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Emerging research approaches benefit to the study of cooked cured ham flavour

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The analysis of the aroma of a food product requires the extraction of its volatile compounds. As the composition of the extract obtained depends on the extraction method, it is important to verify that the odour actually extracted is representative of the product. This verification is particularly relevant in the case of cooked cured ham, as its odour is not very strong as compared to other cured products (dry cured ham or sausage). Extracts were obtained by four different vacuum distillation processes. A sensory comparison of the odour of these extracts with the reference product was performed by ten trained panelists. The odour similarity of the extracts with the reference was assessed in order to select the most representative one, upon defined descriptors. Direct vacuum distillation of ground ham suspended in water was selected as the most representative. Gas chromatography (GC)–olfactometry, a technique of choice to identify potent odorants in food products, was then planned. As one of the key point of this analysis is to correlate the Flame Ionization Detector (FID) detection to the sniffing one, a problem very often underestimated by authors, a GC–olfactometric study of a model mixture was first performed with three selected people. FID and sniffing detection of this model mixture were compared in terms of retention time reproducibility. Standard deviation of retention indices observed for sniffing and FID detection were found in the same range. Retention indices calculated with both detections differed within a confidence interval of ±3 index values. FID and odour detection were found to be correlated within this interval. © 1997 Elsevier Science Ltd

INTRODUCTION

Cured meat products flavour has been studied for a long time in order to identify its specific compounds (Cross & Ziegler, 1965; Gray *et al.*, 1981; Mottram *et al.*, 1984). Several compounds have been identified (Mottram, 1984; Shahidi *et al.*, 1986) but in spite of the numerous studies made on this subject, no single compound or class of compounds has been found to be responsible for the characteristic flavour of cooked cured meat products, nor the involved mechanisms elucidated (Ramarathnam & Rubin, 1994). The overall idea arising from these studies is the importance of focusing efforts on compounds that have a real impact on flavour. Therefore, emerging research approaches facilitate the search of relevant aroma compounds: GC-olfactometry (GC–O) analysis (Acree *et al.*, 1984) after evaluation of sensorial representativeness of the extracts (Abbott *et al.*, 1993; Etievant *et al.*, 1994).

As analyses are made on extracts and not on products, it is important to sensorially evaluate the odour quality of the extract. This evaluation will ascertain that the selected extraction method is as representative as possible of the initial product. This representativeness study, based on sensory evaluation by a trained panel, should be a prerequisite to further analyses (e.g. GC-olfactometry) as clearly demonstrated in various recent studies conducted in this laboratory (Abbott *et al.*, 1993; Moio *et al.*, 1995; Le Quere *et al.*, 1996; Langlois *et al.*, 1996).

Among the problems encountered in flavour analysis, extraction yields and uncontrolled transformation or degradation of compounds during the extraction step interfere with the quality of the extract obtained (Teranishi & Kint, 1993). For example, thermal effects have to be taken into consideration (Spanier & Boylston, 1994). Therefore, cooked cured ham volatiles were extracted under vacuum at a monitored temperature of 30°C, and cold trapped.

Four extraction methods, based on vacuum distillation in different conditions, were compared. The extracts obtained were evaluated by sensory analysis, in

comparison with the product, and the most representative one selected.

Once representativeness of the extract had been assessed, olfactometric study of its volatile components was planned by means of GC-olfactometric analysis. Actually, olfactometric evaluation of GC effluents provides a good idea of the compounds that have a flavour impact (Grosch, 1993; Stahnke, 1995). Thus, this type of analysis was planned with a panel of three persons. Before analysing the extracts, sniffers performance and reproducibility were evaluated with a test mixture. The aim of this preliminary step was to provide information which would help the interpretation of further results to be obtained with the extracts themselves.

The aim of this paper is to report, first on the study of the sensory representativeness of the extracts, and second on the training, with a test mixture, of a panel of three people aimed to further perform the GC–olfactometric study of cooked cured ham extracts to determine the correlation between FID and sniffing signals.

