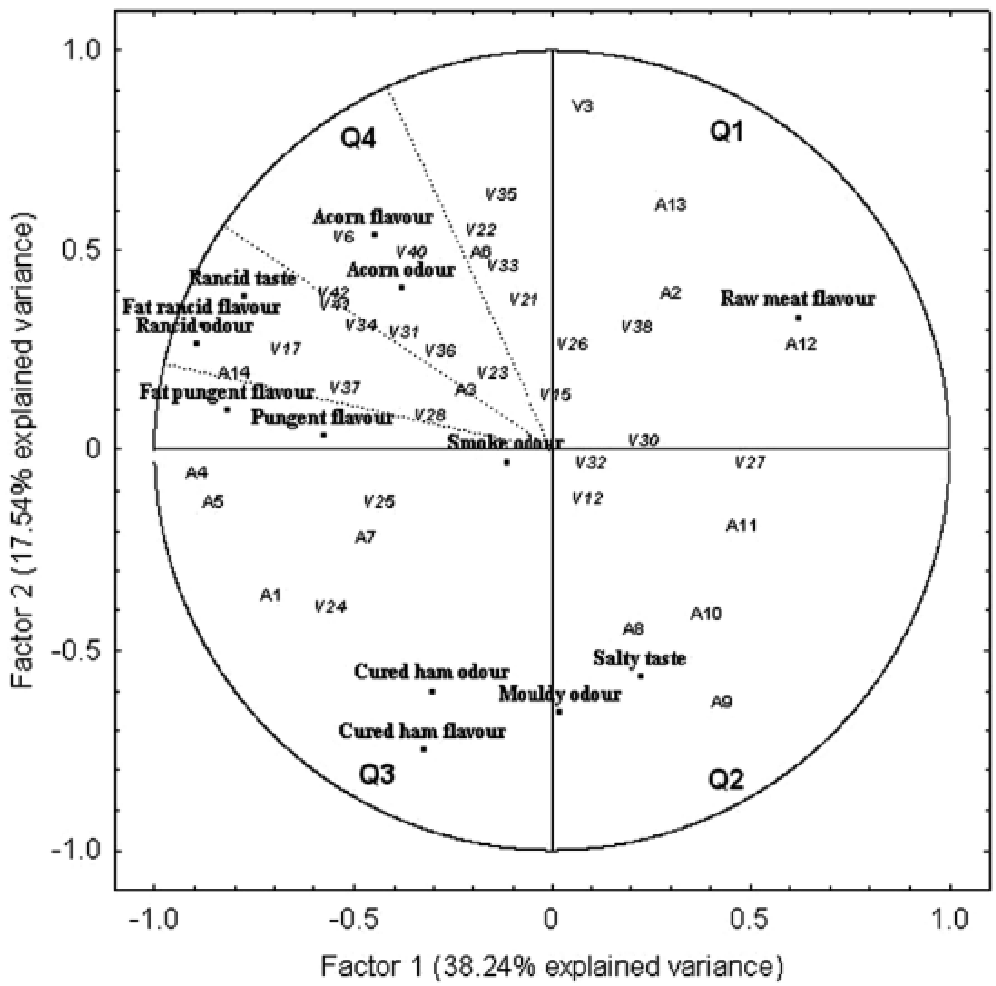
Factor 2 (17.54% explained variance)
Factor 1 (38.24% explained variance)
1.0
0.5
0
-0.5
-1.0
-1.0
-0.5
0
0.5
1.0
Q1
Q2
Q3
Q4
Acorn flavour
Acorn odour
Rancid taste
Fat rancid flavour
Rancid odour
Fat pungent flavour
Pungent flavour
Smoke odour
Raw meat flavour
Salty taste
Mouldy odour
Cured ham odour
Cured ham flavour
V3
V35
V22
V33
V21
V6
V40
V42
V41
V34
V31
V36
V17
V23
V37
V28
V26
V38
V15
V30
V32
V27
V12
V25
V24
A13
A6
A2
A12
A14
A3
A4
A5
A7
A1
A11
A10
A8
A9

FIGURE 1.

