Study on the influences of ultrasound on the flavor profile of unsmoked bacon and its underlying metabolic mechanism by using HS-GC-IMS

Jian Zhang, Wangang Zhang *, Lei Zhou, Ruyu Zhang

Key Laboratory of Meat Processing and Quality Control, MOE, Key Laboratory of Meat Processing, MOA, Jiangsu Synergetic Innovation Center of Meat Processing and Quality Control, College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, PR China

ARTICLE INFO

Keywords:
Ultrasound
GC-IMS
Flavor fingerprint
Enzymatic oxidation
Unsmoked bacon

ABSTRACT

For exploring the influence of ultrasound on the flavor characteristic of unsmoked bacon, sensory evaluation combined with E-nose and headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS) were performed to analyze the overall flavor profile and specific volatile flavor compounds (VFCs), respectively. Furthermore, the metabolic pathway of VFCs affected by ultrasound was also investigated. Results demonstrated that ultrasound improved the flavor characteristic of unsmoked bacon by raising the levels of nonanal, heptanal, octanal, 3-methylbutanal, n-hexyl acetate and n-propyl acetate. Enzymatic oxidation was found to be an important metabolic pathway responsible for the development of flavor characteristic after ultrasound treatment, which could be attributed to the increased activities of lipases and lipoxygenase and the higher concentration of polyunsaturated free fatty acids. The increased level of lipid oxidation after ultrasound treatment was also confirmed by thiobarbituric acid reactive substances. Consequently, ultrasound is an effective approach to enhance the flavor characteristic of unsmoked bacon.

1. Introduction

Bacon is generally processed with the steps of salting, smoked or non-smoked treatment and dry-ripening. Despite smoked treatment imparts a more palatable flavor [1], it can bring health concerns via generating toxic compounds like benzopyrene [2]. With consumers increasingly paying more attention to the concept of safe and healthy food products, unsmoked bacon is going to be a better alternative. However, the insufficient flavor in unsmoked bacon largely impairs its market competitiveness. Therefore, it is a top priority to seek new methods to improve the flavor characteristic of unsmoked bacon.

High intensity ultrasound is regarded as an emerging technology in food field since it possesses the advantages of low energy consumption and high efficiency without environmental pollution [3]. The action mechanism of ultrasound is relied on ultrasonic wave that can cause cavitation phenomenon as well as thermal and mechanical effects, altering the microstructures and physicochemical characteristics of food. Recent years have witnessed the wide application of ultrasound in improving emulsification efficiency [4], prolonging shelf life [5], increasing tenderness [6], and promoting salt penetration in meat and meat products [7]. However, few literatures have reported the effects of ultrasound on the volatile flavor compounds (VFCs) in dry-cured meat products.

The identification of VFCs in meat products can be achieved by several instrumental analytical techniques among which gas chromatography-olfactometry-mass spectrometry (GC-O-MS) and chromatography-mass spectrometry (GC-MS) are most frequently applied methods [8]. Although GC-O-MS can screen the aroma-active substances of complicated matrix, it consumes considerable time to finish repeated operations (e.g. the process of aroma extract dilution analysis) which cannot fully satisfy the rapid detection for flavor compounds [9]. GC-MS is a widely approved method to detect and analyze volatile flavor compounds, but it has some limitations such as complex pretreatment, time-consuming procedure, vacuum conditions for mass spectrometry and no recognition between isomeric and isobaric compounds. Despite two-dimensional GC is emerged in the past decade and compensates for the low separation capacity of GC, this technology is comparatively immature which needs to be further improved [10]. Compared with the above technologies, headspace-gas chromatography-ion mobility spectrometry (HS-GC-IMS) has remarkable predominance in separating and identifying VFCs. HS-GC-IMS not only operates easily and costs lowly, but also incorporates the rapid response
of IMS and the high separation ability of GC, thus making it become a popular and promising technology in food flavor detection [10]. For example, by using GC-IMS, Arroyo-Manzanares et al. [11] successfully differentiated the Iberian hams originated from acorn-fed and feed-fed systems by analyzing different flavor fingerprints for avoiding labeling fraud. With the assistance of GC-IMS, Wang et al. [12] compared the flavor compounds and established the flavor fingerprints of different ages (2, 6 and 12 months) of Jingyuan lamb meat. However, no studies have investigated the change of flavor profile of unsmoked bacon after ultrasound treatment with employing GC-IMS.

To further understand the influences of ultrasound on volatile flavor compounds, HC-GC-IMS technology assisted with sensory evaluation and E-nose measurement was carried out to investigate the change of flavor characteristic in unsmoked bacon after ultrasonic treatment. Besides, the main origin and metabolic pathways of VFCs that were significantly affected by ultrasound treatment were also studied.

2. Materials and methods

2.1. Sample preparation

The process of unsmoked bacon was performed as described by Zhang et al. [13]. Briefly, 20 deboned thigh muscles from Landrace pigs were provided by Yurun Food Co. Ltd. (Nanjing, China) and each of them was manually trimmed with similar weight (590–610 g) and size (25 × 5 × 4 cm³). Then, all raw bacons were chilled for 1 d at 4 °C and were dry-cured using 0.01% nitrite and 3% sodium chloride (g/100 muscle) for 2 d at 85–90% relative humidity (RH) and at 4 °C. After drying, these 20 raw bacons were assigned randomly into 4 groups (5 bacons for each group): one group was randomly selected as control and the other three groups were set as treatment groups. Next, the three treatment groups were performed with ultrasound treatment (Tianhua Ultrasonic Electronic Instrument Co., Ltd, Jining, China) for 1 h at 250, 500 and 750 W, respectively. The frequency of the ultrasonic device was 20 kHz. Finally, all raw bacons were air-drying and ripening in constant climate chambers for 10 d. The RH was decreased by 0.5% per day from 80% to 75% while the temperature increased by 1.5 °C per day from 15 °C to 30 °C. When the process of unsmoked bacon was finished, parts of the final products were immediately provided for sensory evaluation and the others were sampled, vacuum packed and frozen at −80 °C until further analysis.

2.2. Sensory analysis

Sensory evaluation was carried out referring to Bi et al. [14] with slight changes. The sensory panel was consisted of 5 men and 5 women aged from 23 to 30 years old. The panelists have been systematically trained and they possessed rich experience in evaluating the sensory attributes of dry-cured meat products. The descriptive terms were discussed and defined by the panelists during training including caramel, grease, sour, cured, meaty, and overall aroma. After each sensory attribute was familiarly mastered by panelists, the samples of unsmoked bacon were cut into small slices and provided for panelists to perform sensory evaluation. The score of each sensory attribute was ranged from 1 (no flavor) to 9 (strong flavor).