MATERIALS AND METHODS

Materials

5 kg of pork *semimembranosus* muscle from commercial sources (Large White*Pietrain) were sorted out for similar visual appearance and mean pH_{48} value 5.7, standard deviation 0.1. Ten percent (weight/weight) intramuscular brine injection was performed with a pumping needle. The level of sodium nitrite was adjusted to inject 100 mg/kg muscle. No spices were added to the brine in order to avoid their volatile components interference. Alternative tumbling was performed under vacuum at 7°C (working time 15 min at 9 rpm in a cycle of 30 min over 12 h, totalizing 3240 rotations) and the ham was put in a 5 kg sealed cook–in–bag pouch and cooked to a core temperature of 65°C. After cooling to a core temperature of 3°C and a further 24 h temperature stabilization, 100 g slices were made, wrapped in an aluminium sheet, placed individually in a polyethylene bag sealed under vacuum, and frozen to −20°C, to keep oxidation to a minimum. The aluminium foil was used to protect the sample from oligomers migration of the polyethylene bag. The storage at −20°C lasted up to 8 weeks.

Ham was ground frozen at the moment of extraction in a plastic grinder with stainless blades, cooled with liquid nitrogen.

Isolation of volatiles

The volatile constituents were extracted from 100 g of ground ham by vacuum distillation, using four alternative vacuum distillation processes, and collected in glass traps cooled with liquid nitrogen. Two processes consisted of a vacuum distillation directly on the product, and the two other in a simultaneous distillation with added water. All distillations were conducted at a temperature of 30°C.

The vacuum distillation performed directly on the product was divided in a one-step (method A) or two-steps (method B) extraction (Berdague *et al.*, 1991). The first step consisted of a 5 h distillation at 10^3 Pa, 30°C, and the second a 4 h distillation at 10 Pa, 30°C, of the dry residue obtained after the first step.

The vacuum distillation process with added water (Dumont & Adda, 1972), was performed according to two ways of sample preparation. The extraction was made either directly with the ground ham mixed with ultrapure water (milliQ®, 1 w ham : 3 w water) in the distillation flask (method C) or with the water extract (supernatant of the ground ham mixed with water, obtained after two steps of centrifugation at 3500*g* and filtration) of the ham (method D).

After extraction, the contents of the cold traps were acidified to pH 2 with 2N hydrochloric acid. The volatile compounds were then extracted with bidistilled dichloromethane (1 vol. CH_2Cl_2 : 4 vol. extract). The dichloromethane extract was dried over anhydrous sodium sulphate and concentrated to a final volume of 500 μl with a Kuderna-Danish evaporator–concentrator fitted with a Snyder column (Berdague *et al.*, 1991). Before the concentration step, 50 μg of pentanoic acid pentyl ester was added as an internal standard to the GC extracts, but not to the extracts destined to sensory analysis.

Sensory evaluation

The odour of the extracts was assessed by a 10 member panel trained to evaluate cooked ham. The reference sample consisted of diluted ground cooked ham. Test samples consisted of the diluted extracts obtained with the four extraction techniques described above. Test and reference samples were presented in covered opaque glasses thermostated at 20°C. Odour descriptors were generated and learned during five training sessions. For the two evaluating sessions, the test samples were presented with the reference and described for each descriptor on unstructurated scales of 150 mm. Results obtained were statistically analysed with the SAS® statistical package (Dunnett test).

Gas chromatography (GC) of the extracts

Analyses were performed using a Hewlett Packard HP5890 series II gas chromatograph equipped with a flame ionization detector (FID), a splitless–split injector and a DB-FFAP (film thickness 0.25 μm) J&W Scientific fused silica capillary column (30 m × 0.32 mm i.d.). Hydrogen carrier gas was used at a velocity of 37 cms^{-1} at 143°C. The oven temperature was programmed from 40°C to 220°C at a rate of 3°C min^{-1}.

Gas chromatography—olfactometry of a test mixture

Three selected people were trained for GC–Olfactometry analysis with a test mixture containing 12 odorous compounds (allyl isothiocyanate, butanoic acid ethyl ester, L-carvone, cinnamaldehyde, dimethyl disulfide, eucalyptol, eugenol, linalool, menthol, methional, 3-methyl butanoic acid, thymol).