Table 1. Codes and sensory attributes evaluated in white and Iberian hams

Code	Sensory attributes	White	Iberian	p
A1	Red colour	4.73 ± 0.25	6.65 ± 0.43	*0.001*
A2	Homogeneous red colour	3.93 ± 0.18	4.38 ± 0.36	0.261
A3	Subcutaneous fat	4.65 ± 0.36	6.21 ± 0.63	0.059
A4	Fat colour	2.69 ± 0.13	5.61 ± 0.34	*0.000*
A5	Heterogeneous fat colour	2.67 ± 0.13	6.06 ± 0.24	*0.000*
A6	Intramuscular fat	4.11 ± 0.23	5.72 ± 0.67	*0.006*
A7	Crust	3.53 ± 0.19	5.69 ± 0.33	*0.000*
A8	Dried texture	2.78 ± 0.13	3.48 ± 0.29	*0.021*
A9	Melting texture	2.81 ± 0.13	3.36 ± 0.38	0.088
A10	Fibrous texture	2.65 ± 0.10	3.36 ± 0.38	0.128
A11	Elastic texture	2.10 ± 0.08	2.28 ± 0.29	0.391
A12	Sticky texture	2.14 ± 0.12	1.39 ± 0.08	*0.004*
A13	Doughy texture	2.60 ± 0.16	2.83 ± 0.23	0.503
A14	Fat greasy texture	3.15 ± 0.15	5.33 ± 0.27	*0.000*
A15	Cured ham odour	4.93 ± 0.19	5.80 ± 0.29	*0.038*
A16	Rancid odour	1.71 ± 0.07	3.18 ± 0.31	*0.000*
A17	Acorn odour	1.10 ± 0.03	1.27 ± 0.08	*0.017*
A18	Smoke odour	1.02 ± 0.01	1.01 ± 0.01	0.929
A19	Mouldy odour	2.38 ± 0.13	2.48 ± 0.16	0.716
A20	Salty taste	4.47 ± 0.16	4.39 ± 0.21	0.814
A21	Rancid taste	1.22 ± 0.03	2.43 ± 0.39	*0.000*
A22	Cured ham flavour	4.67 ± 0.16	5.15 ± 0.28	0.191
A23	Acorn flavour	1.07 ± 0.02	1.27 ± 0.06	*0.000*
A24	Raw meat flavour	1.93 ± 0.12	1.20 ± 0.09	*0.007*
A25	Pungent flavour	1.69 ± 0.05	2.07 ± 0.12	*0.002*
A26	Fat rancid flavour	1.92 ± 0.06	5.33 ± 0.53	*0.000*
A27	Fat pungent flavour	2.21 ± 0.08	4.29 ± 0.34	*0.000*

Mean intensities of the sensory evaluation and p values of the sensory attributes classifying the hams by their breeds. Note: p values < 0.05 have been written in italics.

Table 2. Codes and relative retention times of the volatile compounds quantified in the hams, mean concentrations in white and Iberian hams and p values of each volatile compound classifying the hams by their breeds (white vs. Iberian).

