International Food Research Journal 28(4): 763 - 779 (August 2021)
Journal homepage: *http://www.ifrj.upm.edu.my*

Characterisation of volatile flavour compounds in Chinese Chahua chicken meat using a spectroscopy-based non-targeted metabolomics approach

[1,2]Zhao, W. H., [1,2]*Wang, G. Y., [1,2]Xun, W., [1,2]Yu, Y. R., [1]Ge, C. R. and [1]*Liao, G. Z.

[1]*Livestock Product Processing and Engineering Technology Research Center of Yunnan Province, Yunnan Agricultural University, Kunming, 650201, China*
[2]*College of Food Science and Technology, Yunnan Agricultural University, Kunming, 650201, China*

Article history

Received: 7 May 2020
Received in revised form: 3 November 2020
Accepted: 15 November 2020

Keywords

Chahua chicken, volatile flavour substances, non-targeted metabolomics, odour activity value (OAV)

Abstract

Chahua chicken is a local chicken breed in China that is famous for its high-quality, sweet, and fresh meat. The present work describes a thorough study of the content and composition of the volatile flavour compounds in raw and cooked meat of 300-day-old Chinese Chahua chickens, as well as the odour characteristics of those flavour substances. The organic flavour compounds from the meat were studied using headspace solid-phase microextraction combined with gas chromatography-mass spectrometry, by applying a metabolomics-based method, and the main active substances were determined by their sensory threshold and odour activity value. Results revealed that 88 and 99 distinct volatile organic compounds (VOCs) were detected in the breast and leg meat, respectively, of raw Chahua chicken; whereas 149 and 151 VOCs were detected in the breast and leg meat, respectively, of cooked Chahua chicken. In general, it was determined that the overall flavour of Chahua chicken strongly depended on aldehydes and alcohols. Ten key aroma-inducing components namely 1-octene-3-ol, (*E*)-2-octenal, 2-methyl-butanal, hexanal, nonanal, octanal, 3-methyl-butanal, heptanal, 2-pentyl-furan, and disulphide-dimethyl contributed to the characteristic flavour of Chahua chicken meat. The results provided fundamental understanding of the composition and odour characteristics of flavour active substances in raw and cooked Chahua chicken meat which could help evaluate the overall volatile flavour characteristics of Chinese Chahua chicken, and inform industrial processors aiming to develop an effective quality standardisation strategies.

Introduction

The concept of metabonomics originated from the metabolic spectral analysis method proposed by Devaux *et al.* (1971). During the rapid development of genomics in the late 1990s, Oliver (1997) and Nicholson *et al.* (1999) proposed the concept of metabonomics which has become a central trend among omics technologies. Metabolomics has been applied to systematically identify the changes to metabolites in cells or organisms, specifically in the response to genetic perturbations or environmental changes (Yeole *et al.,* 2019). Currently, chromatography and its combination technologies have been vastly employed in metabonomics studies owing to their high separation efficiencies and detection sensitivities. In particular, gas chromatography-mass spectrometry (GC-MS) has long been a commonly used analytical technique in metabolomics research because of its superior sensitivity and selectivity, and also due to the extensive databases that are available to assist in metabolite identification (Hong *et al.,* 2012; Mamat *et al.,* 2019).

Volatile substances in food items can stimulate olfactory neurons in the nasal cavity and cause olfaction in the central nervous system. The flavour of meat is composed of taste and odour; so, the first way to judge the flavour of meat is to smell it and develop an intuitive feeling. This is also a common index by which consumers evaluate the quality of meat (Li *et al.,* 2015). Odour components are primarily volatile flavour compounds that are produced by the fat oxidation, thiamine degradation, and Maillard reaction during the processing and cooking of chicken meat. These complex chemical reactions are not unique only to poultry meat, as the flavours of beef, pork, and lamb can also be similar. This phenomenon indicates that there are similar amino acids and sugar flavour precursors in all types of meat; but, the different components of fats reflect different flavours. Poultry meat contains certain fats which produce unique flavours when combined with distinct odour compounds (Smith and Northcutt, 2009).

The study of flavour compounds in meat

*Corresponding author.
Email: ynkmwgy@ynau.edu.cn (Wang, G. Y.) ; liaoguozhou@ynau.edu.cn (Liao, G. Z.)

DOI: https://doi.org/10.47836/ifrj.28.4.13

involves qualitative and quantitative analyses of the volatile organic compounds (VOCs) they contain. Typically, GC-MS is applied for the separation and detection of flavour compounds in samples since it is a rapid and accurate technique for obtaining structural information about the compounds which can be verified based on a mass spectrometry library. There are various comprehensive two-dimensional gas chromatographic methods including combinations with time-of-flight mass spectrometry (GC×GC-TOF/MS) or ion mobility spectrometry (GC-IMS) (Fan *et al.,* 2019; Xi *et al.,* 2020) which can improve the accuracy or widen the scope of detection of scientific research. In a recent report, hundreds of volatile flavour compounds were identified in poultry meat, of which 18 are nonpolar heterocyclic VOCs with medium carbon chain lengths (Sun *et al.,* 2014).

Chahua chicken, also known as Dai nationality chicken, is mainly distributed in the southwestern frontier region of China. In 2000, the Ministry of Agriculture included it in the first list of protected livestock and poultry genetic resources at the national level. It is one of the six famous chicken species in Yunnan Province, and they live mainly in tropical rainforest environments. The name Chahua represents both the egg and meat. Chahua chicken is a domesticated relative of pheasant, with which it shares genetic character, and its meat is particularly delicious and known for its excellent quality. However, to the best of our knowledge, very few studies have focused on Chahua chicken, especially in terms of the characteristic volatile flavour compounds in the meat. Therefore, the present work investigated the main flavour substances in raw and cooked Chahua chicken meat, examined using a headspace solid-phase microextraction GC-MS (HS-SPME-GC-MS) strategy based on metabolomics. The main flavour of active substances in raw and cooked chicken meat were determined based on their sensory thresholds and odour activity values (OAVs). Understanding the composition and odour characteristics of flavour substances in raw and cooked chicken could help improve the flavour characteristics and quality of Chahua chicken throughout the production process, thereby supporting the development of the Chahua chicken industry.

Materials and methods

Biological materials and sample preparation

Chahua chickens were obtained from the experimental chicken farm at Yunnan Agricultural University, Kunming, China. Six 300-day-old Chahua chickens (three males and three females) were selected after being exposed to similar breeding conditions. The procedures for use of animals were approved by the Animal Care Committee of the College of Animal Science and Technology at Yunnan Agricultural University, Kunming, China. Following slaughter, the breast and leg meat were obtained as test samples, and the visible fat and fascia tissue were removed. Once trimmed, the breast and leg meat samples were randomly assigned into two groups, with three breast and three leg samples in each. One group served as the control group, and was not heated. The samples in the other group were each placed into a cooking bag, and heat-treated in an electric constant-temperature water bath until they were fully cooked (3 h) (Wen *et al.,* 2015; Qi *et al.,* 2018). The heated samples were allowed to equilibrate to room temperature, and then all samples were placed at -80°C before being analysed using HS-SPME-GC-MS.

Analytical methods

Chemicals

Hexane (purity > 99.99%) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China), and 2-methyl-3-heptanone (purity > 99.99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Isolation of volatile substances

Following the method described by Li *et al.* (2018), 4 g minced sample were transferred into 20 mL glass headspace vials, which were then sealed and incubated at 50°C for 15 min to equilibrate the system. The SPME fibre (DVB/CAR on PDMS 50/30 μm) was exposed to the headspace for an additional 30 min. The VOCs present in the Chahua chicken samples were detected and measured using an Agilent 7890B gas chromatograph, coupled to an Agilent 5977B mass spectrometer (Agilent Technologies Company Ltd., United States), and separated by a capillary DB-WAX column (30 m × 0.25 mm × 0.25 μm). The carrier gas was helium (99.99% purity), and the injector temperature was 260°C. The temperature of the GC oven was first held at 40°C for 5 min at a flow rate of 1.0 mL/min, increased to 250°C at a rate of 5°C/min, and maintained for 5 min. The gas chromatography parameters were: interface temperature = 260°C; electron energy = EI + 70 eV; full scanning range = 20 - 500 m/z; and MS source and quadrupole temperatures = 230°C and 150°C, respectively. The control group was treated exactly as the experimental group.

Analysis of flavour and aroma compounds

Qualitative analysis

The VOCs were identified preliminarily by matching their mass spectra or retention times with those of known compounds in the National Institute of Standards and Technology (NIST) spectral database. According to literature reports, such matching identification tests are considered reliable if the experimental spectral results have a matching degree that is greater than 800 (on a scale where the maximum value is 1000).