2.3. Electronic nose

The E-nose operation was conducted by using Airsense PEN3 (Airsense Analytics GmbH, Schwerin, Germany). Three grams of minced samples were put in headspace bottles (20 mL) and the bottles with sample were incubated for 30 min at 50 °C in water bath. After incubation, E-nose probe was inserted into the headspace bottle to suck out the headspace gas for measurement. The instrumental parameters were as follows: the times of flush, zero trim, presampling and measurement were 70.0, 10.0, 5.0 and 100 s, respectively. The chamber flow and the initial injection gas flow were all 400 mL/min. Five replicates of each group were determined.

2.4. HS-GC-IMS analysis

The VFCs of unsmoked bacon were detected by using HC-GC-IMS (FlavourSpec®, G.A.S., Dortmund, Germany) equipped with FS-SE-54-CB-1 (15 m × 0.53 mm × 1 μm) according to Hou et al. [15]. A total of 3 g minced samples were put into headspace bottle (20 mL) and incubated for 10 min at 60 °C. After incubation, 500 μL headspace gas of the headspace bottle was automatically drawn into heating injector by a heated syringe needle (85 °C). The chromatographic column was maintained at 60 °C and the running time was 20 min. The carrier gas was high pure nitrogen (99.99%) and its flow rate program was referred to Hou et al. [15]. n-Ketones C4-C9 were used to calculate the retention index (RI). The identification of VFCs was relied on the comparison of drift time and RI in GC-IMS library, and the intensity of VFC was gained in light of the peak volume. Three replicates were measured for each group.

2.5. Lipases extraction and determination

The lipid enzymes were extracted and measured following Zhang et al. [16]. Briefly, 0.2 g minced samples were homogenized in 3 mL phosphate buffer and centrifuged, and then supernatant was taken for next determination. BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA) was used to determine protein concentration.

The 10 μL extracted lipase solution, 280 μL Tris/HCl buffer (0.22 M) and 10 μL 4-methylumbelliferyl-oleate (1 mM) were uniformly mixed and incubated at 37 °C for 30 min. Next the solution was cooled and its fluorescence intensity was measured to determine the activity of neutral lipase.

The 10 μL extracted lipase solution, 280 μL disodium hydrogen phosphate/citric acid buffer (no sodium fluoride) and 10 μL 4-methylumbelliferyl-oleate (1 mM) were uniformly mixed and maintained at 37 °C for 30 min. The reaction was stopped by 10 μL HCl (1 M) and the fluorescence intensity of the solution was measured to determine the acid lipase activity. The measurement steps of phospholipase activity were same to that of acid lipase except the disodium hydrogen phosphate/citric acid buffer (containing sodium fluoride).

A multifunctional microplate reader (M2e, Molecular Devices, San Jose, CA, USA) was applied to determine the fluorescence intensity of three enzymes with λex = 350 nm and λem = 445 nm. Three replicates were measured for each group. One U was the generated amount of 4-methylumbelliferone (μmol) per h at 37 °C and the lipase activity was expressed as U/g protein.

2.6. Free fatty acid (FFA) extraction and determination

The extraction and the determination of FFA were on the basis of Wang et al. [17] with slight changes. Briefly, 3 g minced samples were homogenized with 45 mL extracting solutions (methanol/chloroform = 1/2, v/v) and the mixture was stood for 2 h. After that, the mixture was filtered, washed and centrifuged to gain layered solution. The upper layer of the solution was performed to dryness by using nitrogen blowing instrument (N-EVAP112, Organamation Associates, USA) to gain total lipids. Next, 20 mg total lipids were dissolved in 1 mL chloroform and FFA was eluted by 2% acetic acid solution (diethyl ether as solvent) with Cleanert NH2 solid-phase column (100 mg, 1 mL). Next, the FFA elute was blown to dryness with nitrogen and the concentrated FFA was re-dissolved in 2 mL of 14% boron fluoride-methanol. The FFA solution was subjected to methylation at 60 °C for 30 min. After methylation, the FFA solution was added with 1 mL n-hexane and 1 mL water, shaken and stood for 1 h. The upper layer of the mixture was dried with nitrogen and finally dissolved in 500 μL n-hexane for gas chromatography detection by using GC-2010 Plus (Shimadzu...
Corporation, Japan) equipped with a capillary column (SP-2560; 100 m \( \times \) 0.25 mm ID \( \times \) 0.20 \( \mu \)m). Heptadecanoic acid was used as an internal standard. Four replicates were measured for each group.

The injection port temperature and the detector temperature for the GC were 270 \( ^\circ \)C and 280 \( ^\circ \)C, respectively. The oven temperature was increased from 100 \( ^\circ \)C to 180 \( ^\circ \)C at 10 \( ^\circ \)C/min and maintained for 6 min, then it was increased from 180 \( ^\circ \)C to 200 \( ^\circ \)C at 1 \( ^\circ \)C/min and maintained for 20 min, and finally it was increased from 200 \( ^\circ \)C to 230 \( ^\circ \)C at 4 \( ^\circ \)C/min and maintained for 10.5 min. The carrier gas was nitrogen and split ratio was 1:10.

2.7. Lipoxygenase (LOX) determination

LOX was extracted referring to Zhang et al. [16]. Briefly, 0.2 g minced samples were homogenized in 3 mL phosphate buffer and centrifuged, and then supernatant was gained for next determination. BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA) was used to determine the protein concentration.

LOX activity was assessed by measuring the absorbance increment within 1 min. Briefly, 20 \( \mu \)L enzyme solution, 380 \( \mu \)L citrate buffer (0.05 M) and 40 \( \mu \)L linoleic acid substrate solution were uniformly blended and its absorbance was measured twice at 0 s and 60 s. Three replicates were measured for each group. One U was the increment of absorbance at 234 nm per min. The unit of enzyme activity was U/g protein.

2.8. Thiobarbituric acid reactive substances (TBARS)

TBARS determination was performed following Zou et al. [18] with slight changes. A total of 2 g samples were homogenized (4 \( ^\circ \)C, 12,000 rpm, 3 \( \times \) 15 s) in 10 mL of 17.5% trichloroacetic acid solution (containing 0.1% EDTANa\(_2\)) and centrifuged (4 \( ^\circ \)C, 12,000 rpm, 10 min) for gaining supernatant. Then 2 mL of 0.02 M thiobarbituric acids were added into 2 mL of the supernatant, and the mixed solution was kept for 30 min in a water bath at 95 \( ^\circ \)C. After that, the solution was cooled and its absorbance at 532 nm was determined. Three replicates were measured for each group. On the basis of the standard curve of malondialdehyde (MDA), the TBARS value of sample was calculated and the result was defined as mg MDA/kg muscle.