Code	Rt	Volatile compound	White	Iberian	*p*	Odour threshold	GC-O
V1	0.16	Hexane	0.360 ± 0.029	0.290 ± 0.032	0.199	1.50	Spicy
V2	0.17	Heptane	0.217 ± 0.033	0.230 ± 0.031	0.848	–	Sweety, alkane
V3	0.20	Octane	2.230 ± 0.419	3.000 ± 0.413	0.347	0.94	Sweety, alkane
V4	0.21	2-Propanone	1.718 ± 0.125	2.205 ± 0.324	0.095	500[a]	Fruity, apple, pear
V5	0.27	2-Butanone	0.340 ± 0.036	0.200 ± 0.019	0.025	40	Ethereal
V6	0.29	3-Methylbutanal	0.130 ± 0.017	0.380 ± 0.060	0.000	0.08	Acorn, fruity, cheesy, salty
V7	0.31	2-Propanol	0.072 ± 0.010	0.073 ± 0.010	0.949	26[b]	Alcoholic, dry, buttery-taste
V8	0.32	Ethanol	1.310 ± 0.200	1.520 ± 0.263	0.612	30	Alcohol, sweet
V9	0.34	2-Ethyl furane	0.089 ± 0.037	0.066 ± 0.007	0.739	–	Sweet
V10	0.38	2-Pentanone + 3-pentanone	0.790 ± 0.078	0.483 ± 0.099	0.050	–	Sweet, fruity, green
V11	0.39	2,3-Butanodione	0.362 ± 0.072	0.353 ± 0.096	0.945	–	Vanilla/caramel-like, buttery
V12	0.46	α-Pinene	0.090 ± 0.010	0.080 ± 0.017	0.570	0.018[b]	Sharp, pine
V13	0.51	Methyl benzene	0.100 ± 0.005	0.122 ± 0.007	0.027	0.33	Plastic, glue, strong
V14	0.53	2-Methyl-3-buten-2-ol	0.060 ± 0.008	0.065 ± 0.009	0.744	0.48	Earthy
V15	0.60	Dimethyl disulfide	0.024 ± 0.004	0.018 ± 0.003	0.391	0.012	Cauliflowers, vegetable
V16	0.61	Butyl acetate	0.011 ± 0.001	0.011 ± 0.001	0.804	0.30	Fruity
V17	0.64	Hexanal	1.180 ± 0.197	3.760 ± 0.644	0.000	0.08	Green, grassy, fatty
V18	0.69	2-Methyl propanol	0.110 ± 0.011	0.320 ± 0.040	0.000	1.00	Wine, penetrating
V19	0.75	2-Butanol	0.025 ± 0.006	0.019 ± 0.002	0.159	0.50	Winey
V20	0.78	Ethyl benzene	0.025 ± 0.003	0.023 ± 0.003	0.809	–	Dry, glue, unpleasant
V21	0.90	Butanol	0.035 ± 0.021	0.148 ± 0.008	0.010	0.038	Fruity, medicinal
V22	1.05	2-Heptanone	1.560 ± 0.167	1.240 ± 0.277	0.356	0.30	Spicy, acorn, blue cheese
V23	1.06	Heptanal	1.030 ± 0.329	1.470 ± 0.209	0.489	0.50	Fatty, greasy, ham-like
V24	1.09	Limonene	0.590 ± 0.104	2.680 ± 0.559	0.000	0.25	Citric, fresh
V25	1.21	3-Methylbutanol	1.330 ± 0.114	5.270 ± 1.148	0.000	0.10	Woody, acorn, pleasant green
V26	1.31	2-Pentyl furane	0.490 ± 0.076	0.940 ± 0.218	0.008	0.10	Green fruity
V27	1.43	Octen-3-one	1.250 ± 0.065	0.630 ± 0.081	0.000	0.01	Spicy, mushroom, dirty
V28	1.46	Pentanol	1.250 ± 0.148	1.258 ± 0.060	0.986	0.47	Pungent, strong, balsamic
V29	1.59	(*E*,*E*)-2,4-Dcadienal	0.458 ± 0.062	0.025 ± 0.005	0.000	2.50	Fatty, rancid
V30	1.61	2-Octanone	2.200 ± 0.368	0.720 ± 0.118	0.034	0.51	Fruity, floral, green, fresh
V31	1.63	Octanal	4.280 ± 0.880	7.380 ± 1.104	0.041	0.32	Meat-like, green, fresh
V32	1.84	*E*-2-Heptenal	1.330 ± 0.326	0.710 ± 0.186	0.309	0.05	Green, fatty, fruity, almonds
V33	1.89	2-Heptanol	0.550 ± 0.063	0.700 ± 0.132	0.265	0.01	Oily, sweety
V34	2.09	Hexanol	1.740 ± 0.179	4.100 ± 0.585	0.000	0.40	Fruity, green
V35	2.30	2-Nonanone	1.130 ± 0.219	1.690 ± 0.523	0.258	0.10	Floral, fruity, blue cheese
V36	2.33	Nonanal	2.500 ± 0.444	4.330 ± 0.674	0.043	0.15	Rancid, fatty
V37	2.55	*E*-2-octenal	0.840 ± 0.158	2.470 ± 0.578	0.000	0.004	Leaves, pungent, fatty, fruity
V38	2.76	1-Octen-3-ol	2.720 ± 0.204	1.660 ± 0.213	0.009	0.001	Mushroom-like, earthy, dust
V39	3.02	Decanal	0.260 ± 0.023	0.280 ± 0.034	0.737	0.65	Citrus, waxy
V40	3.11	Benzaldehyde	0.970 ± 0.090	1.780 ± 0.210	0.000	0.06	Bitter almonds, penetrating
V41	3.22	*E*-2-Nonenal	2.030 ± 0.369	3.875 ± 0.922	0.034	0.15	Fatty, waxy
V42	3.47	Octanol	0.420 ± 0.045	0.980 ± 0.115	0.000	0.027	Fatty, sharp
V43	3.94	Butanoic acid	0.569 ± 0.050	0.611 ± 0.092	0.689	0.65	Cheesy, rancid
V44	4.13	Nonanol	0.190 ± 0.013	0.260 ± 0.039	0.078	0.28	Fatty green
V45	4.14	2-Methyl propanoic	6.490 ± 0.464	4.990 ± 0.668	0.115	8.1[a]	Iron, fishy

