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1H NMR-based metabolomics and sensory evaluation characterize taste substances of Jinhua ham with traditional and modern processing procedures

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ABSTRACT

Jinhua ham is one of high quality dry-cured products, and processing methods play a key role in developing the taste attributes of Jinhua ham. To investigate the effect of processing methods on sensory attributes and taste substances of Jinhua ham, this work highlighted the changes of sensory characteristics, texture parameters, water distribution and metabolite profiles, and further discussed the contribution of metabolites to the development of taste and richness in both traditional-processed and modern-processed hams. The modern-processed hams showed higher taste scores and richness intensities than that of traditional processing. The populations of immobilized water significantly increased from raw ham to the later stage of dry-ripening, accompanied by a decrease in the populations of free water during the processing of traditional and modern procedures, while higher populations (more than 2-fold) of free water were shown in the ham of modern processing at the end of post-ripening compared with traditional-processed ham. 1H NMR-based metabolomics revealed that free amino acids, small peptides and organic acids were the most intense response in developing taste and richness intensities of modern-processed ham. Partial least square discriminant analysis and taste-active values further demonstrated that glutamic acid, lactate, glycerol, anserine and creatine were responsible for the higher taste and richness intensities of modern-processed ham.

1. Introduction

Jinhua ham is highly favored by consumers mainly due to its unique organoleptic and flavor characteristics (Zhang, Jin, Wang, & Zhang, 2011; Zhou & Zhao, 2007). During the past few decades, the manufacture of Jinhua ham has generally followed traditional procedure, beginning in winter and ending in the following autumn under natural conditions (Zhang, Zhen, Zhang, Zeng, & Zhou, 2010). However, there are many challenges in the traditional production procedure. For example, the flavor quality of the ham cannot be guaranteed when the abnormal weather such as high temperature (more than 20 °C) occurs. Furthermore, the high residues of sodium content (approximate 10%) appear in final product, which is rejected by consumers (Zhou, Wu et al., 2019b; Zhou, Pan et al., 2019). Consequently, some strategies to reduce sodium content during the modern processing of dry-cured ham should be developed. Modern process shows a shorter processing time (6–8 months) and more refined processing procedure, which only includes five processing steps (raw ham preparation, salting, washing, ripening and post-ripening), compared with the traditional process (Zhou, Pan et al., 2019). The suitability of raw material and the changes of temperature and relative humidity (RH) have been explored to improve their qualities during the processing of dry-cured ham (Arnau, Serra,

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Comaposada, Gou, & Garriga, 2007; Bosse Nee Danz et al., 2018). Furthermore, low dosage of sodium chloride (about 6%) used at the salting stage, and the powerful refrigeration units and rooms for controlling temperature and RH have been developed to guarantee the safe characteristics and flavour attributes in many enterprises during the modern processing of Jinhua ham (Zhou, Pan et al., 2019). However, up to now, the actual substance responsible for the sensory qualities and taste attributes have not yet been well identified and explored during the modern processing of Jinhua ham.

Taste is an important indicator of dry-cured ham, and largely determines consumer preferences for the consumption of the products (Zhang et al., 2018). Amino acids, peptides, organic acids, inorganic salts and nucleotides are the main taste substances in dry-cured meat products (Dashdorj, Amna, & Hwang, 2015; López-Pedrouso et al., 2019; Sforza et al., 2006). Studies have demonstrated that these taste compounds mainly derive from protein degradation and lipid oxidation (Toldrá, 2006). Most researchers agree that lipid oxidation and protein degradation which are the result of the activities of endogenous enzymes, are the major contributors to texture parameters and taste attributes of dry-cured ham, and that a large accumulation of hydrolysis products does not necessarily serve to enhance sensory and taste characteristics in dry-cured ham (Sforza et al., 2006; Toldrá, 2006). Thus, investigating the specific metabolites could be more meaningful to understand the development of taste and sensory attributes in Jinhua ham manufactured by different processing procedures.

Metabonomic technologies show great potential to identify metabolites of dry-cured ham; these identification and quantitation technologies mainly include high performance liquid chromatography, liquid chromatography-mass spectrometer (LC-MS/MS) and high resolution nuclear magnetic resonance (NMR). NMR spectroscopy has unique advantage of enabling rapid identification and quantification of metabolites (Yang, Dai, Ayed, & Liu, 2019), and it has been well applied in scientific field of meat and meat products in recent years (Castejon, Garcia-Segura, Escudero, Herrera, & Cambero, 2015; Xiao, Ge, Zhou, Zhang, & Liao, 2019; Yang et al., 2019). Zhang et al. (2018, 2019) reported that substances including free amino acids, organic acids, nucleic acids and small peptides were systematically identified by NMR in dry-cured ham. However, few literatures focus on comparing the components and profiles of taste substances using 1H NMR and further characterize their contributions to taste and richness intensities of Jinhua ham manufactured by modern and traditional procedures.

Therefore, the aim of the present study was to characterize the components and profiles of taste substances of Jinhua ham using 1H NMR, and to further discuss the contribution of key compounds to taste and richness development of Jinhua ham during the processing of traditional and modern procedures.

2. Materials and methods

2.1. The processing and sampling of Jinhua ham

The hind legs (average weight, 13.0 ± 0.2 kg; pH, 5.8 ± 0.2) of Domestic pigs (Large White × Landrace) were used to the production of Jinhua ham at the Zhejing Provincial Food Company, P.R. China. The processing procedures (traditional and modern procedures) of Jinhua ham were performed according to our previous studies (Zhou, Wu et al., 2019b). Briefly, for the modern processing procedure, hind legs were salted using 0.15 g KNO_3, 0.15 g $NaNO_2$ and 60 g NaCl per kilogram of raw ham, and then the hams underwent post-salting, washing and ripening for 5–6 months in the dry-ripening room, where the RH progressively decreased from 80% to 65% and the ambient temperature increased from 6 °C to 35 °C; for the traditional processing procedure, hind legs were salted using 0.15 g KNO_3, 0.15 g $NaNO_2$ and 80 g NaCl per kilogram of raw ham, and then the hams underwent washing, sun-drying and ripening for 6–8 months in the dry-ripening room, where the temperature and relative humidity depended on the unique local weather and climate. After ripening, these hams with modern and traditional processing further underwent post-ripening for 1–2 months at room temperature (25 °C). The process was terminated when the total weight loss of ham was approximately 40% of the initial weight in both traditional and modern processing. *Bicep femoris* muscles of twenty hams were sampled at the raw ham, the end of drying-ripening and the end of post-ripening in both traditional and modern processing, respectively. Samples were vacuum-packaged and frozen at −80 °C until analyzed.