Quantitative analysis

An internal standard of 2-methyl-3-heptanone was prepared with a concentration of 200 ng/mL for use in semi-quantitative analysis (Pérez-Olivero *et al.,* 2013). The contents of volatile flavour substances in the sample were calculated based on the peak area of each VOC in the sample relative to the peak area of 2-methyl-3-heptanone with known concentration. The concentration of each VOC in the Chahua chicken sample was then calculated using Eq. 1 (Gu *et al.,* 2013):

$$\text{Conc. of VOC}\left(\frac{\text{ng}}{\text{g}}\right) = \frac{\text{Sample peak area}}{\text{Internal standard peak area}} \times \frac{\text{Internal standard conc.(200ng/mL)}}{\text{Sample weight}} \quad \text{(Eq. 1)}$$

Evaluation of the main volatile flavours

The odour activity value (OAV) method was used to evaluate the main flavour components of meat, and the OAV was calculated using Eq. 2 (Gu *et al.,* 2013):

$$\text{OAV} = \frac{C_i}{T_i} \quad \text{(Eq. 2)}$$

where, C_i = relative content of the given volatile flavour compound (ng/g), and T_i = olfactory threshold of the compound (μg/kg). An OAV < 1 indicates that the substance may not significantly contribute to the overall aroma, while an OAV > 1 indicates that the substance may significantly contribute to the overall aroma. Generally, substances with larger OAVs contribute more significantly to the overall aroma (Hausch *et al.,* 2015). Only flavour substances with OAV > 1 are reported in the present work.

Data analysis

All data were pre-processed using Microsoft Excel version 2007 software (Microsoft Office, USA). The results of GC-MS pre-processing generated a data matrix consisting of the retention times (RT), mass-to-charge ratio (*m/z*) values, and peak intensities. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed using SIMCA software (Version 14.1, Umetrics, Umea, Sweden). The reliabilities of the models were rigorously validated by a permutation test (n = 200). The data used were UV (for PCA) or Pareto (for OPLS-DA), and they were scaled before multivariate statistical analysis (Gu *et al.,* 2013; Hausch *et al.,* 2015). Distinct metabolites were identified by their variable importance for projection (VIP) values and p-values. Student's t-tests were conducted using IBM SPSS statistics 19.0 software (SPSS Inc., Chicago, IL, USA) to determine the significance of the results; $p < 0.05$ was considered to be statistically significant. Results were expressed as mean ± standard deviation (SD). The species and content of each VOC were plotted using Origin 2017 (Origin Lab Corp, Northampton, MA, USA), and the cluster heatmap of aroma compounds in Chahua chicken meat was created using TBtools software.

Results and discussion

Establishment and validation of metabolomics model

Based on the HS-SPME-GC-MS data obtained from raw and cooked Chahua chicken meat, the multivariate statistical analysis, orthogonal partial least squares discriminant analysis (OPLS-DA), and regression analysis were performed to establish and validate the metabolomics model. The results enabled the prediction of sample categories (Liu *et al.,* 2017), where the markers, VIP > 1 and $p < 0.05$, served as screening criteria to determine the main aroma components (Zhao *et al.,* 2019).

The unsupervised analysis (PCA-X) of the raw and cooked groups is shown in Figure 1A. The differences between the metabolic sample group and the original state were clear. The natural distribution of the breast and leg meat samples within the raw or cooked meat group displayed no obvious trend, but there was clear separation between the raw and cooked meat groups. To further evaluate the metabolic changes, a supervised multivariate statistical analysis was performed (Johan *et al.,* 2007). OPLS-DA models were established to compare the two groups of Chahua chicken meat samples. The parameters of R2 (cum) and Q2 (cum) represent the interpretability and predictability of each model, respectively. The R2X (cum) was 0.753, the R2Y (cum) was 0.994, and the Q2 (cum) was 0.974, thus indicating that both models were valid. A complete separation between the raw and cooked meat groups was observed (Figure 1B). In addition, permutation

tests (200 iterations) were carried out to prevent the overfitting of the OPLS-DA models (Figure 1C). The abscissa represents the correlation between the random and original group, and the vertical coordinates represent the R2 and Q2 scores. The slope of R2 (0.0, 0.531) was greater than 0, and the *y*-intercept of Q2 (0.0, -0.823) was less than 0.05, thus confirming that the model had sufficient predictability, and did not overfit the data (Liu *et al.,* 2016).

Metabolomics analysis of sample groups

An S-plot of OPLS score plots based on the GC-MS data was constructed to identify the discriminating metabolites (Figure 1D). Variables with higher absolute p[1] and p(corr) values (in the lower left or upper right corner of the plot, respectively) explained the separation between the raw and cooked meat groups. At a statistical significance level of 0.05, discriminating metabolites were identified if: (i) their variable importance for projection (VIP) values > 1, and (ii) there was a significant variation between different concentration (intensity) groups (*i.e., p* < 0.05). A total of 19 metabolites were established as potential markers, and their details are summarised in Table 1. Among these compounds, the VIP (10.5293) value of hexanal, which has flavour features similar to the grass or green plants contributed the most to the flavour of Chahua chicken, thus also increasing its overall aroma. The fruity flavour of the chicken was contributed by 3-methyl-2-butanol (VIP = 6.7640). Another primary component, 1-octen-3-ol (VIP = 5.9219), has been confirmed in many published reports to have a significant effect on the overall meat by increasing the fatty flavour. Betaine has sweet flavour; so, it was concluded that betaine aldehyde cation (VIP = 1.3904) detected in this experiment made an important contribution to the sweet flavour of the chicken meat. The 2-butanone (VIP = 1.1661) and acetaldehyde (VIP = 1.0962) compounds have pungent odours that can stimulate the sense of smell. Nonanal (VIP = 1.9929) and heptanal (VIP = 1.1157) have pleasant meat flavours, and octanal (VIP = 1.0222) has a sweet orange flavour. Benzaldehyde (VIP = 1.0084) is an organic compound formed when a hydrogen of benzene is replaced by an aldehyde group. This compound is most commonly used aromatic aldehyde and it has a special almond smell. Previous findings are in agreement with our results which showed that hexanal was one of the main odour compounds in boiled chicken. It has a herbaceous flavour, and can improve the general meat flavour of the chicken (Fan *et al.,* 2015). All the volatile compounds discussed herein had a unique aroma characteristics, VIP > 1 and *p*-value < 0.05, thus indicating that they each played an important role in the overall flavour of Chahua chicken.

Odour activity value analysis of volatile flavour compounds in chicken meat

The composition and content of volatile flavour compounds in the raw and cooked meat of Chahua chicken were analysed by HS-SPME-GC-MS. As shown in Table 2, more volatile flavour compounds were detected in cooked meat relative to raw meat. In order to distinguish the different groups and aroma components of Chahua chicken more intuitively, a heatmap of characteristic flavour differences was constructed. As shown in Figure 2A and Figure 3, the relative intensity increased from low (green) to high (red). A total of 88 types of volatile flavour compounds were detected in the raw breast meat, consisting of 29 alkanes, 18 alcohols, six aldehydes, five ketones, eight esters, ten acids, and 12 other compounds (sulphur-, nitrogen-, oxygen-, and/or heterocycle-containing compounds). In contrast, a total of 149 volatile flavour compounds were detected in the cooked breast meat group, including 36 alkanes, 25 alcohols, 20 aldehydes, nine ketones, 14 esters, 18 acids, and 27 other compounds (sulphur-, nitrogen-, oxygen-, and/or heterocycle-containing compounds). In addition, 99 volatile flavour compounds were detected in the raw leg meat, including 28 alkanes, 22 alcohols, ten aldehydes, four ketones, seven esters, 13 acids, and 15 other compounds, while the cooked leg meat contained 151 volatile flavour compounds, including 36 alkanes, 28 alcohols, 22 aldehydes, nine ketones, 12 esters, 18 acids, and 26 other compounds. The characteristic aroma compounds of Chahua chicken were evaluated by calculating the OAV value, and comparing it to the threshold value of characteristic odour compounds reported in the literature.

Evaluation of key aroma compounds in chicken meat

The main objective of the present work was to identify the key aroma compounds in Chahua chicken meat. Many previously reported results have shown that amino acids, peptides, nucleotides, and sugars all exist in meat, and the content of inosinic acid is relatively high (Jin *et al.,* 2015). Then, when heated with other compounds during the cooking process, the meat flavour is generated (Kim *et al.,* 2016). In order to further determine the contribution of the metabolites to the flavour and aroma of Chahua chicken samples, HS-SPME-GC-MS combined with multivariate statistical analysis was used to analyse the differences in metabolite content between the two