2.9. Data analysis

SAS 8.0 software was applied to analyze the significant differences among different groups and \( P < 0.05 \) was considered as statistically significant. The E-nose data were conducted for principal component analysis (PCA) by using the matched WinMuster software. GC-IMS data were processed and viewed with GC-IMS Library Search and Laboratory Analytical Viewer (LAV) software. The Reporter, PCA and Gallery plug-ins of LAV were used to compare the 2D- and 3D-spectrogram, the overall flavor differences and the flavor fingerprint differences of identified volatiles in unsmoked bacon among different groups, respectively. Simca 14.1 was employed to build the model of orthogonal partial least squares discrimination analysis (OPLS-DA).

3. Results and discussion

3.1. Sensory analysis

The sensory score plot of different groups is shown in Fig. 1. In comparison with the 0 W group, ultrasonic groups had higher scores in meaty, cured, grease, caramel and overall aroma which were all favorable flavor contributing to the flavor improvement of unsmoked bacon. However, the sour score was the highest in the group of 0 W, which was not beneficial for the acceptance of consumers since excessive sour could produce irritating smell which was the symbol of meat spoilage [19]. These findings suggest that ultrasonic treatment obviously changed the flavor profile of unsmoked bacon. As for ultrasonic groups, the score of each sensory attribute (except sour) was the highest in the 250 W group, and the 500 W group had higher meaty and grease score than the 750 W group. These results indicate that the change of flavor profile was...
affected by the ultrasonic powers among which low power was conductive to flavor improvement. In addition, the score of overall aroma was highest in the 250 W group, followed by the 500 W and 750 W groups, which was resulted from the different concentrations of volatile flavor compounds and their combination effects [20]. To summarize, ultrasound improved the flavor profile of unsmoked bacon and the group of 250 W had the better promoting effect.

3.2. E-nose analysis

The E-nose data were processed by PCA for estimating the overall flavor characteristics of unsmoked bacon under different ultrasonic powers. The PC1 and PC2 explained 85.29% of total variables demonstrating the PCA model was reliable. As illustrated in Fig. 2, the 0 W group was obviously separated with the ultrasonic groups suggesting that ultrasound changed the overall profile of unsmoked bacon. It was worth noting that the 250 W group was located farthest from 0 W group along PC1 axis indicating the 250 W group had the most differences in the overall flavor profile of unsmoked bacon compared with the 0 W group. This result is also consistent with the sensory evaluation which showed that the 250 W group possessed the highest overall aroma score. Moreover, a clearly separation was observed among ultrasonic groups, suggesting that different ultrasonic powers resulted in different overall flavor profiles of unsmoked bacon.

3.3. HS-GC-IMS analysis

3.3.1. 2D- and 3D-topographic plots

For better understanding the flavor profile differences among different groups, volatile flavor compounds (VFCs) in unsmoked bacon were visualized and analyzed by using HS-GC-IMS. Fig. 3 shows the 2D- and 3D-topographic plots of four different ultrasonic groups for preliminary judging the differences of VFCs. In 2D-topographic plots, the abscissa and ordinate were the drift time and retention time of VFCs. The reactive ion peak was expressed by the red perpendicular line and normalized drift time was ranging from 8.25 to 8.31 ms. Each point meant a volatile flavor compound. Different color meant different concentration with red and white representing high and low concentration, respectively. The darker color indicated the higher concentration of the VFC. From the 2D-topographic plots (Fig. 3(A)), the types of VFCs were similar in different groups. However, the color of some points in ultrasonic groups was redder than that of the group of 0 W, suggesting that the concentration of VFCs was evidently changed after ultrasonic treatment. For more intuitively analyzing the differences of VFCs among different groups, 3D-topographic plots were provided. The iron peak intensity of each VFC could be clearly visualized in Fig. 3(B). From the 3D-topographic plots, the iron peak intensities of many VFCs were obviously different among different groups, which also indicated that the flavor profile of unsmoked bacon was influenced by ultrasound.
In addition, the numbers of SHVFCs were affected by ultrasonic powers in each ultrasonic group, forming their own characteristic fingerprints. Most SHVFCs were unique in the SHVFCs of different ultrasonic groups, while “B” area (cyan box) refers to the compounds whose contents are significantly higher in non-ultrasonic group than ultrasonic groups. “C” (green box), “D” (purple box) and “E” (yellow box) areas refer to the compounds whose contents are significantly higher in 250 W group, 500 W group and 750 W group, respectively.

3.3.2. Volatile flavor compounds analysis

For further exploring the differences of flavor profile among different groups, the VFCs in unsmoked bacon were identified by using GC-IMS Library Search. Meanwhile, the fingerprint plots and the peak intensity of identified VFCs were performed by using LAV software. A total of 55 VFCs were designated in four groups and presented in Fig. 4. However, a single VFC with high concentration can form multiple signals presenting as dimer or polymer as a result of the adducts formation between neutral molecules and reactants ions [12]. Besides, the VFC who has higher proton affinity than that of water is also easily to form dimer or polymer [12]. In present study, 10 dimers were recognized including nonanal, 1-octanal, benzaldehyde, heptanal, styrene, 2-heptanone, acetoïn, pentanal, 3-methylbutanal and ethyl acetate. Therefore, 45 VFCs were actually identified in four different groups and their respective peak intensities (calculated by peak volume) are listed in Table 1.

Fig. 4 shows the fingerprint plots of unsmoked bacon, which intuitively reflects the differences of VFCs at four different ultrasonic powers. After carefully observing the color degree of each point and combining the statistical results of each VFC peak intensity (Table 1), five areas (A-E in Fig. 4) were divided to characterize the differential VFCs among different groups. Area “A” showed the VFCs whose concentration was significantly lower in the 0 W group than the ultrasonic groups ($P < 0.05$). Thirty-two VFCs (monomer and dimer were considered as one VFC) were found in area “A” accounting for 71% of total identified VFCs. Only three VFCs including 2-butanone, 2,3-butanediol and acetic acid which were located in area “B” had a significantly higher concentration in the 0 W group than ultrasonic groups ($P < 0.05$). These results indicate that the levels of most VFCs were increased after ultrasound. Among ultrasonic groups, the significantly higher volatile compounds (SHVFCs) in the concentrations of 250, 500 and 750 W groups were illustrated in “C”, “D” and “E” areas, respectively. Although there were intersections in the SHVFCs of different ultrasonic groups, most SHVFCs were unique in each ultrasonic group, forming their own characteristic fingerprints. In addition, the numbers of SHVFCs were affected by ultrasonic powers with the largest in the 250 W group and the smallest in the 750 W group. These results suggest that low ultrasonic power could be more conducive to improve the concentration of VFCs in unsmoked bacon.