2.2. Sensory analysis of ham samples

The sensory analysis was performed according to the description of Zhou, Wang et al. (2019a) with some modifications. The sensory attributes of ham samples including overall taste, saltiness, sweetness, richness, umami, bitterness, sourness and aftertaste attributes were scored by 10 sensory assessors, balanced in terms of gender and owning rich experience. Rating of these attributes' intensities was performed using a linear unstructured 1 mm scale anchored at the scales (0: absence of sensation; 5: maximum of sensation intensity). The results were expressed as the mean of twenty hams of each group.

2.3. Electronic tongue analysis of Jinhua ham

Umami and richness intensities of the water-soluble extraction of Jinhua ham were performed according to the description (Dang, Gao, Ma, & Wu, 2015). Ham samples (10 g) were homogenized at 12000 r for 2 min in 200 mL distilled water. The homogenates were centrifuged at 3000 g for 10 min at 4 °C; the supernatant was collected and filtered with 3 layers of filter paper. Umami and richness intensities of the water-soluble extraction of Jinhua ham were analyzed using TS-SA402B electronic tongue (INSENT Inc., Japan). The sensory intensities of these samples were calculated according to the absolute value of the sensor potential based on the reference solution. The sensor potential of reference solution was defined as 0, and the values more than the intensity (0) of reference solution were considered meaningful. The average values of three times measurements for each sample were analyzed using TS-SA402B Library search software (INSENT, Japan). The results of umami and richness intensities were expressed as the mean values of twenty replicates.

2.4. Texture analysis of Jinhua ham

Textural analysis was performed as described by Lopez-Pedrouso et al. (2018) and Zhou, Wang, Cai et al. (2019). The ham samples (20 × 10 × 10 mm) were measured by texture analyzer (TA-XT Plus; Stable Micro Systems, Godalming, UK) equipped the special probe (P 50). The measurement of adhesiveness, hardness, springiness and cohesiveness of the ham samples was performed according to the description of Zhou, Wang, Cai et al. (2019a). All measurements were performed at 25 °C. The results of texture parameters were expressed as the mean values of twenty replicates.

2.5. Moisture content analysis of Jinhua ham

The moisture content was determined according to Gou, Comaposada, and Arnau (2004). Briefly, 5 g of the minced *Bicep femoris* muscles were dried at 105 ± 2 °C to a constant weight, and the moisture content was defined as gram per 100 g muscles. The results of moisture content were expressed as the mean values of twenty replicates.

2.6. Water distribution analysis of Jinhua ham

Water distribution analysis of ham samples was performed by Low field nuclear magnetic resonance (LF-NMR), as previously described by Garcia et al. (2015) and Zhou, Wang, Cai et al. (2019a). Sample preparation and measurement were performed according to the description

of Zhou, Wang, Cai et al. (2019a) at 30 °C. The procedure of Carr-Purcell-Meiboom-Gill sequence (CPMG) was used to measure transverse relaxation times (T2) and transverse relaxation data were analyzed by the algorithm of biexponential fittings. Transverse relaxation times (T2) and distribution populations of bound water (P2b), immobilized water (P21) and free water (P22) were calculated according to the measurements of twenty replicates.

2.7. The preparation of metabolites

The preparation of ham metabolites (n = 7) for each group (raw ham, traditional-processed ham and modern-processed ham) were performed according to the description (Zhang et al., 2018). Briefly, *biceps femoris* muscle samples (400 mg) of each ham was homogenized at 12000 r for 3 min in 600 μL of methanol/water (2:1, v/v). The extracts were centrifuged at 12000 g for 10 min at 4 °C. The methanol of the supernatants was removed by vacuum freeze-drying. The extraction was dissolved in 600 μL of 0.1 M K_2HPO_4/NaH_2PO_4 buffer (pH 7.4) containing 50% D_2O, 0.01% NaN_3 and 0.001% sodium trimethylsilylpropionate and then were centrifuged at 12000 g at 4 °C for 10 min. The 550 μL supernatants of each extraction were transferred into a 5 mm outer diameter NMR tube and 2, 2-dimethyl-2-silapentane-5-sulfonate was also added to quantify these metabolites.

2.8. Data analysis of 1H NMR spectra

1H NMR spectra of extraction were collected at 298 K on a Bruker Avance 600 MHz Spectrometer equipped with ultra-low temperature detection probe under the operating condition of 600.13 MHz using the standard Bruker pulse sequence NOESYGPPR1D (RD-90°-t1–90°-tm90°-acquisition). The Free Induction Decay signal was automatically zero filled, and Fourier transform in processing module and baseline correction of data was performed in in Chenomx NMR Suite 8.1 (Chenomx Inc., Edmonton, Canada). All these spectra were analyzed against Chenomx Compound Library. The residual water (δ 4.7–5.5) signals of the spectral regions were removed. A total of 32 metabolites were quantified according to the internal standard from 1H NMR spectra. All metabolite concentration information were normalized by weight across all replicate samples before being used in the later on multivariable analysis.

2.9. Characterizing the key taste compounds

Components which could contribute to the discrimination between modern-processed ham and traditional-processed ham were identified by partial least square discriminant analysis (PLS-DA). The candidate taste components were further calculated the taste-active values (TAVs) so as to confirm their contributions to taste and richness intensities, according to these descriptions (Haseleu, Lubian, Mueller, Shi, & Koenig, 2013; Kranz, Viton, Smarrito-Menozzi, & Hofmann, 2018; Liu, Xia, Wang, & Chen, 2019; Zhang, Ayed, Wang, & Liu, 2019).