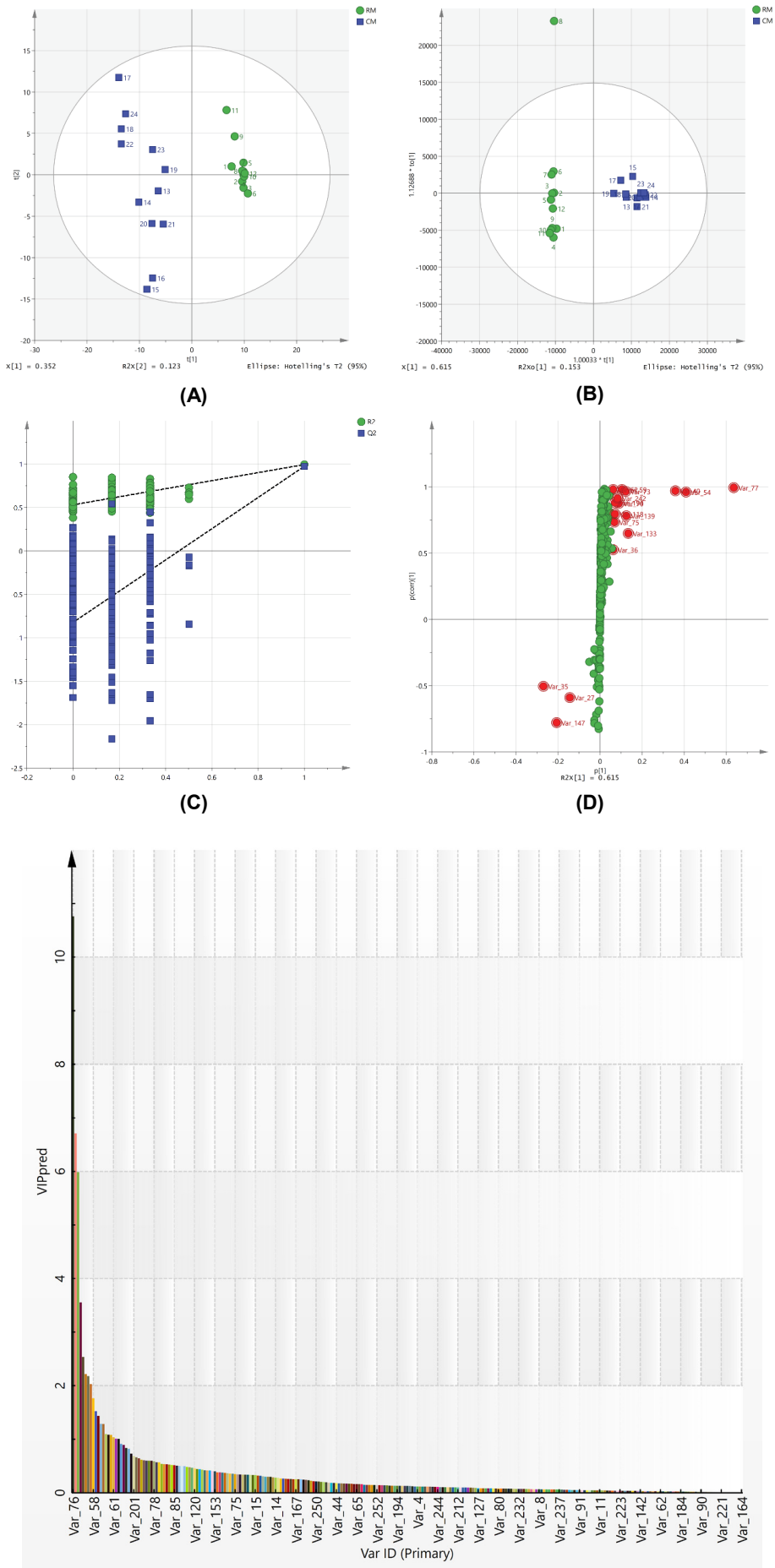

Figure 1. Identification of distinct active flavour substances in Chahua chicken meat via multivariate statistical analysis: PCA scores (A), OPLS-DA score plots (B), Permutations plots (C), S-plots (D), and VIP predictor plots (E). RM = raw meat; and CM = cooked meat.

Table 1. Identification of potential flavour markers in Chahua chicken meat.

Var ID	Compound	Similarity	CAS	RT (min.)	VIP pred.	*P*-value
Var_76	Hexanal	962	66-25-1	8.0773	10.5293	1.42E-16
Var_58	1-Pentanol	967	71-41-0	13.7187	1.7221	2.41E-13
Var_61	Octanal	967	124-13-0	14.6826	1.0222	6.66E-13
Var_72	Nonanal	964	124-19-6	17.7010	1.9983	2.10E-12
Var_39	1-Octen-3-ol	972	3391-86-4	19.3268	5.9219	1.69E-11
Var_53	3-Methyl-2-butanol	968	598-75-4	15.7642	6.7640	1.76E-10
Var_193	Guanidine	898	506-93-4	14.9291	1.1927	4.09E-10
Var_241	Betaine aldehyde cation	854	7418-61-3	11.3666	1.3904	2.11E-09
Var_69	Toluene	965	108-88-3	6.71138	1.4592	4.21E-09
Var_117	2-Butanone	940	78-93-3	3.46869	1.1661	2.90E-07
Var_138	8(9)-EpETE	930	851378-93-3	12.9191	2.0818	7.50E-06
Var_146	Carbon disulphide	926	75-15-0	2.15163	3.4185	9.27E-06
Var_74	Heptanal	962	111-71-7	11.4155	1.1157	0.0002
Var_132	2,2,4,6,6-Pentamethylheptane	937	13475-82-6	5.01896	2.2052	0.0007
Var_27	2,2'-Oxybis-ethanol	982	111-46-6	14.5249	2.3908	0.0020
Var_35	Dimethylsilanediol	977	1066-42-8	24.2308	1.0091	0.0053
Var_41	Benzaldehyde	971	100-52-7	20.9881	1.0084	1.60E-19
Var_68	Acetaldehyde	965	75-07-0	2.0428	1.0962	9.06E-07
Var_70	1-Hexanol	964	111-27-3	16.7060	1.0108	0.0071

groups, and the key metabolites were discussed. As shown in Table 1, 19 markers were selected because of their VIP > 1 and *p*-value < 0.05. Previous studies determined that hexanal, 1-octene-3-ol, nonanaldehyde, caprylic acid, and other volatile compounds have distinct effects on the flavour of chicken meat (Frank *et al.,* 2019). Hexanal is the main volatile decomposition product of linoleic acid (LA), which is *n*-6 polyunsaturated fatty acid (PUFA). It is usually associated with an unpleasant taste, used to track lipid oxidation and odour generation in food. Since dehydrated dairy products commonly have a cardboard odour, it is recommended for use as a potential marker to evaluate milk quality (Aronson *et al.,* 2007). Some scholars have pointed out that sulphur compounds may play an important role as flavour components of meat. Carbon disulphide was detected in the present work (VIP pred. = 3.4185). When mixed with other sulphur compounds, carbon disulphide produces a rotten radish flavour. To date, 621 volatile organic compounds including aldehydes, alcohols, and hydrocarbons have been identified in chicken (Takakura *et al.,* 2014). As shown in Figure 3A, most of thecompounds detected in Chahua chicken meat were alkanes, alcohols, and aldehydes. Therefore, in order to analyse the differences of characteristic aroma compounds in Chahua chicken meat, a heat map was generated according to the content of each flavour compound (Figure 2B). The tree diagram on the left is clustered based on the distribution of the aroma components in different groups, and the distribution within the same branch represents an analogous situation. The results of the heat mapping illustrated that the similarity between the RB and RL was greater than that between CB and CL. In addition, 19 primary aroma components were grouped into three categories. The first group, comprised of 2, 2'-oxybis-ethanol and carbon disulphide, had higher content in raw meat; whereas the content of the second group of substances including toluene, heptanal, acetaldehyde, and 8(9)-EpETE was higher in CB; and the third set of

Table 2. Composition and content of volatile flavour substances in the muscle of Chahua chicken (ng/g).