The total 45 VFCs identified in unsmoked bacon could be classified into six categories including hydrocarbons (2), aldehydes (14), ketones (6), alcohols (14), acids (4) and esters (5). As demonstrated in Table 1, the peak intensity of total VFCs was significantly higher in ultrasonic groups than the 0 W group, which is in line with the result of de Lima Alves et al. [21] who revealed that ultrasound improved the level of flavor compounds in fermented sausage. Among ultrasonic groups, the 250 W group had the highest total VFCs intensity, followed by the 500 W and 750 W groups. In addition, the 250 W group had the highest total aldehydes, total ketones, total alcohols and total hydrocarbons while the peak intensity of each category (except ketones) was lowest in the 0 W group suggesting that these two groups had the most differences in the peak intensity of VFCs. These results are in accordance with the findings of sensory evaluation and E-nose.

3.3.3. OPLS-DA

For screening the significantly differential VFCs between the 0 W and ultrasonic groups, the model of OPLS-DA was built. Considering the 250 W group had the highest peak intensity of total VFCs, the highest score of overall aroma and the greatest differences of flavor profile in E-nose analysis compared with the 0 W group, the 250 W group was thus selected as the representative of ultrasound groups for comparing the flavor profile differences with the 0 W group with the help of OPLS-DA model.

Firstly, the score plot of OPLS-DA is demonstrated in Fig. 5(A). The $Q^2$, $R^2X$ and $R^2Y$ were 0.997, 0.94 and 0.999 respectively suggesting the model was excellent. The groups of 0 W and 250 W were clearly separated in score plot suggesting a significant flavor differences between these two groups. Next, the plot of permutation test was performed for validating OPLS-DA reliability. In Fig. 5(B), the intercept of $Q^2$ regression line was negative and original $Q^2$ value was greater than any permuted Y vector, indicating the model was highly reliable [13]. At last, S-plot and variable importance for the projection (VIP) plot were applied to screen the significantly differential VFCs. In general, the compound with VIP > 1 was considered as significantly differential VFC [13]. As shown in Fig. 5(D), eight compounds (nonanal, heptanal, octanal, n-hexyl acetate, 3-methylbutanal, n-propyl acetate, styrene and 2-octanol) were screened as significantly differential VFCs which were marked with red star in S-plot (Fig. 5(C)).
Table 1
Intensity of volatile flavor compounds identified by GC-IMS in unsmoked bacon at different ultrasonic powers.

| No. | Compounds                             | Intensity (a.u.) |
|-----|---------------------------------------|-----------------|
| 1   | Nonanal                               | 13680.2 × 205.07<sup>tc</sup> |
| 2   | 2-Octanal                              | 2250.7 × 38.07<sup>tc</sup>  |
| 3   | Phenylacetaldehyde                     | 911.67 × 122.65<sup>tc</sup> |
| 4   | Octanal                                | 2450.31 × 128.52<sup>tc</sup> |
| 5   | Benzaldehyde                           | 2619.28 × 226.87<sup>tc</sup> |
| 6   | Heptanal                               | 4512.18 × 36.58<sup>tc</sup>  |
| 7   | Menthone                               | 961.15 × 9.38<sup>tc</sup>   |
| 8   | Hexanone                               | 3741.12 × 39.20<sup>tc</sup>  |
| 9   | Pentanal                               | 3033.91 × 79.21<sup>bc</sup>  |
| 10  | 2-Methylbutanal                        | 5817.53 × 306.85<sup>bc</sup> |
| 11  | Methylpropanol                         | 2413.46 × 37.98<sup>bc</sup>  |
| 12  | Isobutanol                             | 1707.76 × 33.17<sup>bc</sup>  |
| 13  | Propanal                               | 2407.35 × 288.68<sup>bc</sup> |
| 14  | Butanal                                | 1641.39 × 143.69<sup>bc</sup> |
| 15  | Total ketones                          | 42414.09 × 573.51<sup>bc</sup> |
| 16  | 2-Hexanone                             | 472.14 × 105.78<sup>bc</sup>  |
| 17  | 2-Heptanone                            | 927.56 × 56.65<sup>bc</sup>  |
| 18  | Acetoin                                | 18030.09 × 1617.22<sup>a</sup> |
| 19  | 2,3-Pentanediene                       | 779.20 × 15.15<sup>bc</sup>  |
| 20  | 2-Butanone                             | 1842.85 × 44.33<sup>bc</sup>  |
| 21  | 2,3-Butanediene                        | 1282.67 × 105.23<sup>bc</sup> |
| 22  | Total ketones                          | 23334.51 × 172.02<sup>bc</sup> |
| 23  | Terpinene                              | 1848.48 × 241.71<sup>bc</sup> |
| 24  | Phenylethanol                          | 2900.32 × 258.63<sup>bc</sup> |
| 25  | 1-Octanol                              | 723.43 × 16.95<sup>bc</sup>   |
| 26  | 2-Octanone                             | 1509.56 × 70.94<sup>bc</sup>  |
| 27  | 1-Octen-3                              | 1592.34 × 260.31<sup>bc</sup> |
| 28  | Methylbenzenemethanol                  | 270.66 × 30.68<sup>bc</sup>   |
| 29  | n-Hexanal                              | 585.20 × 71.35<sup>bc</sup>   |
| 30  | 2,3-Butanediol                         | 2164.36 × 143.70<sup>bc</sup> |
| 31  | 2-Hexanone                             | 1062.79 × 14.42<sup>bc</sup>  |
| 32  | 1-Pentanone                            | 339.84 × 10.88<sup>bc</sup>   |
| 33  | 1-Butanone                             | 925.85 × 37.03<sup>bc</sup>   |
| 34  | 1-Propanol                             | 1566.54 × 26.47<sup>bc</sup>  |
| 35  | Ethanol                                | 3488.38 × 126.53<sup>bc</sup> |
| 36  | 3-Octanone                             | 340.53 × 21.86<sup>bc</sup>   |
| 37  | Total alcohols                         | 19300.43 × 322.56<sup>bc</sup> |
| 38  | Hexanolic acid                         | 728.3 × 51.61<sup>bc</sup>    |
| 39  | Pentanolic acid                        | 432.17 × 23.45<sup>bc</sup>   |
| 40  | Acetic acid                            | 1221.26 × 13.59<sup>bc</sup>  |
| 41  | Propanolic acid                        | 393.94 × 10.88<sup>bc</sup>   |
| 42  | Butyric acid                           | 925.85 × 37.03<sup>bc</sup>   |
| 43  | Propanoic acid                         | 1226.25 × 24.57<sup>bc</sup>  |
| 44  | Propanoic acid                         | 3150.03 × 21.86<sup>bc</sup>  |
| 45  | Total acids                            | 19300.43 × 322.56<sup>bc</sup> |
| 46  | Hexanoic acid                          | 359.10 × 31.49<sup>bc</sup>   |
| 47  | Methyl benzoate                        | 775.42 × 63.66<sup>bc</sup>   |
| 48  | n-Hexyl acetate                        | 342.27 × 43.45<sup>bc</sup>   |
| 49  | Ethyl acetate                          | 2927.07 × 24.57<sup>bc</sup>  |
| 50  | Propanoic acid                         | 3237.98 × 30.68<sup>bc</sup>  |
| 51  | Butyl butyrate                         | 12469.26 × 592.06<sup>bc</sup> |
| 52  | Ocimene                                | 1167.21 × 192.66<sup>bc</sup> |
| 53  | Ocimene                                | 2039.07 × 81.32<sup>bc</sup>  |
| 54  | Total hydrocarbons                     | 3207.18 × 267.34<sup>bc</sup> |
| 55  | Total hydrocarbons                     | 138354.62 × 498.03<sup>bc</sup> |
| 56  | Total alcohols                         | 103562.98 × 1840.00<sup>bc</sup> |

<sup>A-D</sup>Identical letters in the same row indicate that there was no significant difference in different groups (P > 0.05).