For this part of the study, the Hewlett Packard HP5890 series II gas chromatograph was equipped with a J&W scientific on-column injector and a 2 m uncoated, deactivated fused silica pre-column (0.32 mm i.d.) was connected to the DB-FFAP column using a press-fit glass connector. A splitting system was installed at the end of the column to divide the effluent with a 1:1 split ratio between the detector and the sniffing port, attached to the GC as described by Abbott *et al.* (1993). The temperature was programmed from 40 to 220°C at a rate of $5°Cmin^{-1}$. FID detection of compounds and olfactory results were recorded simultaneously, using hardware and software devices developed in this laboratory (P. Mielle & R. Almanza, Coconut® INRA 1987–1993). The rate of 5°C/min was chosen in order to perform the evaluation of all the components of the test mixture within a sniffing duration of 30 min, as longer sessions can fatigue the sniffers (Acree, 1993).

To facilitate correlation between odours and FID signals, retention indices for each compound were calculated according to Van den Dool and Kratz (1963), using a C_{10} to C_{24} *n*-alkanes solution, which was chromatographed prior to each analysis.

RESULTS AND DISCUSSION

Representativeness of the extracts

Cooked cured ham volatiles were extracted with the four methods described above. Extractions were made in triplicate for chromatography profiles and in duplicate for sensory analysis, plus some extra extractions for the training sessions.

The mean gas chromatography profiles (Fig. 1) were found relatively different from one method to the other in terms of number of compounds and quantities. According to these results, more volatile compounds,