Compound	Breast		Leg	
	Raw	Cooked	Raw	Cooked
Alkane				
Toluene	—	107.21 ± 2.94[a]	—	88.29 ± 2.60[b]
Acetonitrile	2.06 ± 0.14[c]	6.74 ± 1.02[b]	2.19 ± 0.29[c]	10.70 ± 0.86[a]
Methylene chloride	4.03 ± 0.76[a]	2.37 ± 0.17[b]	4.70 ± 0.78[a]	2.31 ± 0.12[b]
Hexadecane	1.45 ± 0.27[d]	13.02 ± 1.35[b]	9.51 ± 1.19[c]	33.19 ± 1.44[a]
Decamethylcyclopentasiloxane	15.73 ± 1.77[c]	27.02 ± 1.42[a]	28.07 ± 1.97[a]	24.35 ± 1.73[b]
Dodecane	16.70 ± 1.28[c]	51.31 ± 3.15[a]	3.26 ± 0.27[d]	40.51 ± 1.77[b]
Tridecane	7.16 ± 1.48[c]	25.9 ± 1.84[a]	7.74 ± 1.18[c]	13.35 ± 0.27[b]
Octamethylcyclotetrasiloxane	15.16 ±1.55[c]	34.92 ± 0.77[a]	10.81 ± 0.81[d]	32.13 ± 2.07[b]
Hexamethylcyclotrisiloxane	13.04 ±1.78[c]	15.87 ± 1.97[b]	14.15 ± 0.82b[c]	25.76 ± 0.25[a]
n-Hexane	17.37 ±0.95[a]	3.85 ± 0.65[c]	2.70 ± 0.58[c]	8.03 ± 0.44[b]
Octane	1.68 ±0.25[c]	16.82 ± 1.03[a]	2.25 ± 0.48[c]	15.34 ± 0.96[b]
2,2,4,6,6-Pentamethylheptane	148.66 ± 1.71[d]	373.86 ± 3.40[b]	172.01 ± 5.71[c]	559.76 ± 6.84[a]
Pentane	0.24 ± 0.04[c]	34.03 ± 2.85[a]	1.61 ± 0.30[c]	22.36 ± 1.72[b]
2,4-Dimethylheptane	0.86 ± 0.08[c]	1.15 ± 0.13[b]	1.33 ± 0.26[b]	2.67 ± 0.10[a]
2,2,6-Trimethyloctane	1.07 ± 0.24[c]	10.00 ± 1.28[b]	7.43 ± 1.11[b]	66.33 ± 2.64[a]
Octamethyltrisiloxane	1.64 ± 0.13	1.26 ± 0.18	1.66 ± 0.72	1.30 ± 0.24
2,2,3,4-Tetramethylpentane	0.80 ± 0.08[c]	7.57 ± 0.50[a]	3.34 ± 0.21[b]	3.68 ± 0.75[b]
Decamethyltetrasiloxane	1.26 ± 0.07[b]	4.57 ± 0.47[a]	1.69 ± 0.32[b]	1.41 ± 0.38[b]
Pentyloxirane	—	12.88 ± 1.81	—	13.79 ± 1.09
Heptane	0.37 ± 0.06[d]	2.40 ± 0.58[a]	0.90 ± 0.10[c]	1.43 ± 0.23[b]
4,7-Dimethylundecane	6.72 ± 0.69[b]	19.05 ± 2.18[a]	—	1.64 ± 0.22[c]
2,4,6-Trimethyloctane	0.44 ± 0.02[b]	1.36 ± 0.04[a]	0.56 ± 0.10[b]	0.52 ± 0.03[b]
4-Ethyl-2,2,6,6-tetramethylheptane	1.30 ± 0.15[d]	14.16 ± 0.56[a]	4.29 ± 0.17[c]	12.90 ± 0.80[b]
2,2-Dimethylundecane	1.60 ± 0.30[b]	4.45 ± 0.83[a]	0.36 ± 0.02[c]	0.69 ± 0.13[c]
2,6,10-Trimethylpentadecane	0.56 ± 0.01[c]	3.71 ± 0.61[b]	3.38 ± 0.87[b]	12.20 ± 1.20[a]
3,7-Dimethylnonane	0.56 ± 0.11[b]	1.36 ± 0.23[a]	1.27 ± 0.29[a]	1.60 ± 0.24[a]
2,6,10-Trimethyldodecane	3.45 ± 0.25[c]	7.14 ± 0.93[a]	5.02 ± 0.75[b]	7.60 ± 0.48[a]
Octylcyclohexane	0.52 ± 0.03[c]	1.24 ± 0.23[b]	0.57 ± 0.14[c]	1.73 ± 0.15[a]
3,5-Dimethylheptane	6.98 ± 0.61[c]	45.66 ± 3.01[a]	37.25 ± 0.91[b]	44.17 ± 2.95[a]
Hexyloxirane	—	1.24 ± 0.19[b]	—	1.61 ± 0.22[a]

2,3,5-Trimethylhexane	—	0.41 ± 0.08[b]	—	0.66 ± 0.10[a]
Neopentane	4.30 ± 0.80[c]	95.61 ± 3.37[a]	6.74 ± 0.94[c]	24.86 ± 2.01[b]
3-Ethyl-2,2-dimethyloxirane	—	4.06 ± 0.62	—	4.14 ± 1.21
Isopropylcyclobutane	—	0.53 ± 0.03	—	0.51 ± 0.07
2,3,4-Trimethylhexane	—	2.90 ± 0.43[a]	—	1.53 ± 0.39[b]
2,3,4-Trimethyldecane	0.52 ± 0.09[b]	1.36 ± 0.44[a]	0.45 ± 0.10[b]	1.44 ± 0.37[a]
Alcohol				
1,4-Butanediol	0.51 ± 0.03[b]	3.46 ± 0.46[a]	0.46 ± 0.11[b]	3.60 ± 0.95[a]
10-Chloro-1-decanol	—	0.50 ± 0.04[c]	0.68 ± 0.05[b]	2.37 ± 0.26[a]
1-Octen-3-ol	19.75 ± 1.41[c]	1869.65 ± 20.90[b]	20.9 ± 0.57[c]	1975.09 ± 16.05[a]
2,3,4-Trimethyl-1-pentanol	1.68 ± 0.26[a]	1.40 ± 0.26[a]	0.25 ± 0.03[b]	1.75 ± 0.11[a]
2,3-Butanediol	0.15 ± 0.04[c]	1.30 ± 0.12[a]	0.34 ± 0.03[b]	0.17 ± 0.03[c]
2-Amino-4,6-dihydroxypyrimidine	—	4.05 ± 0.72[a]	—	1.36 ± 0.25[b]
2-n-Propyl-1-heptanol	0.76 ± 0.18[b]	5.03 ± 0.61[a]	0.90 ± 0.07[b]	4.65 ± 0.31[a]
2-Decanol	0.03 ± 0.00[d]	10.18 ± 0.45[a]	0.55 ± 0.10[c]	1.51 ± 0.08[b]
2-Mercaptoethanol	—	1.01 ± 0.16[b]	—	1.52 ± 0.28[a]
2-Octen-1-ol	—	1.40 ± 0.39	—	1.69 ± 0.18
3-Hexanol	1.37 ± 0.39[a]	—	0.24 ± 0.05[b]	—
3-Methyl-2-butanol	3.88 ± 0.50[c]	2400.22 ± 23.27[b]	1.49 ± 0.33[c]	2530.59 ± 14.52[a]
3-Methyl-3-buten-1-ol	2.78 ± 0.46[a]	0.53 ± 0.19[c]	1.49 ± 0.26[b]	0.36 ± 0.05[c]
4-Ethylcyclohexanol	0.16 ± 0.03[c]	5.33 ± 1.16[b]	—	8.33 ± 1.03[a]
6-Methyl-2-heptanol	0.31 ± 0.03[c]	2.62 ± 0.64[a]	0.35 ± 0.08[c]	1.07 ± 0.19[b]
α-Terpineol	0.77 ± 0.05[b]	2.68 ± 0.40[a]	0.26 ± 0.02[c]	2.44 ± 0.24[a]
2,2'-Oxybis-ethanol	—	3.18 ± 0.28[c]	206.93 ± 4.23[a]	13.26 ± 1.68[b]
Z-2-Dodecenol	—	5.48 ± 0.73[a]	—	2.38 ± 0.34[b]
(E)-4-Hexen-1-ol	—	1.37 ± 0.12[a]	—	0.88 ± 0.04[b]
Methanethiol	—	17.77 ± 0.96	—	17.70 ± 1.15
Ethanol	16.79 ± 1.05[c]	17.96 ± 1.10[c]	62.45 ± 1.01[b]	769.92 ± 3.62[a]
3-Methyl-1-butanol	4.81 ± 0.25[a]	1.48 ± 0.33[b]	1.46 ± 0.40[b]	1.33 ± 0.15[b]
1-Butanol	0.54 ± 0.14[b]	5.17 ± 0.33[a]	0.70 ± 0.16[b]	4.14 ± 1.16[a]
1-Hexanol	1.57 ± 0.35[c]	47.94 ± 5.24[b]	3.24 ± 0.87[c]	59.69 ± 2.46[a]
1-Pentanol	4.47 ± 0.99[c]	207.53 ± 5.81[a]	3.15 ± 0.85[c]	134.42 ± 5.18[b]
1-Octanol	1.31 ± 0.27[c]	23.16 ± 1.17[a]	1.37 ± 0.34[c]	18.20 ± 0.67[b]

(S)-2-Octanol	—	—	16.13 ± 1.29[a]	0.87 ± 0.03[b]
2-Ethyl-1-hexanol	—	—	4.45 ± 0.41	6.47 ± 0.86
3,5,5-Trimethyl-1-hexanol	—	—	0.25 ± 0.06[b]	0.73 ± 0.04[a]
Aldehyde				
(E)-2-Octenal	—	12.50 ± 1.88	—	12.46 ± 0.48
(E)-4-Decenal	—	3.71 ± 0.29	—	2.99 ± 0.99
(Z)-2-Heptenal	—	1.53 ± 0.09[b]	—	6.22 ± 1.26[a]
2-Methylbutanal	23.84 ± 1.71[c]	58.40 ± 0.87[a]	33.49 ± 1.87[b]	22.49 ± 0.40[c]
2-Ethyl-2-hexenal	—	9.47 ± 0.59[a]	—	2.27 ± 0.26[b]
3-Ethylbenzaldehyde	—	0.40 ± 0.01	—	0.28 ± 0.06
5-Ethylcyclopent-1-enecarboxaldehyde	—	7.57 ± 0.29[b]	—	11.79 ± 0.57[a]
Benzaldehyde	3.98 ± 0.72[b]	43.87 ± 2.96[a]	0.66 ± 0.14[b]	47.17 ± 1.99[a]
Propanal	—	27.65 ± 1.72	—	29.94 ± 1.67
Pyruvaldehyde	—	0.57 ± 0.22[b]	0.24 ± 0.02[c]	0.71 ± 0.03[a]
Butanal	—	6.08 ± 0.96	—	5.50 ± 0.54
Hexanal	—	6642.70 ± 10.47[a]	4.31 ± 0.98[c]	5684.17 ± 9.71[b]
Nonanal	0.75 ± 0.11[c]	261.23 ± 2.01[a]	0.69 ± 0.13[c]	147.42 ± 4.15[b]
Pentadecanal	—	0.40 ± 0.11[c]	0.61 ± 0.10[b]	13.08 ± 0.76[a]
Betaine aldehyde cation	1.35 ± 0.38[c]	81.09 ± 3.65[a]	1.27 ± 0.27[c]	65.51 ± 3.07[b]
Pentanal	—	4.36 ± 0.44[a]	—	2.55 ± 0.15[b]
Acetaldehyde	17.79 ± 0.36[c]	82.61 ± 2.06[a]	2.92 ± 0.21[d]	37.97 ± 1.43[b]
2-Methylpropanal	—	4.34 ± 0.71[a]	—	2.68 ± 0.47[b]
Octanal	0.16 ± 0.05[c]	55.69 ± 1.96[a]	0.44 ± 0.03[c]	42.85 ± 2.10[b]
1-Naphthaldehyde	—	—	2.38 ± 0.13[a]	1.69 ± 0.20[b]
3-Methylbutanal	—	1.48 ± 0.33[a]	—	0.79 ± 0.05[b]
Heptanal	—	135.97 ± 3.03	—	130.42 ± 4.11
Ketone				
1-(Acetyloxy)-2-propanone	—	11.29 ± 1.36[a]	—	7.06 ± 1.34[b]
1-(p-Tolyl)butan-1-one	0.26 ± 0.02[c]	0.68 ± 0.17[b]	0.24 ± 0.04[c]	1.15 ± 0.17[a]
2,3-Butanedione	40.55 ± 1.45[b]	16.50 ± 1.89[d]	46.22 ± 1.60[a]	26.32 ± 0.76[c]
2,3-Pentanedione	—	7.82 ± 0.91[b]	—	17.65 ± 0.43[a]
2-Butanone	3.46 ± 0.58[c]	58.59 ± 2.76[a]	3.00 ± 0.44[c]	40.10 ± 2.00[b]
2-Octanone	0.33 ± 0.07[c]	7.82 ± 0.79[a]	—	5.96 ± 1.10[b]