2.10. Statistical analysis

All values were expressed as the mean ± standard deviation. The umami, richness, water content, relaxation time (T2), T2 populations, taste compound profiles and taste-active values were analyzed by Duncan's multiple range test in one-way analysis of variance of SAS 8.0 (SAS Institute Inc., Cary, NC, USA). Student's t-test model was also performed to compare these parameters at the same sampling point between traditional and modern processing. The significant level was set as 0.05. Hierarchical cluster analysis and principle component analysis (PCA) were also performed to characterize the components and contents of metabolites among modern-processed and traditional-processed hams. Partial least square discriminant analysis (PLS-DA) was used to further characterize the key taste compounds among modern-processed and traditional-processed hams using SMICA 14.0.

3. Results and discussion

3.1. Sensory characteristics of ham samples

Sensory characteristics of *biceps femoris* muscle derived from the ham samples at the end of post-ripening are shown in Fig. 1. The sensory scores of sweetness, sourness, aftertaste and bitterness did not show obvious difference between traditional-processed ham and modern-processed ham, while significantly higher scores in overall taste, richness and umami were shown in the ham of modern processing than that of traditional processing. Interestingly, there was a significant decrease in the saltiness scores of modern-processed ham than that of traditional-processed ham, which could be attributed to the fact that these hams of modern processing were salted using lower NaCl content, compared with the production of traditional processing (Zhou, Wang et al., 2019a). Consistently, the intensities of umami and richness of ham samples obviously increased from the raw ham to the end of post-ripening in both traditional and modern processing; significantly higher intensities of umami and richness in modern-processed ham observed by electric tongue were also shown at the later stage of dry-ripening and the end of post-ripening compared with the samples of traditional-processed ham. The sensory results indicated that the ham manufactured by modern processing would have more intense overall taste, richness and umami attributes than that of traditional-processed ham.

The attributes of overall taste, richness and umami are extremely significant quality characteristics of dry-cured ham (Hersleth, Lengard, Verbeke, Guerrero, & Næs, 2011; Zhang et al., 2018). Processing technology and procedures have a significant effect on organoleptic qualities of dry-cured ham (Toldrá, 2006; Zhou, Wu et al., 2019b). In our study, the sensory results showed that overall taste, richness and umami were more significant than sweetness, sourness, aftertaste and bitterness in describing the changes of organoleptic attributes of Jinhua ham between traditional and modern processing. The difference of overall taste, richness and umami intensities could be attributed to the fact that the ham of modern processing accumulated higher profiles in key taste substances which could enhance the intensities of overall taste, richness and umami.

3.2. The changes of texture parameters in both traditional-processed and modern-processed hams

The profiles of hardness, adhesiveness, springiness and cohesiveness are shown in Table 1 during the processing of traditional procedure and modern procedure in Jinhua ham. As shown in Table 1, the values of hardness, adhesiveness and cohesiveness significantly increased from raw ham to the later stage of dry-ripening, whereas the values of springiness correspondingly decreased; no obvious difference was observed in hardness, springiness and cohesiveness from the later stage of dry-ripening to the end of post-ripening in both traditional-processed ham and modern-processed ham, while the increased profile of adhesiveness was found from the later stage of dry-ripening to the end of post-ripening in both traditional and modern processing. Interestingly, no obvious difference was shown in the values of hardness, springiness and cohesiveness in traditional-processed and modern-processed hams at the later stage of dry-ripening and at the post-ripening stage, indicating that these ham samples of traditional processing and modern processing showed similar textural profiles.

Texture is an important organoleptic characteristic to evaluate the quality of dry-cured ham (Tomovic et al., 2013; Zhou, Wang et al., 2019a). Proper texture of dry-cured ham is very popular with consumers and texture parameters of dry-cured ham mainly include hardness, adhesiveness, springiness and cohesiveness, which is closely related with the changes of salt penetration, moisture content and ripening time during the processing of dry-cured ham (Arnau et al., 2007; Fulladosa,