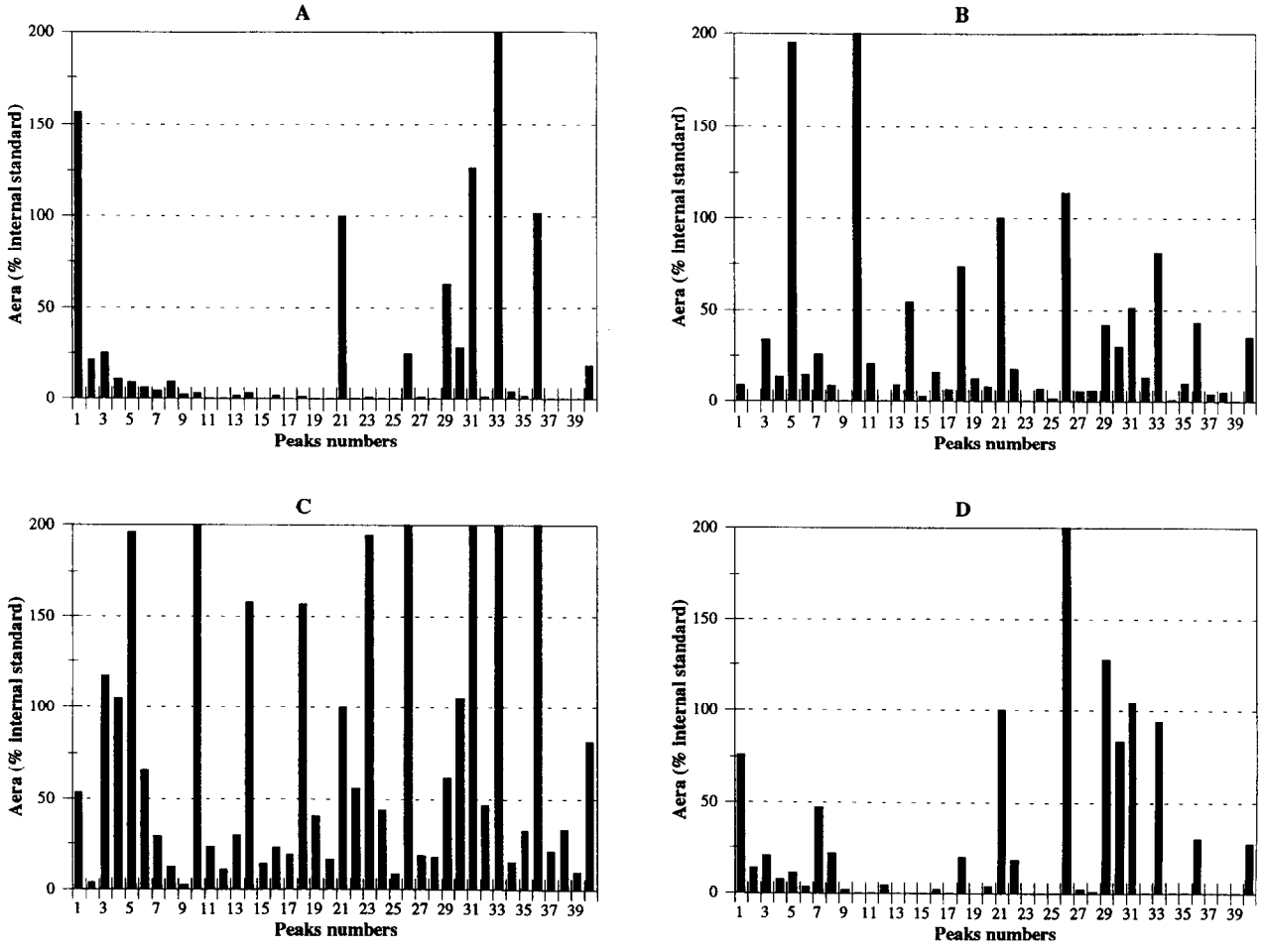

Fig. 1. GC profiles of major compounds from extracts obtained by one-step (A) and two-steps (B) dry reduced pressure distillation and direct (C), or water extract (D) water co-distillation under reduced pressure. Y-axis represents % of internal standard (peak #21).

and in higher amounts, were obtained with direct vacuum distillation of ground ham suspended in water (method C). The two-step vacuum distillation (method B) also extracted many compounds but in lower amounts.

The odour of the extracts, with cooked cured ham presented as a reference, was evaluated in order to select the extract with the most representative odour. The intensity of odours according to five descriptors previously generated during training sessions (cooked cured ham, cooked pork meat, andouillette (cooked pork intestines speciality), cooked meat product, solvent) was evaluated and scored on unstructured scales in comparison to the reference product (Etievant *et al.*, 1993). The descriptive session was repeated twice, with extracts from session 1 and 2 coming from different duplicates. The scores obtained for the extracts were compared to the scores of the reference for each descriptor, in terms of distance between the scores (the shorter the distance, the closer the extract to the product).

Results of the two evaluating sessions were not significantly different. Only two descriptors were found significantly discriminant: cooked cured ham ($\alpha = 1\%$) and cooked pork meat ($\alpha = 5\%$). These two descriptors were supposed to be particularly relevant to this study, as the product has a high score for cooked cured ham descriptor and a low score for cooked pork meat descriptor. When the difference between the product and its extracts was tested, the two extracts obtained with methods A and D were found to be significantly different from the product. Methods B and C (two-step vacuum distillation and direct vacuum distillation with water, respectively) were selected for not being significantly different from the reference for both discriminant descriptors ($\alpha = 5\%$).

In order to corroborate these results, cooked pork meat and cooked cured ham volatiles were extracted by both methods. The odours of products and extracts were compared by triangle test, product by product and extract by extract. The products were judged significantly different as well as their respective extracts from both methods ($\alpha = 1\%$). This result assessed that when a difference was detected between the products, it was also detected between the extracts.

Finally, direct vacuum distillation of ground ham suspended in water (method C) was selected according to results of representativeness and also because of a more efficient extraction yield (as measured on the GC profiles, Fig. 1) and a shorter extraction time (5 h compared to 2 days with method B).

GC–olfactometry set up

Once a representative extract is obtained, a GC–olfactometry technique such as Aroma Extract Dilution Analysis (AEDA), (Acree *et al.*, 1984; Grosch, 1993) is an interesting way of studying the different compounds present in the extract, in order to pinpoint the molecules