6-Methyl-2-heptanone	—	5.82 ± 0.45	—	5.90 ± 0.57
Acetone	141.88 ± 0.15[c]	179.58 ± 4.66[a]	169.01 ± 3.37[b]	125.74 ± 4.25[d]
Cyclopentanone	—	3.26 ± 0.56[a]	—	1.50 ± 0.32[b]
Ester				
11-cis-Vaccenylacetate	—	0.47 ± 0.06[a]	—	0.26 ± 0.05[b]
Acetic acid (butyl ester)	—	6.40 ± 1.22	—	5.70 ± 1.22
Butyrolactone	5.90 ± 0.71[a]	6.26 ± 0.95[a]	1.48 ± 0.32[c]	3.42 ± 0.33[b]
Carbamodithioic acid (diethyl-, methyl-ester)	0.62 ± 0.10[d]	1.55 ± 0.25[a]	0.73 ± 0.12[c]	1.21 ± 0.19[b]
Dibutyl phthalate	0.25 ± 0.05[b]	0.39 ± 0.15[ab]	0.42 ± 0.06[a]	0.29 ± 0.05[ab]
Dimethyl diethyl malonate	0.24 ± 0.05[c]	0.70 ± 0.17[a]	0.54 ± 0.01[b]	—
Dimethylsilanediol	67.16 ± 2.61[d]	102.41 ± 3.55[b]	74.37 ± 3.05[c]	227.46 ± 2.31[a]
Ethyl acetate	1.41 ± 0.14[c]	26.83 ± 1.50[a]	—	11.15 ± 1.31[b]
Formic acid (hexyl ester)	—	3.03 ± 0.84	—	3.14 ± 0.59
Formic acid (pentyl ester)	—	6.54 ± 0.86[a]	—	3.81 ± 1.01[b]
Glycerol1-myristate	—	0.88 ± 0.05[a]	—	0.29 ± 0.03[b]
n-Caproic acid vinyl ester	—	10.69 ± 0.57[a]	—	4.63 ± 1.16[b]
Spironolactone	1.52 ± 0.32[b]	2.35 ± 0.24[a]	0.67 ± 0.58[c]	1.60 ± 0.36[b]
Thiopropionic acid (S-ethyl ester)	0.27 ± 0.05[b]	0.37 ± 0.09[a]	0.27 ± 0.01[b]	—
Acid				
Methoxyacetic acid	6.36 ± 1.34[a]	1.57 ± 0.31[c]	3.24 ± 0.67[b]	4.21 ± 0.87[b]
(E)-2-Octenoicacid	—	0.19 ± 0.01[b]	—	0.32 ± 0.01[a]
12(13)-Epoxy-9Z-octadecenoicacid	—	0.29 ± 0.04	0.56 ± 0.11	1.82 ± 0.15
3-Methylbutanoic acid	1.62 ± 0.13[a]	0.64 ± 0.02[c]	1.27 ± 0.28[b]	0.44 ± 0.05[c]
8(9)-EpETE	16.08 ± 1.24[c]	779.37 ± 6.21[a]	2.01 ± 0.04[d]	155.50 ± 5.32[b]
Acetic acid	18.63 ± 0.95[a]	12.45 ± 1.55[b]	12.73 ± 1.41[b]	7.58 ± 1.36[c]
Butanoic acid	1.39 ± 0.21	1.49 ± 0.39	1.37 ± 0.37	1.36 ± 0.34
Citraconic acid	—	5.86 ± 0.81[a]	—	1.60 ± 0.24[b]
Cyclohexane sulfamic acid	0.22 ± 0.03[c]	2.19 ± 0.15[a]	0.34 ± 0.05[c]	0.70 ± 0.07[b]
Glafenine	—	7.67 ± 1.09[b]	—	10.10 ± 0.73[a]
Hexanoic acid	4.15 ± 1.07[c]	8.11 ± 0.13[a]	1.51 ± 0.35[d]	5.91 ± 0.63[b]
L-Pipecolic acid	—	2.78 ± 0.61	0.36 ± 0.05	1.92 ± 0.12
Methylmalonic acid	—	1.26 ± 0.18	—	1.19 ± 0.09
Methylphosphonic acid	—	0.37 ± 0.10	0.42 ± 0.11	0.56 ± 0.16

N-Tigloylglycine	0.37 ± 0.03[c]	1.24 ± 0.15[a]	0.44 ± 0.12[c]	0.69 ± 0.22[b]
O-Succinyl-L-homoserine	0.67 ± 0.04[b]	5.41 ± 0.61[a]	0.51 ± 0.07[b]	5.87 ± 0.74[a]
Propanoic acid	2.36 ± 0.56[a]	1.81 ± 0.09[b]	0.26 ± 0.06[c]	2.29 ± 0.17[a]
S-Ethyl-DL-homocysteine	—	3.10 ± 0.49[a]	—	1.55 ± 0.31[b]
Other				
2-Ethylfuran	—	56.79 ± 2.45[a]	—	26.4 ± 0.67[b]
2-Pentylfuran	—	51.92 ± 1.77[a]	—	29.06 ± 2.20[b]
3-Methylfuran	—	0.34 ± 0.05	—	0.36 ± 0.09
Furan	—	6.44 ± 1.27	—	5.37 ± 0.75
trans-Linalool oxide (furanoid)	—	5.71 ± 0.89[a]	—	2.58 ± 0.20[b]
Ethyl ether	10.85 ± 1.60	—	11.57 ± 1.05	—
Diethyl carbitol	0.83 ± 0.03[b]	1.30 ± 0.27[a]	0.22 ± 0.05[c]	1.57 ± 0.29[a]
(E)-1,3-Pentadiene	2.85 ± 0.56	29.77 ± 1.12	1.32 ± 0.30	—
1,2-Diamino-2-methylpropane	—	0.69 ± 0.01	—	0.69 ± 0.07
1-Hexadecylamine	—	0.61 ± 0.08	0.58 ± 0.07	1.43 ± 0.17
2-Aminopyridine	—	2.64 ± 0.40[a]	—	1.23 ± 0.34[b]
3-Methylthiophene	—	21.04 ± 2.25[a]	—	14.54 ± 1.62[b]
6-Chloropurineriboside	—	0.51 ± 0.08	0.33 ± 0.03	0.82 ± 0.04
Allomatrine	2.57 ± 0.26[b]	4.18 ± 0.91[a]	2.46 ± 0.52[b]	3.32 ± 0.45[ab]
Carbetamide	0.45 ± 0.09[b]	1.03 ± 0.11[a]	0.40 ± 0.06[b]	0.95 ± 0.20[a]
Carbon disulphide	503.02 ± 7.29[b]	34.27 ± 2.20[c]	643.02 ± 4.32[a]	37.8 ± 1.72[c]
Dimethyl sulphide	—	2.30 ± 0.48[b]	—	5.13 ± 0.77[a]
Dimethyl disulphide	—	0.72 ± 011[a]	—	1.02 ± 0.03[b]
D-Lyxose	0.50 ± 0.10	6.26 ± 1.15	—	4.26 ± 0.92
D-Mannosamine	0.17 ± 0.03[c]	2.56 ± 0.50[b]	0.16 ± 0.04[c]	4.46 ± 0.83[a]
Guanidine	2.76 ± 0.49[c]	85.34 ± 2.69[a]	2.82 ± 0.17[c]	41.36 ± 1.36[b]
Hydrogen sulphide	—	14.23 ± 0.63	—	14.51 ± 1.81
N,N-Dibutylformamide	5.22 ± 0.82[a]	3.57 ± 0.60[b]	4.71 ± 0.82[a]	2.27 ± 0.17[c]
N-Benzylaniline	2.35 ± 0.28[b]	5.30 ± 0.74[a]	2.54 ± 0.10[b]	5.26 ± 0.96[a]
N-Methylpropionamide	—	7.72 ± 0.74	—	2.43 ± 0.13
Tetradecylamine	1.15 ± 0.17[a]	1.28 ± 0.37[a]	0.70 ± 0.16[b]	1.46 ± 0.15[a]
Trimethylamine N-oxide	—	1.46 ± 0.15	0.39 ± 0.01	0.77 ± 0.02

Values are mean ± standard error (n = 6). Means in the same rows with different superscript letters differ significantly ($p < 0.05$). "—" indicates not detected.