3.3.4. Significantly differential VFCs analysis

The eight significantly differential VFCs included 4 aldehydes, 2 esters, 1 hydrocarbon, and 1 alcohol. It was noticeable that aldehyde numbers accounted for 50%, indicating that they were the most affected categories by ultrasound. In addition, the eight VFCs could be derived from three metabolic pathways including lipid oxidation, Strecker degradation and acetic acid reaction.

The first metabolic pathway was lipid oxidation with four VFCs (nonanal, heptanal, octanal and 2-octanal) being involved [22]. Nonanal, octanal and heptanal were all linear aldehydes and were originated from the oxidative degradation reaction of unsaturated fatty acids [22]. Nonanal contributed to fruity and sweet aroma [161] as one of the characteristic aroma in Jinhuua ham [23]. In present study, nonanal had the highest intensity among all identified VFCs and its peak intensity was increased by 103.79% in the 250 W group compared with the 0 W group. Heptanal was responsible for cured and fatty aroma [24] and its peak intensity was increased by 97.13% after ultrasonic treatment at 250 W. It was also identified in smoked bacon which could contribute to sensory attributes [25]. Octanal was associated with meaty, fresh and green aroma [26] and 168.39% growth was observed in its peak intensity in the 250 W group. Octanal was a common flavor compound in dry-cured meat products and was reported as the most abundant flavor in French dry-cured hams [26]. Once nonanal, heptanal and octanal possessed a low flavor thresholds and had relatively high peak intensity, and thus we speculated that they were the major contributors to flavor improvement of unsmoked bacon after ultrasonic treatment. 2-Octanal was originated from oleic acid oxidation but it had limited contribution to meat flavor due to its high threshold [27-28].

The second metabolic pathway was Strecker degradation and two VFCs (3-methylbutanal and styrene) were involved with this pathway [29,30]. 3-Methylbutanal possessed a fruity and cheesy aroma, and had...
a relatively high concentration in present study. It could be a major contributor to flavor development after ultrasound treatment due to a low flavor threshold [26]. Styrene was derived from the transformation of phenylalanine which was an aromatic hydrocarbon as well known for high threshold [30], thus exerting little contribution to the flavor improvement.

The third metabolic pathway was esterification reaction by which n-hexyl acetate and n-propyl acetate were generated [31]. Esters were formed by the action of alcohols and carboxylic acids and were linked with fruity, sweet or fatty odor. n-Propyl acetate was considered as a main aroma compound in Jinhua ham and its intensity was increased with the extended processing period. n-Hexyl acetate exerted a key action for the characteristic aroma of heated yeast fermented pork [31,32]. Since ester compounds had low threshold [31], the significantly increased peak intensities of n-hexyl acetate and n-propyl acetate in present study might well be conductive to flavor development.

Based on the above analysis, nonanal, heptanal, octanal, 3-methylbutanal, n-hexyl acetate and n-propyl acetate could be the crucial contributors to the improvement of overall aroma of unsmoked bacon after ultrasound treatment.

3.4. Metabolic mechanism exploration of ultrasound on flavor improvement

Lipid is a crucial precursor of VFCs formation in meat products and the degree of lipid oxidation is closely related to distinctive flavor formation [33]. In present study, considering the half of the eight significantly differential VFCs were derived from lipid oxidation, thus the lipid oxidation origin could be the crucial pathway for improving the flavor characteristic of unsmoked bacon by ultrasonic treatment. Lipid oxidation is consisted of enzymatic oxidation and autooxidation, and enzyme-oxidation has been considered as a key pathway for the flavor improvement of dry-cured meat product [16,17,34,35]. Besides, ultrasound could impact the activities of endogenous enzymes by mechanical action and cavitation effect of rat and beef skeletal muscles [36,37]. Based on the above arguments, the effects of ultrasound on enzymatic oxidation were further explored for better understanding the metabolic mechanism of flavor improvement.

Table 2

| Enzymes          | Stage | 0 W  | 250 W | 500 W | 750 W |
|------------------|-------|------|-------|-------|-------|
| Phospholipase    | M     | 59.79±3.23 | 107.39±3.97 | 83.14±3.10 | 79.45±3.41 |
|                  | N     | 15.34±1.36  | 22.32±2.61  | 18.75±1.52 | 18.31±1.52 |
| Acid lipase      | M     | 16.70±1.18 | 38.96±2.61 | 23.05±1.52 | 22.81±1.48 |
|                  | N     | 11.89±2.28 | 22.80±1.58 | 18.20±1.35 | 16.45±1.23 |
| Neutral lipase   | M     | 51.56±1.56 | 96.39±1.56 | 72.65±1.56 | 70.34±1.56 |
|                  | N     | 38.55±0.50 | 30.56±0.50 | 30.15±0.50 | 30.69±0.50 |

Different letters in the same row mean significant difference in different ultrasonic groups (P < 0.05); M means the 0 d of dry-ripening (after ultrasound treatment) and N means the ending of dry-ripening.

Fig. 5. OPLS-DA plots of the 0 W and 250 W groups. 4(A): OPLS-DA score plot (0 W, cyan dots; 250 W, green dots); 4(B): permutation plot; 4(C): S-plot (volatile flavor compound marked in red means its VIP > 1); 4(D): VIP(pred) (variable importance for predictive components) plot (the volatile flavor compound whose VIP < 1 is not shown).
end of dry-ripening stage, the remaining activities of three lipases were higher than all the 0 W group had higher remaining activity of neutral lipase than all crucial enzymes for flavor improvement of ultrasonic groups. However, than the 0 W group at this stage, indicating these two lipases were content and the increase of salt content during the dry-ripening period the fact that ultrasound with proper power could promote the release of its weaker tolerance to the rising salt content after ultrasound treatment. of neutral lipase activity in ultrasonic groups might well be attributed to ultrasonic groups at the end of dry-ripening stage. Ultrasound could compared with the 0 W group ( ).