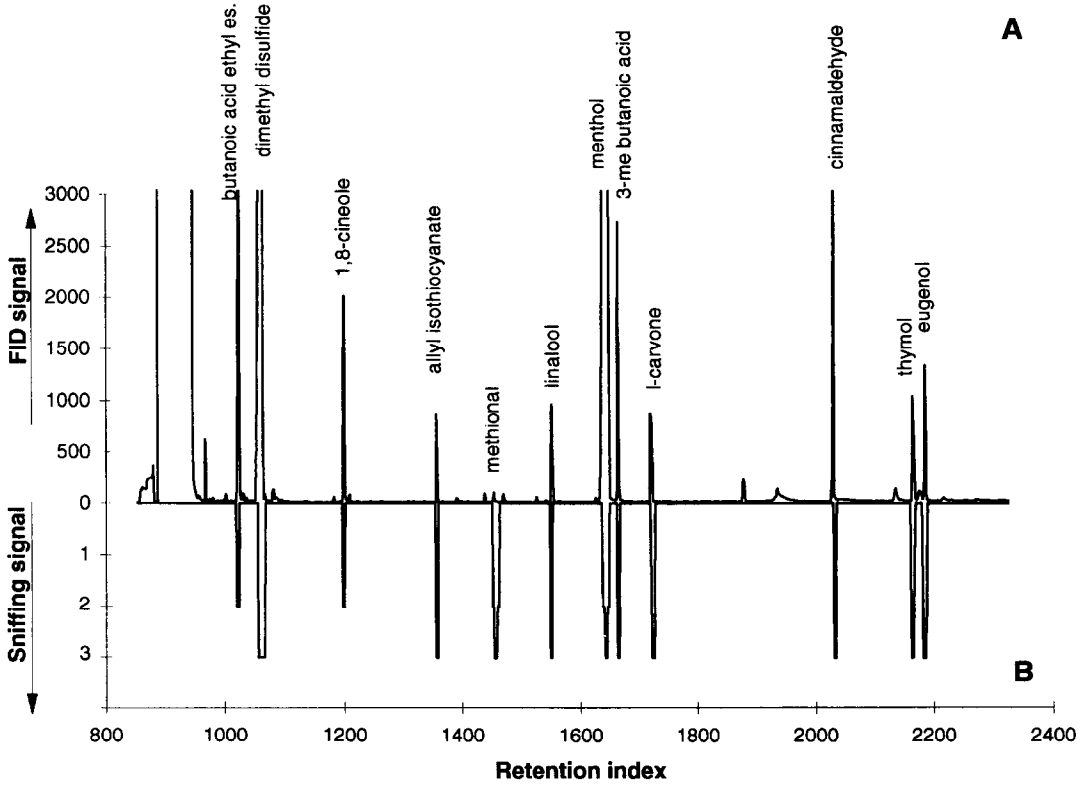

Fig. 2. GC profile of the test mixture, detected by FID (A) or sniffing (B), with identification of the odorous compounds. Values for positive Y-axis represent arbitrary FID signal (A), and for negative Y-axis number of sniffing detections (B). The three sniffers evaluated the sample twice, and a value of 1 is attributed when an odour is detected twice by the same person.

Table 1. Retention indices of the compounds of the test mixture detected by FID (three repetitions) or by sniffing (three sniffers × two repetitions).

Compounds	Odour (Fenaroli, 1994)	Odour descriptors (sniffers)	FID indices (S.D.)	Sniff indices (S.D.)	(FID-Sniff) index difference
Butanoic acid ethyl ester	Fruity pineapple undernote	Fruity[1, 2]	1023 (2)	1021 (1)	2
Dimethyl disulfide	Intense, onion, cabbage	Fermented[1], plastic[2], unknown[3]	1063 (3)	1057 (1)	6
1,8-cineole	Camphorous, fresh	Eucalyptus[1], mint[2]	1197 (2)	1195 (4)	2
Allyl isothiocyanate	Strong, pungent	Sulfurous[1],sewer[2], cheese[3]	1356 (0)	1356 (5)	0
Methional	Onion, soup like, meat like	Cooked potatoes[1, 2], grassy[3]	1454 (2)	1452 (5)	2
Linalool	Floral, woody, faint citrus	Orange[1], flower[2], licorice[3]	1550 (1)	1542 (3)	8
Menthol	Mint like	Mint[1, 2], mint sweets[3]	1646 (1)	1642 (3)	4
3-methyl butanoic acid	Disagreable, persistant	Rotten peas[1], excrement[2], cheese[3]	1664 (1)	1663 (2)	1
L-carvone	Hebaceous, spearmint	Mint[1], chewing gum[2, 3]	1719 (1)	1717 (3)	2
Cinnamaldehyde	Pungent, spicy, cinnamon	Cinnamon[1, 3], unknown[2]	2031 (2)	2029 (3)	2
Thymol	Herbaceous, medicinal	Thyme[1, 3], eucalyptus[2]	2165 (2)	2163 (1)	2
Eugenol	Intense, spicy, clove	Spicy[1, 2], cloves[3]	2186 (2)	2185 (1)	1

[1,2,3] identification of sniffer.

that have an actual sensorial impact. As correlation between FID and odour retention indices is a key point of this method, correlation values between both detections were first determined, during the initial training of a panel of three sniffers. The sniffers were first selected for their performance with an olfactory test developed by the team of C. Rouby (Laboratoire de Physiologie Neurosensorielle, UCB Lyon I, F-69622 Villeurbanne cedex). The composition of the test mixture was elaborated to detect potent anosmias (Labows & Wysolcki, 1984 and laboratory results) and to get sniffers used to the sniffing process.