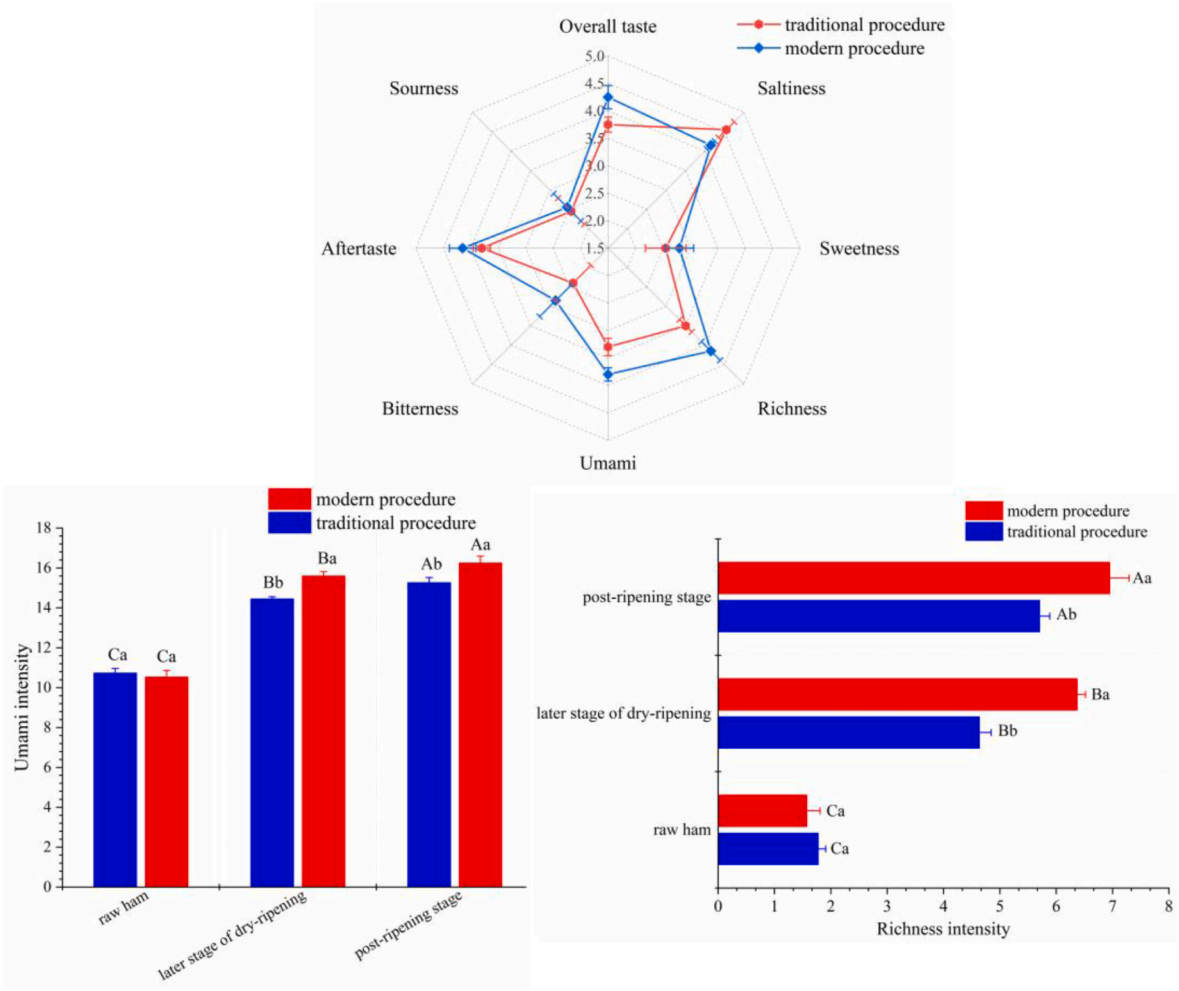

Fig. 1. Sensory intensities of the *biceps femoris* muscle of Jinhua ham. $^{A-C}$ Identical letters indicate that there is no significant difference in different processing stages ($P > 0.05$); $^{a-b}$ Identical letters indicate that there is no significant difference at the same processing stage ($P > 0.05$).

Table 1
The changes of texture parameters of Jinhua ham.

	Hardness (N)	Adhesiveness (N•s)	Springiness	Cohesiveness
raw ham	208.69 ± 20.37B	−11.39 ± 2.07C	0.57 ± 0.08A	0.32 ± 0.04B
end of dry-ripening (T)	9824.04 ± 744.64Aa	−46.48 ± 2.02Bb	0.46 ± 0.05Ba	0.48 ± 0.07Aa
end of dry-ripening (M)	9586.37 ± 547.93Aa	−65.64 ± 4.45Ba	0.42 ± 0.06Ba	0.45 ± 0.02Aa
end of post-ripening (T)	10047.41 ± 682.75Aa	−72.85 ± 2.62Aa	0.45 ± 0.03Ba	0.46 ± 0.03Aa
end of post-ripening (M)	10233.65 ± 723.32Aa	−80.81 ± 9.36Aa	0.39 ± 0.09Ba	0.42 ± 0.06Aa

T, M represent the sampling of traditional-processed ham and modern-processed ham, respectively. $^{A-C}$ Different letters indicate significant difference in different processing stages ($P < 0.05$); $^{a-b}$ different letters indicate significant difference in different groups ($P < 0.05$).

Serra, Gou, & Arnau, 2009). In detail, a soft texture of dry-cured ham means that the product does not reach sufficient hardness, which is closely related to salt concentration and moisture loss (Ruiz-Ramirez, Arnau, Serra, & Gou, 2005). Adhesiveness is associated with proteolysis and water distribution of dry-cured ham (Morales, Arnau, Serra, Guerrero, & Gou, 2008). In the present study, the values of hardness from 208.69 N in raw ham increased to 10047.41 N in traditional-processed ham and to 10233.65 N in modern-processed ham, and no obvious difference was observed in these parameters of hardness, adhesiveness, springiness and cohesiveness at the final product, which indicated that modern-processed procedure did not change the development of textural parameters of Jinhua ham.

3.3. The changes of moisture and water distribution in both traditional-processed and modern-processed hams

The changes of moisture content and water distribution of Jinhua ham during the processing of traditional and modern procedures are shown in Fig. 2. The moisture content of *biceps femoris* muscle samples decreased significantly from the raw ham to the end of post-ripening in both traditional and modern processing ($P < 0.001$); no obvious difference was observed at the final product of traditional processing and modern processing ($P > 0.05$), and the moisture content of *biceps femoris* muscle was approximately 49.74% and 52.19% at the final product of traditional and modern processing procedures, respectively.

Bound water (T2b) with relaxation time around 0–5 ms is closely associated with macromolecules, immobilized water (T21) is a major component characterized by relaxation time around 20–60 ms, and extramyofibrillar water (T22, free water) is a slower relaxing component (T22) with a relaxation time around 100–400 ms (Garcia et al., 2015).