3.4.1. Lipase activity

Changes of lipase activity, FFA concentration, LOX activity and TBARS investigating the effects of ultrasound on enzymatic oxidation, the good index to characterize the degree of lipid oxidation. Therefore, for -

3.4.2. Free fatty acid

For verifying whether ultrasound increased the level of lipid hydrolysis with the action of lipases, the composition and the concentration of FFAs were determined. A total of 15 FFAs were identified in unsmoked bacon and they were consisted of 7 saturated fatty acids (SFAs), 4 monounsaturated fatty acids (MUFTAs) and 4 polyunsaturated fatty acids (PUFTAs). As shown in Table 3, the concentration of total FFAs was significantly increased for all ultrasonic groups in comparison with the 0 W group ( ) indicating that ultrasound did improve the FFAs concentration. This result could be explained by the increased acid lipase and phospholipase activities. As for , no significant differences were observed among all groups (except ) but ultrasonic groups had two more MUFAs (C17:1 and C20:1) than the 0 W group. Noticeably, the concentration of was remarkably improved after ultrasound treatment compared with the 0 W group. Meanwhile, the 250 W group had the highest concentration of while there were no obvious differences between the 500 W and 750 W groups, which are in line with the results of acid lipase and phospholipase activities. PUFTAs are the most important origin of VFCs since they are more prone to be oxidized due to their higher unsaturation than SFA and MUFA . Therefore, the higher concentration of PUFTAs might exert greater contribution to flavor generation. In addition, it is worth noting that the FFAs results are also consistent with the peak intensity of four significantly differential VFCs (nonanal, heptanal, octanal, and 2-octanol) derived from lipid oxidation.

3.4.3. LOX activity

For validating the increase of oxidation degree of FFAs, LOX activity was further evaluated as demonstrated in Fig. 6. At the 0 d of dry-ripening stage (green columns in Fig. 6), a significant increased level in LOX activities was observed in all ultrasonic groups than the 0 W group ( ). This result suggests that ultrasound treatment could enhance the LOX activities which is beneficial for the oxidation reaction of PUFA during dry-ripening period. At the end of dry-ripening stage (orange columns in Fig. 6), the remaining LOX activities of each group were all evidently decreased which could be attributed to the inhibition effects of increasing salt content and decreasing water content during dry-ripening [36]. However, each ultrasonic group still had significantly higher remaining LOX activity than the 0 W group, suggesting that the increased LOX activity in ultrasonic groups contributed to the FFA ketones, etc.) with the involvement of LOX [16]. In addition, TBARS is a good index to characterize the degree of lipid oxidation. Therefore, for investigating the effects of ultrasound on enzymatic oxidation, the changes of lipase activity, FFA concentration, LOX activity and TBARS after ultrasound were determined.

3.4.1. Lipase activity

Table 2 shows the activities of phospholipase, and acid and neutral lipases of all groups at two main stages. At the 0 d of dry-ripening stage (just finished ultrasound treatment for ultrasonic groups), the three lipases activities were all significantly increased in ultrasonic groups compared with the 0 W group ( ), which could be explained by the fact that ultrasound with proper power could promote the release of lipase from lysosome and thus enhance the lipase activities [36]. At the end of dry-ripening stage, the remaining activities of three lipases were all obviously decreased in each group due to the decrease of water content and the increase of salt content during the dry-ripening period [36]. It was noticeable that the remaining activities of phospholipase and acid lipases were still significantly higher in the ultrasonic groups than the 0 W group at this stage, indicating these two lipases were crucial enzymes for flavor improvement of ultrasonic groups. However, the 0 W group had higher remaining activity of neutral lipase than all ultrasonic groups at the end of dry-ripening stage. Ultrasound could accelerate the salt penetration resulting in an inhibition effect on the lipase activity, and different types of enzymes had different sensitivity in salt content [36]. Thus, we speculated that the greater decrease degree of neutral lipase activity in ultrasonic groups might well be attributed to its weaker tolerance to the rising salt content after ultrasound treatment. Among ultrasonic groups, for both at 0 d and at the end of dry-ripening stage, the three lipases activities were the highest in 250 W group (except neutral lipase activity in final product) while there were no significant differences between the 500 W and 750 W groups ( ).

Table 3 Concentration of free fatty acids (µg/mg lipid) at different ultrasonic powers.

| No. | Name       | 0 W | 250 W | 500 W | 750 W |
|-----|------------|-----|-------|-------|-------|
| 1   | C10:0      | 1.24 ± 0.11 A | 1.17 ± 0.07 A | 1.14 ± 0.07 A | 0.31 ± 0.06 A |
| 2   | C11:0      | 4.10 ± 0.07 A | 3.99 ± 0.22 A | 4.00 ± 0.31 A | 3.93 ± 0.43 A |
| 3   | C12:0      | 2.91 ± 0.12 A | 2.76 ± 0.10 A | 2.57 ± 0.20 A | 2.66 ± 0.11 A |
| 4   | C14:0      | 9.99 ± 0.31 A | 9.83 ± 0.66 A | 10.21 ± 0.57 A | 10.57 ± 0.54 A |
| 5   | C16:0      | 44.10 ±      | 44.78 ±      | 51.14 ±      | 58.21 ±      |
| 6   | C18:0      | 1.26 ±       | 2.13 ±       | 8.11 ±       | 2.63 ±       |
| 7   | C20:0      | 28.80 ±      | 8.70 ±      | 31.56 ±      | 30.69 ±      |
| 8   | C22:0      | 0.76 ±       | 0.33 ±       | 3.15 ±       | 1.83 ±       |
| 9   | C24:0      | 1.64 ± 0.30 A | 1.79 ± 0.04 A | 2.00 ± 0.17 A | 1.91 ± 0.13 A |
| 10  | C16:1      | 0.21 ± 0.04 A | 0.27 ± 0.03 A | 0.35 ± 0.11 A | 0.30 ± 0.11 A |
| 11  | C17:1      | ND          | 0.26 ± 0.04 A | 0.29 ± 0.03 A | 0.19 ± 0.01 B |
| 12  | C18:19c    | 5.39 ± 0.53 A | 6.44 ± 2.58 A | 7.56 ± 1.22 A | 9.31 ± 1.76 A |
| 13  | C20:1      | ND          | 0.15 ± 0.11 A | 0.24 ± 0.06 A | 0.27 ± 0.19 A |
| 14  | C12:0      | 5.60 ±       | 7.12 ±       | 8.44 ±       | 10.07 ±      |
| 15  | C18:20c    | 6.08 ± 1.10 A | 17.35 ± 1.12 A | 14.75 ± 1.30 A | 17.25 ± 1.30 A |
| 16  | C18:3c     | 0.54 ± 0.16 C | 3.32 ± 0.40 C | 1.54 ± 0.50 B | 0.88 ± 0.23 C |
| 17  | C20:4c     | 0.80 ± 0.13 C | 1.51 ± 0.18 C | 1.09 ± 0.14 B | 1.18 ± 0.18 B |
| 18  | C24:1c     | 11.02 ±      | 24.21 ±      | 16.94 ±      | 17.71 ±      |
| 19  | Total all  | 109.40 ±     | 124.34 ±     | 128.00 ±     | 126.79 ±     |

\( \sum \text{SFA} \) total saturated fatty acids; \( \sum \text{MUFA} \) total monounsaturated fatty acids; \( \sum \text{PUFA} \) total polyunsaturated fatty acids. Identical letters in the same row indicate that there was no significant difference in different groups ( ).