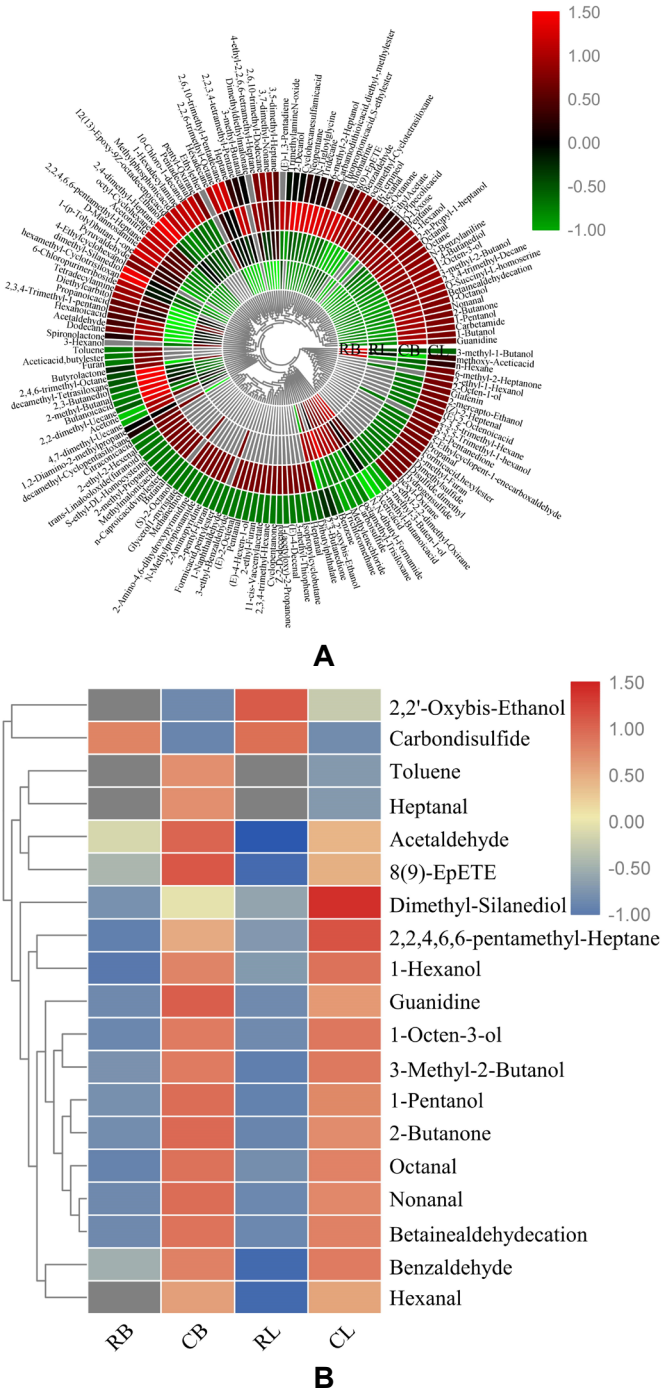

Figure 2. Heat map analysis of aroma compounds in Chahua chicken meat. Heat map of all aroma compounds (A), and heat map analysis of characteristic aroma compounds (B). RB = raw breast meat; RL = raw leg meat; CB = cooked breast meat; and CL = cooked leg meat.

aroma components were higher in CB and CL, and this group included 13 substances such as dimethylsilanediol and 2,2,4,6,6-pentamethylheptane. However, a comprehensive evaluation of aroma components cannot be based on the content of these substances alone; it must also consider the olfactory threshold of human sensory organs. Accordingly, the mechanisms responsible for inducing the effects of aroma on overall flavour still merit discussion. In the present work, only the compounds detected above their threshold values were analysed and further discussed.

Alkanes

Alkanes generally have a high aroma threshold value, meaning that they can cause an olfactory reaction only at a high concentrations; therefore, alkanes typically have little effect on the

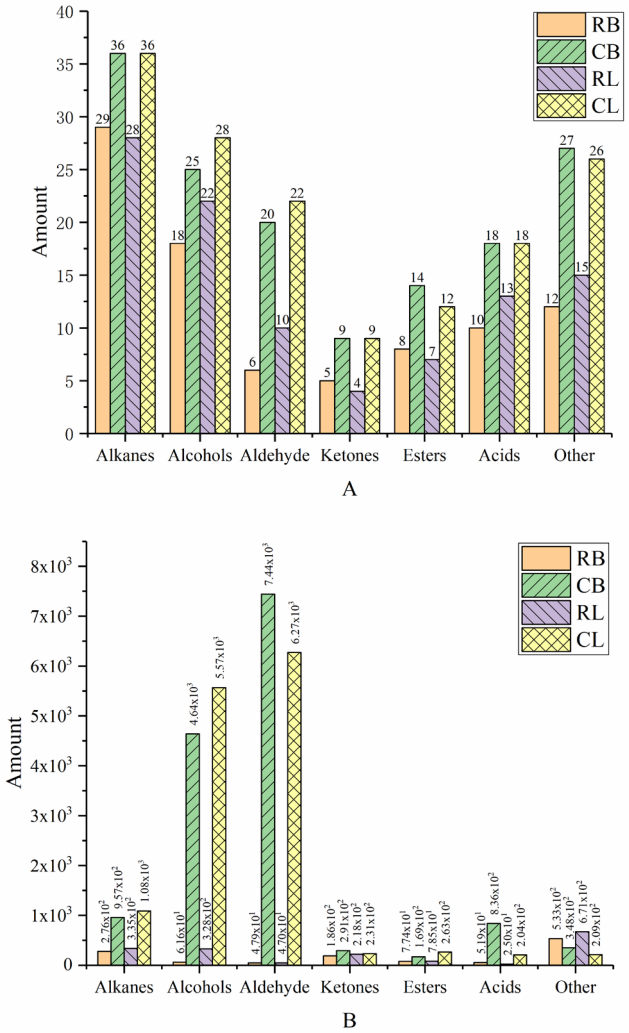

Figure 3. Types (A) and contents (B) of volatile flavour substances in Chahua chicken meat before and after heat treatment. RB = raw breast meat; RL = raw leg meat; CB = cooked breast meat; and CL = cooked leg meat.

overall flavour of food. The main flavour compounds of chicken are not alkanes, but they do play a fundamental role in generating the overall flavour of the chicken (Yu and Chen, 2010). Additionally, alkanes can also be precursors of aldehydes and ketones which have greater potential impact on the overall flavour. The content and types of alkanes in the cooked meat group were significantly higher than those found in the raw meat group, but the carbon atoms in those alkanes were relatively stable due to the lack of unsaturated bonds. Although a greater number of alkane compounds were detected in each sample group relative to other types of compounds, they did not contribute much to the overall flavour of Chahua chicken due to their higher threshold values.

Alcohols

Alcohols are one of several important classes of chemicals that contribute to the characteristically strong and sharp taste of Chahua chicken meat. Alcohol compounds can be formed via secondary hydroperoxidation of fatty acids, action of lipoxygenase on fatty acids, or reduction of carbonyl compounds (Wang *et al.,* 2015). The higher the threshold value of a saturated or unsaturated alcohol, the smaller its contribution to the overall flavour, and vice versa (Drumm and Spanier, 1991). It is clear from Figure 3A that the alcohol content in the cooked breast and leg meat groups reached 4640.40 and 5566.49 ng/g, respectively. Alcohols with higher molecular weights are often precursors of well-known flavour and aroma-active esters (Rodrigues *et al.,* 2008). As shown in Table 3, 1-octene-3-ol was the only alcohol with an OAV > 1. The OAVs of 1-octene-3-ol in cooked breast and leg meat were 1869.65 and 1975.09, respectively. These results showed that 1-octene-3-ol was the main flavour component contributing to the overall flavour of Chahua chicken. Linoleic acid, arachidonic acid, or other polyunsaturated fatty acids can be oxidised directly to form

Table 3. Threshold and OAV of volatile flavour substances in Chahua chicken meat.