The evaluation of the performance of the three sniffers and the comparison of the calculated retention indices obtained by FID and odour detection were performed by analysis of a test mixture containing twelve identified odorous compounds. FID and sniffing detection are presented in Fig. 2.

The detection signals given by the three sniffers were comparable for the twelve compounds of the mixture. Only two sniffers out of three detected the odours of butanoic acid ethyl ester and 1,8-cineole. The result for 1,8-cineole could be explained by a specific anosmia known in 33% of the human population (Labows & Wysolcki, 1984). For the other compound, individual detection threshold differences might explain the observed results.

As odour description is subjective and depends on personal cultural references, the odour descriptors given by the sniffers did not often correspond to the descriptors found in literature (Fenaroli, 1994), and differences in description were also found between the sniffers. However, each sniffer always gave the same descriptor to each compound, supporting that sniffers gave reproducible results in detection and description.

Mean results of the test mixture evaluation (Table 1) show that standard deviations of the retention indices observed by FID were consistent with usual laboratory results when using polar GC columns (between 1 and 3 index values). For the sniffing results (3 persons*2 sniffing sessions), standard deviations of the retention indices were found between 1 and 5 index values. Thus, standard deviations were found to be in a comparable range with both detection methods.

Correlation between FID and sniffing detection was tested by comparison of the mean retention indices obtained. The confidence interval observed for FID and sniffing detection was calculated to be of ± 3 retention index ($\alpha = 5\%$). Therefore, retention indices determined by FID and sniffing were not significantly different for the 12 compounds detected ($\alpha = 5\%$, t-test), distributed over a wide range of retention indices (from 1000 to 2200).

CONCLUSION

A better understanding of the sensory quality of cooked cured ham implies the determination of the flavour compounds involved. An important part of the study is to ascertain that the compounds identified are actually pertinent to the flavour of the product, and to what extent. GC–olfactometry is nowadays considered as a powerful tool to identify the key-odorant impact compounds (Grosch, 1993). However, in order to perform a pertinent GC–O analysis, it is essential to first recover an extract as representative of the product as possible.

Thus, the first step of the study was to extract volatile compounds from cooked cured ham. Four alternative vacuum distillation methods were tested. The odour

representativeness of the extracts was assessed by sensory analysis. Direct vacuum distillation of ground ham suspended in water was found the most representative one and was retained for the future GC–O analyses. Moreover, this method extracted more volatile compounds than the three others (dry vacuum distillation at 10^3 Pa, or 10 Pa, or vacuum distillation of a water extract of ham), as observed by gas chromatography. As the extract showing an odour representative of cooked cured ham was obtained by distillation in presence of water and not by direct vacuum distillation, the compound(s) implicated in the aroma of cooked cured ham might be rather hydrophilic.

In the perspective of GC–O analysis of cooked cured ham extracts using AEDA (Grosch, 1993), a panel of three sniffers, selected for their olfactory performances, was trained to GC–O techniques with a mixture containing known odorous compounds. Retention indices for the FID traces and the simultaneous aromagrams were recorded. Mean retention indices calculated at the FID and the sniffing port were significantly correlated with a confidence interval of ± 3 index value ($\alpha = 5\%$), for retention indices from 1000 to 2200.

Therefore, it could be anticipated that odours detected by GC–olfactometric study of the volatiles extracted from a product will be correlated with the FID signal with a confident interval of ± 3 retention index for each compound, when separated on a polar GC column. This correlation, a problem very often underestimated in previous studies, and particularly accute when using a polar GC column, was very important to ascertain before further identification of the potent odorants by GC–MS (Mass Spectrometry). For cooked cured ham, this will be the purpose of our next studies.

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