These results imply that lipase activity was also influenced by ultrasonic power and that excessive power had a contrary effect on lipase activity. To sum up, ultrasound improved the lipases activities in particular for acid lipase and phospholipase activities, which might well lead to the improvement of lipid hydrolysis and further promote the generation of free fatty acids.
oxidation and flavor improvement. The above results also explained the results that ultrasonic groups had significant higher peak intensities of nonanal, heptanal, octanal and 2-octanol compared with the 0 W group (Table 1). In addition, the 250 W group had the highest LOX activities, which is in concert with the highest peak intensity of nonanal, heptanal, octanal and 2-octanol in the 250 W group (Table 1).

3.4.4. TBARS

TBARS is the most used index reflecting lipid oxidation degree. For further proving the increase of lipid oxidation after ultrasonic treatment, TBARS in final products were determined and shown in Fig. 7. The TBARS of all ultrasonic groups was distinctively higher than that of the 0 W group (P < 0.05) confirming that ultrasound improved the level of lipid oxidation. The same findings were also found in other literature which reported that ultrasound increased the TBARS of spiced beef and Italian salami [5,39]. To summarize, ultrasonic treatment significantly increased the lipases’ activities and thus promoted the generation of PUFA’s which were further oxidized to more VFCs with the action of LOX activity.

4. Conclusion

Ultrasound treatment remarkably improved the overall flavor characteristic of unsmoked bacon by sensory evaluation, E-nose and GC-IMS analysis. Six volatile flavor compounds (nonanal, octanal, heptanal, 3-methylbutanal, n-hexyl acetate and n-propyl acetate) were considered as crucial contributors closely related to flavor profile improvement. Enzymatic oxidation was confirmed as an important metabolic pathway responsible for the development of flavor characteristic of unsmoked bacon after ultrasonic treatment. Further research should be paid attention to explore the mechanism of ultrasound on autodissociation occurred in unsmoked bacon.

CRediT authorship contribution statement

Jian Zhang: Conceptualization, Data curation, Methodology, Software, Formal analysis, Writing – original draft, Writing – review & editing. Wangang Zhang: Supervision, Resources. Lei Zhou: Validation. Ruyu Zhang: Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was financially supported by Supported by China Agriculture Research System of MOF and MARA, and the earmarked fund for

Jiangsu Agricultural Industry Technology System (JATS(2020)425).

References

[1] E.P. Emmerson, Improving the sensory and nutritional quality of smoked meat products BT-Procesed Woods meats, in: J.P. Kerry, J.P. Kerry (Eds.), Woodhead publishing series in food science, technology and nutrition, Woodhead Publishing, 2011, pp. 527–545.
[2] E. Ledesma, M. Rendueles, M. Diaz, Benzo(a) pyrene penetration on a smoked meat product during smoking time, Food. Addit. Contam. A 31 (10) (2014) 1688–1698.
[3] T.S. Awad, H.A. Moharram, O.E. Shaltout, D. Aker, M.M. Yousef, Applications of ultrasound in analysis, processing and quality control of food: A review, Food Res. Int. 48 (2) (2012) 410-427.
[4] L. Zhou, J. Zhang, Y. Yin, W. Wang, Y. Yang, Effects of Ultrasound-Assisted Emulsification on the Emulsifying and Rheological Properties of Myofibrillar Protein Stabilized Pork Fat Emulsions, Foods 10 (6) (2021) 1201.
[5] J. Zhang, Y. Zhang, Z. Zou, W. Zhang, Effects of ultrasound-assisted cooking on quality characteristics of spiced beef during cold storage, Food Sci. Technol. 136 (2021) 110359, https://doi.org/10.1016/j.jfsts.2021.10359.
[6] E. Pena-Gonzales, A.D. Alarcon-Rojjo, I. Garcia-Galicia, L. Carrillo-Lopez, M. Huerta-Jimenez, Ultrasound as a potential process to tenderize beef: Sensory and technological parameters, Ultrasom. 53 (2019) 134–141.
[7] D.-C. Kang, A.-R. Wang, G.-H. Zhou, W.-G. Zhang, S.-M. Xu, G.-P. Guo, Power ultrasound on mass transport of beef: Effects of ultrasound intensity and NaCl concentration, Innov. Food Sci. Emerg. 35 (2016) 36–44.
[8] H. Song, J. Liu, GC-O-MS technique and its applications in food flavor analysis, Food Res. Int. 114 (2018) 187–198.
[9] W. Grosch, Detection of potent odorants in foods by aroma extract dilution analysis, Trends Food Sci. Tech. 4 (3) (1993) 68–73.
[10] S. Wang, H. Chen, B. Sun, Recent progress in food flavor analysis using gas chromatography–ion mobility spectrometry (GC–IMS), Food Chem. 315 (2020) 126358, https://doi.org/10.1016/j.foodchem.2019.126358.
[11] N. Arroyo-Manzanares, A. Martin-Gomez, N. Jurado-Campos, R. Garrido-Delgado, C. Arce, L. Arce, Target vs spectral fingerprint data analysis of Iberian ham samples for avoiding labelling fraud using headspace–gas chromatography–ion mobility spectrometry, Food Chem. 246 (2018) 65–73.
[12] F. Wang, Y. Gao, H. Wang, B. Xi, X. He, X. Yang, W. Li, Analysis of volatile compounds and flavor fingerprint in Jingyuan lamb of different ages using gas chromatography–ion mobility spectrometry (GC–IMS), Meat Sci. 175 (2021) 108449, https://doi.org/10.1016/j.meatsci.2021.108449.
[13] J. Zhang, W. Zhang, L. Xing, Effects of ultrasound on the taste components from aqueous extract of unsmoked bacon, Food Chem. 365 (2021) 130411, https://doi.org/10.1016/j.foodchem.2021.130411.
[14] Y. Bi, G. Zhou, D. Pan, Y. Wang, Y. Dang, J. Liu, M. Jiang, J. Cao, The effect of coating incorporated with black pepper essential oil on the lipid deterioration and aroma quality of Jinhua ham, J. Food Meas. Charact. 13 (4) (2019) 2740–2750.
[15] H. Hou, C. Liu, X. Lu, D. Fang, Q. Hu, Y. Zhang, L. Zhao, Characterization of flavor frame in shiitake mushrooms (Lentinula edodes) detected by HS-GC-IMS coupled with electronic tongue and sensory analysis: Influence of drying techniques, Food Sci. Technol. 146 (2021), 111402.
[16] L. de Lima Alves, J.Z. Donadel, D.R. Athayde, M.S. da Silva, B. Klein, M. B. Fagundes, C.R. de Menezes, J.S. Barin, P.C.B. Campagnol, R. Wagner, A. J. Cichoski, Effect of ultrasound on proteolysis and the formation of volatile compounds in dry fermented sausages, Ultrason. Sonochem. 67 (2020) 105161, https://doi.org/10.1016/j.ultrasone.2019.105161.
[17] T. Wang, Y.T. Jiang, J.X. Cao, Y.J. Chen, X. Sun, X. Zeng, D. Pan, C. Ou, N. Gan, Study on lipoylsis-oxidation and volatile flavour compounds of dry-cured goose with different curing salt content during production, Food Chem. 190 (2016) 33–40.
[18] Z. Zou, D. Kang, R. Liu, J. Q. Gou, W. Zhang, Effects of ultrasound assisted cooking on the chemical profiles of taste and flavor of spiced beef, Ultrason. Sonochem. 46 (2018) 36–45.
[19] D. Dave, A.E. Ghaly, Meat spoilage mechanisms and preservation techniques: a critical review, Am. J. Agric. Biol. Sci. 6 (4) (2011) 486–510.
[20] A. Maggiolino, J.M. Lorenzo, R. Marino, A. della Malva, P. Centoducati, P. De Palo, Foal meat volatile compounds: Effect of vacuum ageing on semimembranosus muscle, J. Sci. Food Agr. 99 (4) (2019) 1660–1667.
[21] L. de Lima Alves, I.G. Donadel, D.R. Athayde, M.S. da Silva, B. Klein, M. B. Fagundes, C.R. de Menezes, J.S. Barin, P.C.B. Campagnol, R. Wagner, A. J. Cichoski, Effect of ultrasound on proteolysis and the formation of volatile compounds in dry fermented sausages, Ultrason. Sonochem. 67 (2020) 105161, https://doi.org/10.1016/j.ultrasone.2020.105161.
[22] E. Sabio, M.C. Vidal-Aragón, M.J. Bernalte, J.L. Gata, Volatile compounds present in six types of dry-cured ham from south European countries, Food Chem. 61 (4) (1998) 493–502.
[23] D. Liu, G. Zhou, X. Xu, Study on key odor compounds of Jinhua ham, Journal of Nanjing Agricultural University 32 (2) (2009) 173–176.
[24] M. Narváez-Rivas, E. Gallardo, M. León-Camacho, Analysis of volatile compounds from Iberian hams: a review, Graus Aceites 63 (4) (2012) 432–454.
[25] E. Saldana, L. Saldarriaga, J. Cabreya, R. Siche, J.H. Behrens, M.M. Selani, M.A. de Almeida, I.D. Silva, J.S. Silva Pinto, C.J. Contreras-Castillo, Relationship between volatile compounds and consumer-based sensory characteristics of bacon smoked with different Brazilian woods, Food Res. Int. 119 (2019) 839–849.
[26] D.L. García-González, R. Aparicio, R. Aparicio-Ruiz, Volatile and amino acid profiling of dry cured hams from different swine breeds and processing methods, Molecules 18 (4) (2013) 3927–3947.