Aroma compound	Threshold	Odour character	RB	CB	RL	CL
1-Octen-3-ol	1.00	Mushroom, oil, and fermentation	19.75	1869.65	20.9	1975.09
(*E*)-2-Octenal	3.00	Green, nutty, and fatty	—	4.17	—	4.15
2-Methylbutanal	1.00	Coffee, cocoa, and similar chocolate	23.84	58.4	33.49	22.49
Hexanal	4.50	Grass, green, and tallow	—	1476.16	0.96	1263.15
Nonanal	1.00	Vanilla, fat, and wax	0.75	261.23	0.69	147.42
Octanal	0.70	Fat, orange, and honey	0.23	79.56	0.63	61.21
3-Methylbutanal	0.20	Apple flavour	—	7.4	—	3.95
Heptanal	3.00	Oil and fat	—	45.32	—	43.47
2-Pentyfuran	6.00	Beans, fruits, earth, and similar vegetables	—	8.65	—	4.84
Dimethyl disulphide	0.06	—	—	12	—	17

"—" indicates not detected. RB = raw breast meat; RL = raw leg meat; CB = cooked breast meat; and CL = cooked leg meat. Threshold (Leffingwell and Associates, 2001; Fan *et al.,* 2015).

1-octene-3-ol (Lorenzo and Domínguez, 2014). This alcohol is mainly present in mushroom, oil, and fermentation flavours (Shi *et al.,* 2013); so, it can enhance the fat flavour of the meat.

Aldehydes

Aldehydes are highly volatile and important flavour compounds in cooked meat. The concentration of aldehydes in cooked meat is high and the threshold is low; so, they can have a significant effect on the overall aroma of the chicken. In fact, if these VOCs are removed, the aroma of chicken essentially disappears (Bravo-Lamas *et al.,* 2018). Based on Table 3, (*E*)-2-acetaldehyde, 2-methylbutyraldehyde, hexanal, nonanal, octanal, 3-methylbutyraldehyde, and heptanal are the main aldehyde flavour compounds in the cooked meat. The higher OAVs indicated that they have an important contribution to the aroma of Chahua chicken meat. Aldehydes are commonly produced by the oxidation of lipids during the meat cooking process. Among them, hexanal and heptanal are mainly produced by the oxidation of linoleic acid or arachidonic acid. Acetaldehyde and nonanaldehyde are produced by the oxidation of oleic acid, and these compounds have a pleasant meat flavour (Yang *et al.,* 2017). Hexanal has the flavour of grass, green plants, and beef, and contributed the most to the flavour of cooked chicken meat analysed in the present work. In the cooked breast meat group, the content of nonanal, octyl aldehyde, and heptanaldehyde reached 261.23, 79.56, and 45.32 ng/g, respectively. Moreover, the OAVs of the three compounds were 147.42, 61.21, and 43.47, respectively, thus highlighting the important contribution of these flavour substances to Chahua chicken meat. The 2-methyl butyraldehyde has the aroma characteristics of coffee and cocoa, which can provide an attractive chocolate-like aroma to meat. The 3-methyl butyraldehyde has an apple flavour, which was not detected in raw meat. However, after heat treatment, the concentration of 3-methyl butyraldehyde in the cooked breast and leg meat reached 1.48 and 0.79 ng/g, respectively. Due to its low threshold, this compound enhanced the overall flavour of meat.

Ketones

Ketones can be produced following the Maillard reaction or by oxidative degradation of fat. They are relatively stable, and generally have a floral and lasting fragrance (Whitfield and Mottram, 1992). It is clear from Figure 3 that nine different ketones were detected in the cooked breast and leg meat, and their concentrations were 291.36 and 231.38 ng/g, respectively. When compared with the raw meat groups, there was no significant difference in the content of ketones, thus indicating that the aroma of ketones was long-lasting and stable. However, due to the lower content and higher threshold of ketones relative to aldehydes, they had no significant contribution to the flavour of the meat.

Furans

Furans are the products of sugar cracking and Maillard reaction during chicken heating process, and they are important heterocyclic compounds that add flavour to Chahua chicken. As shown in Table 1, furans were not detected in raw meat, but five furan compounds were detected in the cooked meat groups. Among them, 2-*n*-pentylfuran played an important

role (OAV > 1) in the flavour of cooked breast and leg meat. Its threshold value was relatively low, thus allowing it to present the flavours of beans, fruits, soil, and vegetables. This compound is the main flavour substance produced during the cooking process.

Nitrogen- and sulphur-containing compounds

Compounds containing nitrogen and sulphur are mainly produced by the Maillard reaction. The heteroatoms are typically introduced between the carbonyl group of reducing sugar, and the amino group of amino acids. Through a series of complex reactions, nitrogen-containing heterocyclic compounds and black char-like substances are formed. The sulphur-containing compounds produced in the reaction process have typical meat aromas, and have been widely used in the production of meat flavourings. They embody characteristics of baking flavours, and contribute to the basic flavour of meat (Lotfy *et al.,* 2015). Sulphur-containing compounds generally have low thresholds and strong meat flavour characteristics; thus, they represent key components of meat flavour. It is clear from Table 2 that dimethyl disulphide was detected in the cooked breast and leg meat groups. The OAVs of dimethyl disulphide in the breast and leg meat were 12.00 and 17.00, respectively, thus indicating that it played an important role in the formation of the overall flavour of the cooked Chahua chicken. Dimethyl disulphide has a malodorous odour, which may be related to the degradation reaction caused by high temperature. Carbon disulphide (VIP pred. = 3.4185) has a rotten egg flavour, although high-purity carbon disulphide has a sweet and ethereal flavour, which can improve the fragrance of Chahua chicken meat.

Other compounds

In addition, a certain number of esters and acids were detected in the present work which also potentially had an effect on the flavour profile of Chahua chicken. Esters are formed by esterification reactions between carboxylic acids and alcohols, and their contents and intensities typically increase during post-mortem aging of meat (Pugliese *et al.,* 2015). Volatile esters in Chahua chicken meat are usually related to the sweet and fruity flavour, whereas acids impact the overall aroma of meat by contributing to the cheesy and fatty odours. Along with some unpleasant odour notes, acids also contribute to the astringency, especially bitterness and rancidity. The threshold value of acetic acid was as high as 22,000 ng/g; so, it makes little contribution to the overall flavour formation. Ethyl acetate was detected in the cooked breast and leg meat groups, and it has a sweet taste and auxiliary modification. However, its threshold value was relatively high; so, it had little effect on the overall flavour of the meat. Since the volatility of acids is lower than many of the other chemical components, only a small quantity of acids was detected in the present work. In general, acids and esters played a coordinating and balancing role in terms of the overall flavour of Chahua chicken.

Conclusions

Investigating the changes in the types and contents of VOCs in raw and cooked breast and leg meat of Chinese Chahua chicken samples provided the basis for evaluating the overall characteristics of their volatile flavour compounds. The HS-SPME-GC-MS analysis revealed that 88 and 99 VOCs were detected in the raw breast and leg meat, respectively, while 149 and 151 VOCs were detected in the cooked breast and leg meat groups, respectively. Ten key aroma components were identified as the most important contributors to the aroma of the Chahua chicken, including 1-octene-3-ol, (*E*)-2-octenal, 2-methylbutanal, hexanal, nonanal, octanal, 3-methylbutanal, heptanal, 2-pentylfuran, and dimethyl disulphide. Ultimately, it was determined that alcohols and aldehydes are the main substances affecting the flavour of Chinese Chahua chicken. The combined analysis method evaluating OPLS-DA models based on metabonomics was used to screen potential markers and explain flavour characteristics from multiple perspectives. The resulting description of the characteristic flavour compounds was very complex; therefore, it was necessary to integrate all available knowledge to evaluate the meat quality, including GC-MS and electronic nose. Overall, the results of the present work elucidated the characteristic aroma components of Chahua chicken meat which help clarify the flavour characteristics of Chahua chicken, and provide the understanding required for industrial processors to develop effective quality standardisation strategies.

Acknowledgement

The present work was financially supported by the National Natural Science Foundation of China (31960511), the Major R&D Projects of Science and Technology Plan Projects of Yunnan Province (2016ZA008), and the International Science and Technology Cooperation Project of Key R&D Programs in Yunnan Province (2018IA101).

References

Aronson, D. B., Bosch, S., Gray, D. A., Howard, P. H. and Guiney, P. D. 2007. A comparative human health risk assessment of *p*-dichlorobenzene-based toilet rimblock products versus fragrance/surfactant-based alternatives. Journal of Toxicology and Environmental Health, Part B 10(7): 467-526.

Bravo-Lamas, L., Barron, L. J. R., Farmer, L. and Aldai, N. 2018. Fatty acid composition of intramuscular fat and odour-active compounds of lamb commercialized in northern Spain. Meat Science 139: 231-238.

Devaux, P. G., Horning, M. G. and Horning, E. C. 1971. Benzyloxime derivatives of steroids. A new metabolic profile procedure for human urinary steroids human urinary steroids. Analytical Letters 4(3): 151-160.

Drumm, T. D. and Spanier, A. M. 1991. Changes in content of lipid autoxidation and sulphur-containing compounds in cooped beef during storage. Journal of Agricultural and Food Chemistry 39(2): 336-343.

Fan, H., Fan, W. and Xu, Y. 2015, Characterization of key odorants in Chinese Chixiang aroma-type liquor by gas chromatography-olfactometry, quantitative measurements, aroma recombination, and omission studies. Journal of Agricultural and Food Chemistry 63(14): 3660-3668.