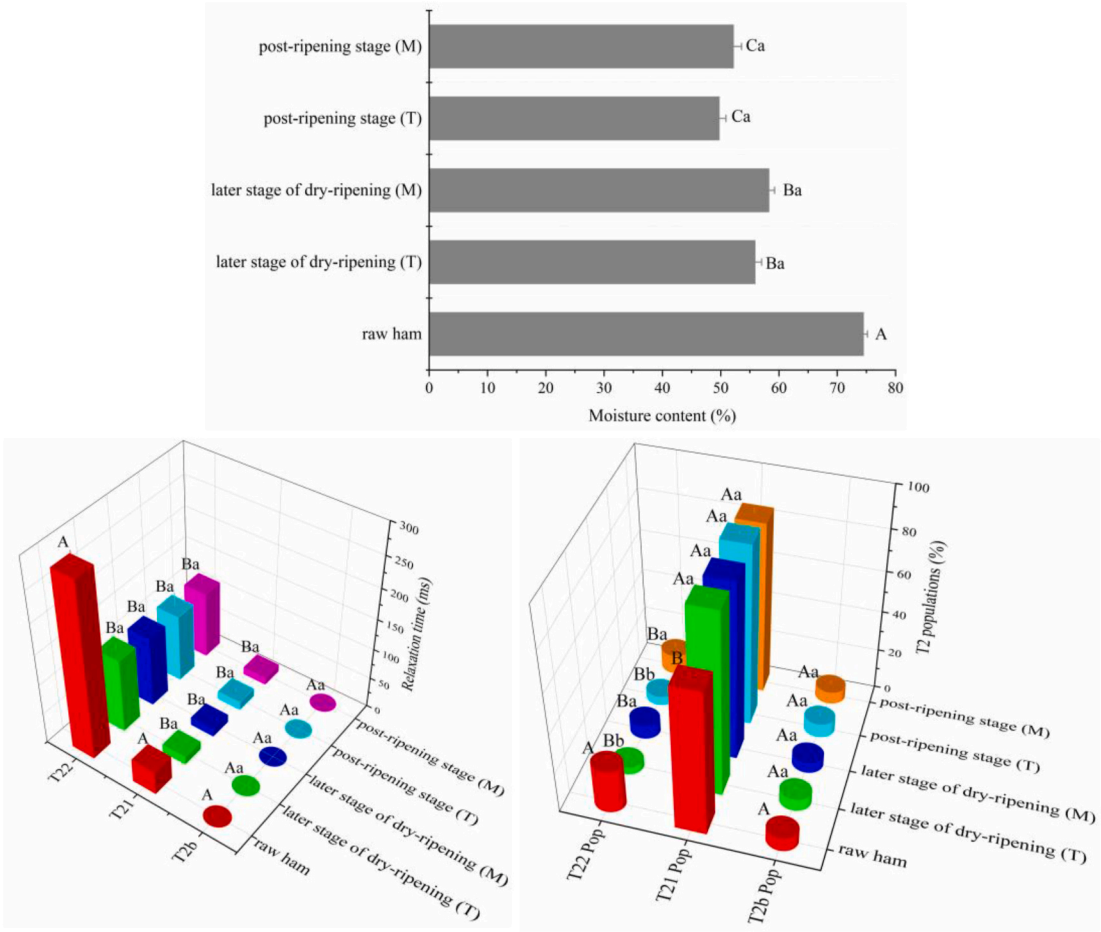

Fig. 2. The changes of moisture content and water distribution of Jinhua ham. $^{A-C}$ Identical letters indicate that there is no significant difference in different processing stages ($P > 0.05$); $^{a-b}$ Identical letters indicate that there is no significant difference at the same processing stage ($P > 0.05$).

The transversal relaxation times and corresponding populations of low-field NMR gained from these samples of traditional- and modern-processed hams are shown in Fig. 2. The relaxation times of water were significantly affected by ripening time. The transverse relaxation time of immobilized water (T21) and the transverse relaxation time of free water (T22) of traditional-processed and modern-processed hams significantly decreased from raw ham to the later stage of dry-ripening ($P < 0.001$), but there was no significant change from the later stage of dry-ripening to the end of post-ripening in both traditional-processed and modern-processed hams. The transverse relaxation time of bound water (T2b) did not show obvious difference from raw ham to the end of post-ripening in both traditional-processed and modern-processed hams. For the changes of corresponding populations of water, the populations of immobilized water (T21 Pop) significantly increased from raw ham to the later stage of dry-ripening ($P < 0.001$), while the populations of free water (T22 Pop) significantly decreased from raw ham to the later stage of dry-ripening; there was no significant change in the populations of immobilized water (T21 Pop) and free water (T22 Pop) from the later stage of dry-ripening to the end of post-ripening in both traditional-processed and modern-processed hams. No obvious changes in the populations of bound water (T2b Pop) were observed during the whole processing of traditional procedure and modern procedure, while significantly higher populations in T22 Pop were shown in modern-processed ham than in traditional-processed ham at the final product.

Moisture content and water distribution play a role in developing sensory attributes including texture and juiciness (García, Rodríguez, de Ávila Hidalgo, & Bertram, 2016). High content in moisture usually results in soft texture of dry-cured ham and these products with soft texture commonly are rejected by consumers (Morales et al., 2008). In our results, the moisture content of traditional-processed and modern-processed hams decreased from raw ham to the end of post-ripening, and the content of moisture decreased to 49.74% and 52.19% at final products, respectively. The change could be explained by the fact that the dehydration of muscle tissues occurred during the salting and ripening stage (Bajd, Gradišek, Apih, & Serša, 2016; Bosse Nee Danz et al., 2018). Some studies have demonstrated that the decrease of moisture content showed a great contribution to the increase of hardness of dry-cured ham (Alino et al., 2009). Thus, in our study, the increase of hardness could be attributed to the decrease of moisture content and redistribution of water populations during the processing of Jinhua ham. The mobility of water resulted in the loss of water and a shift from P22 populations to P21 populations in our study. The changes in water populations of ham in the present study agreed well on the reports of fermented sausages (Garcia et al., 2015). This shift can be mainly explained by the fact that the formation of protein network induced by salting and ripening holds more immobilized water (Garcia et al., 2015; Sun, Holley, & Safety, 2011). Some studies demonstrated that the populations of free water are related to the development of juiciness of dry-cured ham (Bertram, Aaslyng, & Andersen, 2005). In our study, higher populations of T22 Pop in modern-processed ham could contribute to the higher juiciness scores of modern-processed ham, which is confirmed by previous study of Zhou, Wu et al. (2019b).