[27] C.M. Sánchez-Peña, G. Luna, D.L. García-González, R. Aparicio, Characterization of French and Spanish dry-cured hams: influence of the volatiles from the muscles and the subcutaneous fat quantified by SPME-GC, Meat Sci. 69 (4) (2005) 635–645.

[28] L.J. van Gemert, Compilations of odour threshold values in air, water and other media, Boelens Aroma Chemical Information Service, Huizen, The Netherlands, 2003.

[29] M. Flores, C.C. Grimm, F. Toldrà, A.M. Spanier, Correlations of sensory and volatile compounds of Spanish “Serrano” dry-cured ham as a function of two processing times, J. Agr. Food Chem. 45 (6) (1997) 2178–2186.

[30] F.J. Hidalgo, R. Zamora, Amino acid degradations produced by lipid oxidation products, Crit. Rev. Food Sci. 56 (8) (2016) 1242–1252.

[31] D. Liu, Lu. Bai, X.i. Feng, Y.P. Chen, D. Zhang, W. Yao, H. Zhang, G. Chen, Y. Liu, Characterization of Jinhua ham aroma profiles in specific to aging time by gas chromatography-ion mobility spectrometry (GC-IMS), Meat Sci. 168 (2020) 108178, https://doi.org/10.1016/j.meatsci.2020.108178.

[32] X. Li, S.Q. Liu, Effect of thermal treatment on aroma compound formation in yeast fermented pork hydrolysate supplemented with xylose and cysteine, J. Sci. Food Agr. 2021.

[33] D.S. Mottram, Flavour formation in meat and meat products: a review, Food Chem. 62 (4) (1998) 415–424.

[34] C.S. Vestergaard, C. Schivazappa, R. Virgili, Lipolysis in dry-cured ham maturation, Meat Sci. 55 (1) (2000) 1–5.

[35] S. Wu, J. Yang, H. Dong, Q. Liu, X. Li, X. Zeng, W. Bai, Key aroma compounds of Chinese dry-cured Spanish mackerel (Scomberomorus niphonius) and their potential metabolic mechanisms, Food Chem. 342 (2021) 128381, https://doi.org/10.1016/j.foodchem.2020.128381.

[36] H. Feng, G.V. Barbosa-Cánovas, J. Weiss, Ultrasound technologies for food and bioprocessing Vol. 1 (2011) p. 599.

[37] A. Wang, D. Kang, W. Zhang, C. Zhang, Y. Zou, G. Zhou, Changes in calpain activity, protein degradation and microstructure of beef M. semitendinosus by the application of ultrasound, Food Chem. 245 (2018) 724–730.

[38] M.M. Campo, G.R. Nute, S.I. Hughes, M. Enzer, J.D. Wood, R.I. Richardson, Flavour perception of oxidation in beef, Meat Sci. 72 (2) (2006) 303–311.

[39] L. de Lima Alves, M. Stefanello da Silva, D.R. Martins Flores, D. Rodrigues Athayde, A. Roggia Ruviaro, D. da Silva Brum, V.S. Fagundes Batista, R. de Oliveira Mello, C. Ragagnin de Menezes, P.C. Battanello Campagnol, R. Wagner, J. Smanioto Barin, A.J. Cichoski, Effect of ultrasound on the physicochemical and microbiological characteristics of Italian salami, Food Res. Int. 106 (2018) 363–373.