Odour threshold values (mg/kg) and sensory descriptions obtained by GC-olfactometry (GC-O).

Note: Rt, relative retention time. [a] Fazzalari, 1978; [b] Naga

Table 3. Stepwise ridge linear regression: sensory attributes explained by volatile compounds

Attributes	Selected volatiles	Regression
Acorn odour	V40 (benzaldehyde); V22 (2-heptanone); V6 (3-methylbutanal)	$R = 0.85$; adjusted-$R^2 = 0.70$
Acorn flavour	V6 (3-methyl butanal); V34 (hexanol); V25 (3-methyl butanol); V35 (2-nonanone)	$R = 0.90$; adjusted-$R^2 = 0.77$
Rancid odour	V17 (hexanal); V28 (pentanol); V34 (hexanol)	$R = 0.79$; adjusted-$R^2 = 0.60$
Rancid taste	V6 (3-methylbutanal); V34 (hexanol); V42 (octanol)	$R = 0.91$; adjusted-$R^2 = 0.82$
Fat rancid flavour	V42 (octanol), V6 (3-methylbutanal);V24 (limonene)	$R = 0.93$; adjusted-$R^2 = 0.86$
Fat pungent flavour	V42 (octanol); V24 (limonene)	$R = 0.87$; adjusted-$R^2 = 0.78$

Table 4. Statistical correlation between sensory attributes qualifying the entire hams and the volatile compounds quantified in the four parts of the hams. Minimum correlation was fixed at 0.60 ($p < 0.05$)

Sensory attribute	BF		SF		SM		ST	
	Volatile compounds	*R* (min, max)	Volatile compounds	*R* (min, max)	Volatile compounds	*R* (min, max)	Volatile compounds	*R* (min, max)
Acorn odour	22, 24, 30, 40	>0.60 *<0.87*	6	0.62	37, 40	>0.60 *<0.89*	40	>0.60 <0.75
Acorn flavour	6, 25, 35	>0.60 <0.81					25, 34, 35	>0.60 <0.84
Rancid odour	17, 24, 34, 36	>0.60 *<0.85*	35, 42	>0.60 *<0.82*	17, 20, 21	>0.60 *<0.82*		
Rancid taste	17, 34, 36	>0.60 <0.94	42	0.74	6, 21, 35	>0.60 <0.90	6, 34, 42, 17	>0.60 *<0.91*
Fat rancid flavour	24, 27, 34	>0.60 <0.86	30, 42	>0.60 <0.91	17, 22, 21, 25, 33, 34, 35	>0.60 *<0.97*	6, 21	>0.60 <0.81
Fat pungent flavour	24, 27	>0.60 <0.80	17, 33, 42	>0.60 <0.91	21, 24, 35	>0.60	6, 24	>0.60 <0.73
Cured ham odour			24, 26, 35	>0.60 <0.74				
Cured ham flavour					24, 31	>0.60 <0.62		
Raw meat flavour			21	0.73	17, 31	>0.60 <0.78	21, 27, 36	>0.60 <0.82

The numbers correspond to the volatile codes of Table 2. Note: BF, *biceps femoris* muscle; SF, subcutaneous fat; SM, *semimembranosus* muscle; ST, *semitendinosus* muscle; R, regression coefficient.