3.4. Metabolite components of Jinhua ham

Representative 600-MHz ^{1}H NMR spectra for *biceps femoris* muscle of raw ham, traditional-processed ham and modern-processed ham are shown in Figure S1. From these spectra, 32 metabolites were identified, which mainly included free amino acids, organic acids, small peptides, nucleic acids and sugars. These components were further quantified based on the known concentration of 2, 2-dimethyl-2-silapentane-5-sulfonate standard. To compare the changes of each metabolite, hierarchical cluster analysis heatmap (Fig. 3) was performed to explore the information of metabolite profiles. Samples from raw ham, traditional-processed ham and modern-processed ham displayed different color distributions, indicating that there were characteristic differences in the profiles of metabolite among three groups. Taking the Euclidean distance into consideration, the identified metabolites were classified into 2 main classes, that the metabolites of raw ham clustered into one group, and the metabolites of traditional-processed and modern-processed hams clustered into another group, which indicated that these samples of traditional-processed ham and modern-processed ham showed similar metabolites in terms of the components and profiles. Furthermore, Principle component analysis (PCA) was also employed to characterize the 32 metabolites identified in these hams and to further evaluate the effects of processing procedures on metabolite profiles of Jinhua ham. The score plot of PCA (Fig. 2) showed that 94.3% of the variability was explained by the first three principal components, accounting for 81.8%, 9.5% and 3.0% of the total variance, respectively. These metabolites of fumarate, anserine and ADP were the major contributors of first principal components of PCA model; lactate/lactic acid, creatine, glycerol, niacinamide, creatine phosphate, adenosine, glycine, hypoxanthine and glucose were the key contributors of secondary principal components of PCA model; valine, acetic acid, tyrosine, phenylalanine, glutamate/glutamic acid, tryptophan, methionine, leucine, isoleucine and carnosine were the important contributors of third principal components of PCA model. Overall, the results of PCA and hierarchical cluster analysis heatmap indicated that free amino acids, organic acids, small peptides, nucleic acids and sugars were main components of metabolites of Jinhua ham (Zhang et al., 2018), and that these differential components and profiles were the source of variation in response to processing procedures.

3.5. Partial least square discriminant analysis (PLS-DA) and taste-active values of key taste substances

To get more information on dry-cured ham with different processing procedures (traditional- and modern-processing procedures), the relationship between metabolites and taste scores and richness intensities was conducted by PLS-DA. X-matrix was designed as the profile of metabolites, and Y-matrix was set as taste scores and richness intensities in the PLS-DA model (Fig. 4). The PC1 and PC2 of PLS-DA model explained 99.1% of the variance in the X-matrix and 98.6% of the variance in the Y-matrix. These results indicated that the PLS-DA model well explained these variates including sensory attributes (taste scores and richness intensities) and most of metabolites. Variable Importance for the Projection plots (VIP) were employed to evaluate the contribution of metabolites towards improving taste and richness of the dry-cured ham. As is shown in Fig. 4, several metabolites including glutamate/glutamic acid, alanine, leucine, lactate, glycerol, anserine, creatine and creatine phosphate showed a high VIP-value (more than 1), indicating that these parameters could have a key contribution to Y-variables (taste scores and richness intensities). Furthermore, the coefficients plots showed that taste and richness (Fig. 5) were strongly correlated with glutamate/glutamic acid, alanine, leucine, lactate, glycerol, anserine, creatine and creatine phosphate, respectively.

In order to further evaluate the contributions of these metabolites on taste and richness, these key metabolites were characterized by the analysis of taste-active values and taste attributes. As shown in Fig. 6, the taste-active values of lactate, glutamate and alanine were over 30, indicating that these metabolites showed great contributions to the taste and richness of Jinhua ham. In addition, these metabolites including leucine, anserine, and glycerol also showed the taste-active values with more than 1, meaning that they had significant effect on the taste and richness of dry-cured ham. However, the taste-active values of creatine were less than 1 in both traditional-processed ham and modern-processed ham, indicated that it did not directly contributed to the bitterness of Jinhua ham. The taste attributes of these metabolites were further characterized according to previous studies (Haseleu et al., 2013; Kranz et al., 2018; Liu et al., 2019; Zhang, Ayed, Wang, & Liu, 2019). As shown in Fig. 6, lactate was the major contributor of sourness of dry-cured ham; glutamate was the main component of umami taste; glycerol and alanine contributed to the sweet taste; anserine showed a positive effect on meaty taste. Interestingly, lactate, anserine, glycerol and creating showed significantly higher taste-active values in these samples of modern-processed ham than that of traditional-processed ham. These differences in taste-active values could be the main source of the variations of taste and richness between modern-processed ham and traditional-processed ham.

The taste and richness development of dry-cured ham was related to the profile of free amino acids, small peptides, organic acids and nucleic acids (Dashdorj et al., 2015). Furthermore, free amino acids and small peptides were identified as the major components of taste substances of dry-cured ham (Sforza et al., 2006). The taste and richness attributes of dry-cured ham could be associated with the amount of free amino acid

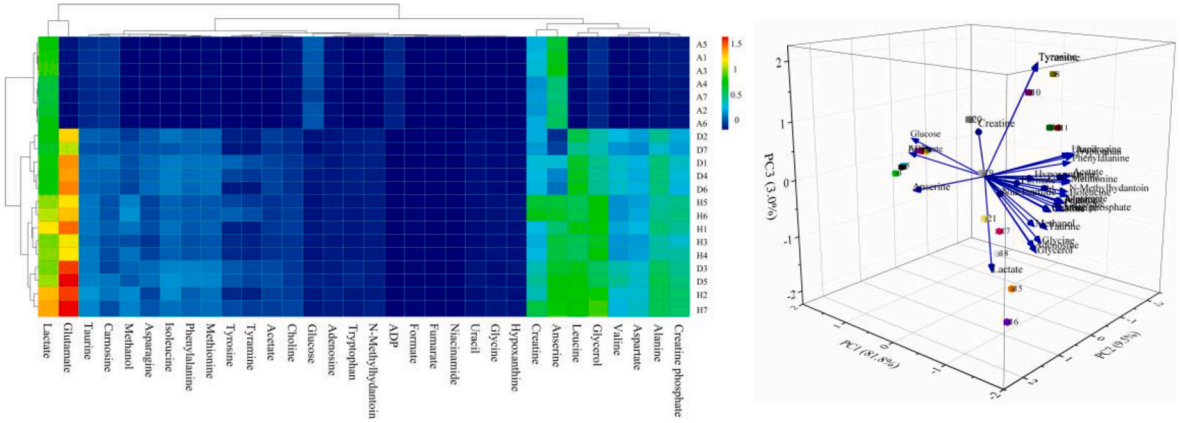

Fig. 3. Hierarchical cluster analysis heatmap and principal component analysis (PCA) on the metabolites. A1, A2, A3, A4, A5, A6 and A7 represent the samples of raw ham; D1, D2, D3, D4, D5, D6 and D7 represent the traditional-processed ham samples of the end of post-ripening; H1, H2, H3, H4, H5, H6 and H7 represent the modern-processed ham samples of the end of post-ripening.