Fan, Q., Wang, X. L., Zhao, Y. F., Zheng, F. P., Li, H. H., Zhang, F. Y., … and Chen, F. 2019. Characterization of key aroma compounds in Laobaigan Chinese Baijiu by GC×GC-TOF/MS and means of molecular sensory science. Flavour and Fragrance Journal 34(6): 514-525.

Frank, D., Hughes, J., Piyasiri, U., Zhang, Y., Kaur, M., Li, Y., … and Stark, J. 2019. Volatile and non-volatile metabolite changes in 140-day stored vacuum packaged chilled beef and potential shelf life markers. Meat Science 161: article ID 108016.

Gu, S. Q., Wang, X. C., Tao, N. P. and Wu, N. 2013. Characterization of volatile compounds in different edible parts of steamed Chinese mitten crab (*Eriocheir sinensis*). Food Research International 54(1): 81-92.

Hausch, B. J., Lorjaroenphon, Y. and Cadwallader, K. R. 2015. Flavor chemistry of lemon-lime carbonated beverages. Journal of Agricultural and Food Chemistry 63(1): 112-119.

Hong, Z. Y., Lin, Z. B., Liu, Y., Tan, G. G., Lou, Z. Y., Zhu, Z. Y., … and Zhang, L. M. 2012. Innovative microwave-assisted oximation and silylation procedures for metabolomic analysis of plasma samples using gas chromatography-mass spectrometry. Journal of Chromatography A 1254: 14-22.

Jin, G. F., He, L. C., Li, C. L., Zhao, Y. H., Chen, C., Zhang, Y. H., … and Ma, M. H. 2015. Effect of pulsed pressure-assisted brining on lipid oxidation and volatiles development in pork bacon during salting and drying-ripening. LWT - Food Science and Technology 64(2): 1099-1106.

Johan, T., Holmes, E. and Torbjörn, L. 2007. Chemometrics in metabonomics. Journal of Proteome Research 6(2): 469-479.

Kim, Y. H. B., Kemp, R. and Samuelsson, L. M. 2016. Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. Meat Science 111(1): 168-172.

Leffingwell and Associates. 2001. Flavor-base 10th ed. Retrieved from website: http://www.leffingwell.com/flavbase.htm

Li, D. P., Zhang, J. B., Song, S. J., Feng, L. G. and Luo, Y. K. 2018. Influence of heat processing on the volatile organic compounds and microbial diversity of salted and vacuum-packaged silver carp (*Hypophthalmichthys molitrix*) fillets during storage. Food Microbiology 72: 73-81.

Li, X. L., He, L. P., Yang, Y., Liu, F. J., Cao, Y. and Zuo, J. J. 2015. Effects of extracellular polysaccharides of *Ganoderma lucidum* supplementation on the growth performance, blood profile, and meat quality in finisher pigs. Livestock Science 178: 187-194.

Liu, H. Y., Garrett, T. J., Su, Z. H., Khoo, C. and Gu, L. W. 2017. UHPLC-Q-Orbitrap-HRMS-based global metabolomics reveal metabolome modifications in plasma of young women after cranberry juice consumption. The Journal of Nutritional Biochemistry 45: 67-76.

Liu, H. Y., Tayyari, F., Edison, A. S., Su, Z. H. and Gu, L. W. 2016. NMR-based metabolomics reveals urinary metabolome modifications in female Sprague-Dawley rats by cranberry procyanidins. The Journal of Nutritional Biochemistry 34: 136-145.

Lorenzo, J. M. and Domínguez, R. 2014. Cooking losses, lipid oxidation and formation of volatile compounds in foal meat as affected by cooking procedure. Flavour and Fragrance Journal 29(4): 240-248.

Lotfy, S. N., Fadel, H. H. M., El-Ghorab, A. H. and Shaheen, M. S. 2015. Stability of encapsulated beef-like flavourings prepared from enzymatically hydrolysed mushroom proteins with other precursors under conventional and

microwave heating. Food Chemistry 187: 7-13.

Mamat, S. F., Azizan, K. A., Baharum, S. N., Mohd Noor, N. and Aizat, W. M. 2019. The influence of two solvent extraction ratios in GC-MS-based metabolomics of different *Garcinia mangostana* Linn. fruit tissues. International Food Research Journal 26(5): 1447-1457.

Nicholson, J. K., Lindon, J. C. and Holmes, E. 1999. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica 29(11): 1181-1189.

Oliver S. G. 1997. Yeast as a navigational aid in genome analysis. 1996 Kathleen Barton-Wright Memorial Lecture. Microbiology 143: 1483-1487.

Pérez-Olivero, S. J., Trujillo, J. P. and Conde, J. E. 2013. Minor volatile compounds in white wines from Canary Islands, Madeira, and Pico (Azores) by headspace solid-phase microextraction-gas chromatography-mass spectrometry: a qualitative study. International Scholarly Research Notices 2013: article ID 529306.

Pugliese, C., Sirtori, F., Škrlep, M., Piasentier, E., Calamai, L., Franci, O. and Čandek-Potokarb, M. 2015. The effect of ripening time on the chemical, textural, volatile and sensorial traits of *Bicep femoris* and *Semimembranosus* muscles of the Slovenian dry-cured ham *Kraški pršut*. Meat Science 100: 58-68.

Qi, J., Wang, H. H., Zhou, G. H., Xu, X. L., Li, X., Bai, Y. and Yu, X. B. 2018. Evaluation of the taste-active and volatile compounds in stewed meat from the Chinese yellow-feather chicken breed. International Journal of Food Properties 20(S3): S2579-S2595.

Rodrigues, F., Caldeira, M. and Câmara, J. S. 2008. Development of a dynamic headspace solid-phase microextraction procedure coupled to GC-qMSD for evaluation the chemical profile in alcoholic beverages. Analytica Chimica Acta 609(1): 82-104.

Shi, X. X., Zhang, X. M., Song, S. P., Tan, C., Jia, C. S. and Xia, S. Q. 2013. Identification of characteristic flavor precursors from enzymatic hydrolysis-mild thermal oxidation tallow by descriptive sensory analysis and gas chromatography-olfactometry and partial least squares regression. Journal of Chromatography B 913-914: 69-76.

Smith, D. P. and Northcutt, J. K. 2009. Pale poultry muscle syndrome. Poultry Science 88(7): 1493-1496.

Sun, L. X., Chen, J. P., Li, M. Y., Liu, Y. X. and Zhao, G. M. 2014. Effect of star anise (*Illicium verum*) on the volatile compounds of stewed chicken. Journal of Food Process Engineering 37(2): 131-145.

Takakura, Y., Osanai, H., Masuzawa, T. and Wakabayashi, H. 2014. Characterization of the key aroma compounds in pork soup stock by using an aroma extract dilution analysis. Bioscience, Biotechnology, and Biochemistry 78(1): 124-129.

Wang, S., He, Y., Wang, Y. Y., Tao, N. P., Wu, X. G., Wang, X. C., … and Ma, M. J. 2015. Comparison of flavor qualities of three sourced *Eriocheir sinensis*. Food Chemistry 200: 24-31.

Wen, S. Y., Zhou, G. H., Li, L., Xu, X. L., Yu, X. B., Bai, Y. and Li, C. B. 2015. Effect of the degree of cooking doneness on *in vitro* digestion of pork proteins: a peptidomic perspective. Journal of Agricultural and Food Chemistry 63(1): 250-261.

Whitfield, F. B. and Mottram, D. S. 1992. Volatiles from interactions of Maillard reactions and lipids. Journal of Critical Reviews in Food Science and Nutrition 31(1-2): 1-58.

Xi, Y. M., Ying, S. J., Shao, C. R., Zhu, H. X., Yan, J. S. and Shi Z. D. 2020. Metabolomic profiling of goslings with visceral gout reveals a distinct metabolic signature. British Poultry Science 61(3): 258-265.

Yang, Y., Zhang, X., Wang, Y., Pan, D. D., Sun, Y. Y. and Cao, J. X. 2017. Study on the volatile compounds generated from lipid oxidation of Chinese bacon (unsmoked) during processing: aroma generated from lipid oxidation in bacon (unsmoked). European Journal of Lipid Science and Technology 119(10): article ID 1600512.

Yeole, R. D., Rane, V. P., Ahirrao, V. K., Chavan, R. P., Patel, A. M., Deshpande, P. K., … and Patil, K. R. 2019. Identification of metabolites of novel Anti-MRSA fluoroquinolone WCK 771 in mouse, rat, rabbit, dog, monkey and human urine using liquid chromatography tandem mass spectrometry. Biomedical Chromatography 33(7): article ID e4532.

Yu, H. Z. and Chen, S. S. 2010. Identification of characteristic aroma-active compounds in steamed mangrove crab (*Scylla serrata*). Journal of Food Research International 43(8): 2081-2086.

Zhao, H. A., Cheng, N., Wang, Q., Zhou, W. Q., Liu, C. Y., Liu, X. Y., … and Cao, W., 2019. Effects of honey extracted polyphenols on serum antioxidant capacity and metabolic phenotype in rats. Food and Function 10: 2347-2358.