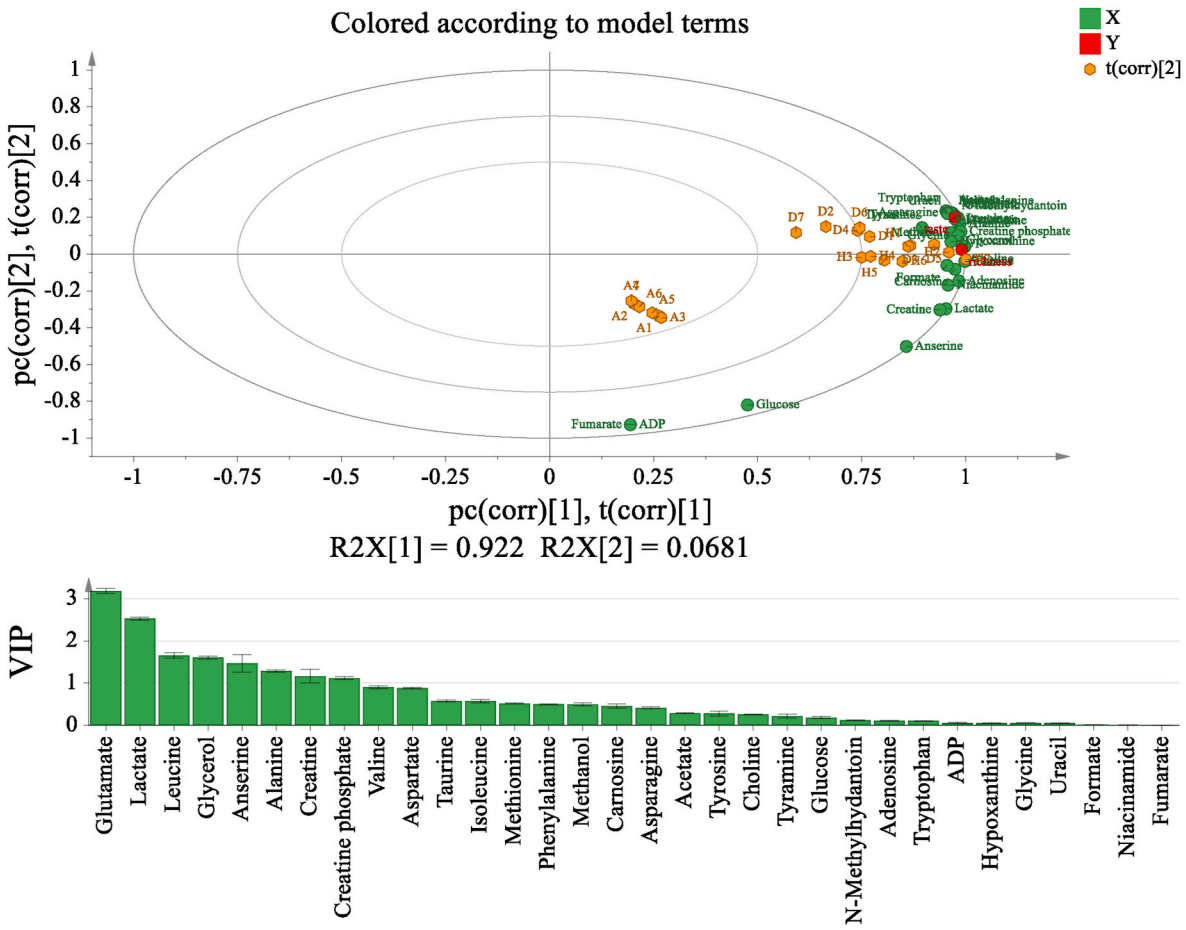

Fig. 4. Partial least square discriminant analysis (PLS-DA) assesses the correlation between taste scores, richness intensities and metabolites profiles.

(Kęska & Stadnik, 2017). Sweet taste is associated with the content of threonine, serine, alanine, glycine and proline; glutamic acid and aspartic acid are responsible for the umami taste of dry-cured ham, whereas the content of phenylalanine, isoleucine, leucine and methionine is related to bitter taste of dry-cured ham (Kęska & Stadnik, 2017; Sforza et al., 2006). It has been well documented that 13 amino acids (tyrosine, leucine, phenylalanine, isoleucine, alanine, glutamic acid, valine, methionine, tryptophan, aspartate, asparagine, taurine and glycine) were identified and quantified by 1H NMR, and these amino acids significantly increased from the raw ham to the final products in both traditional-processed and modern-processed hams. In meat and meat products, glutamic acid is one of the main amino acids, accounting for 20% of all amino acids in meat proteins. Consistently, in our results, the most abundant free amino acids were glutamic acid, alanine, aspartic acid, valine and leucine; the content of glutamic acid and aspartic acid accounted for 20.25% of all amino acids in both traditional-processed and modern-processed hams. According to taste classification, the content of umami amino acids including aspartic acid and glutamic acid showed largest increase and followed by sweet amino acids (alanine, glycine and valine) at the final products in both traditional-processed and modern-processed hams. These results indicated that the increase of umami and sweet amino acids could be responsible for the improvement of taste and richness from raw ham to post-ripening of Jinhua ham (Kęska & Stadnik, 2017). However, in terms of profiles and kinds of free amino acids, there were no obvious difference between traditional-processed and modern-processed hams, indicating that free amino acids were not the main source of difference of taste and richness between traditional-processed ham and modern-processed ham.

Carnosine (histidine-derived dipeptide) and anserine (β-alanyl-l-1methylhistidine) have been described as taste compounds (Jung et al., 2013). They are widely distributed in the skeletal muscle and are significantly positive correlation to sensory characteristics of muscle food. Suyama and Shimizu (1982) demonstrated that carnosine had a notable buffer effect. Studies also reported that carnosine could enhance the taste attributes of muscle foods (Djenane, Martínez, Sánchez-Escalante, Beltrán, & Roncalés, 2004). Carnosine and anserine could prolong the taste sensation in oral cavity (Dashdorj et al., 2015). In addition, both carnosine and anserine are able to break unsaturated aldehydic products and minimize the rancidity in dry-cured meat products (Gianelli, Flores, & Toldrá, 2003). Furthermore, some studies demonstrated that anserine was related to the meaty taste and richness of muscle food, for examples, the existence of anserine significantly increased the richness and taste attributes of beef broth (Pereira-Lima, Ordoñez, de Fernando, & Cambero, 2000). These studies indicated that anserine and carnosine played a role in developing the taste attributes of dry-cured ham (Dashdorj et al., 2015). In our results, higher content of anserine was shown in modern-processed ham than in traditional-processed ham, which could contribute to the higher richness intensities and taste scores of Jinhua ham with modern-processed procedure.

Organic acids play also a role in developing the taste attributes of dry-cured ham. In our study, the content of lactate, creatine phosphate and creatine was relatively higher than others in both modern-processed ham and traditional-processed ham. Lactate is the product of glycolysis, and has an important effect on meat quality and on pH development (Bertram, Oksbjerg, & Young, 2010). Furthermore, lactate was the main contributor of sourness and richness, which depended on its concentration (Dashdorj et al., 2015). In the present study, higher content of lactate was shown in modern-processed ham than in

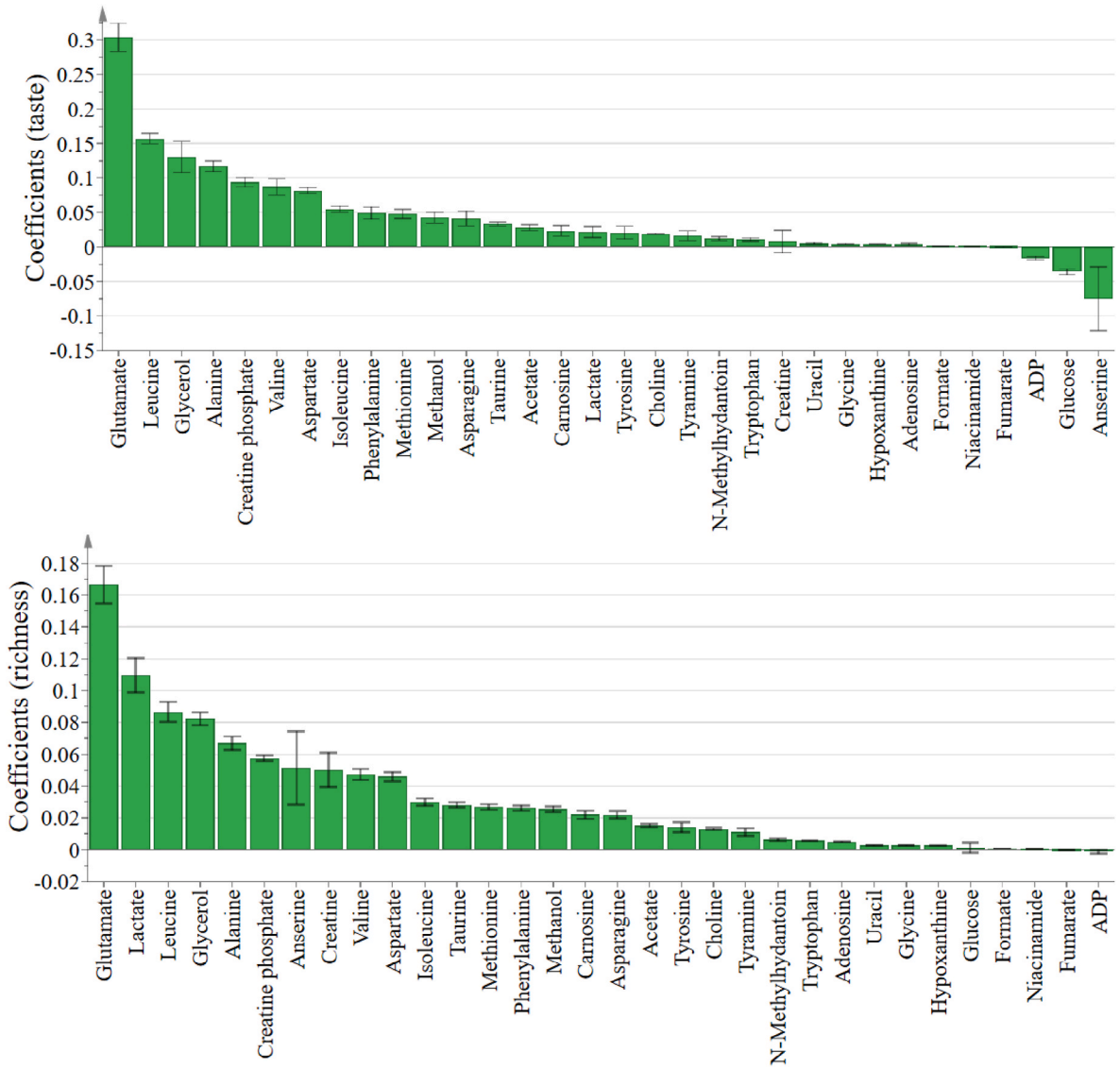

Fig. 5. The changes of coefficients of taste, richness and metabolites.

traditional-processed ham, which could be attributed to the shorter processing procedure of modern-processed ham. Creatine and creatine phosphate played a vital role in the energy delivery process of muscle cells (Jung et al., 2013). In terms of taste attributes, creatine has been identified as taste-active compounds in dry-cured ham (Zhang et al., 2018) and stewed beef broth (Pereira-Lima et al., 2000). Schlichtherle-Cerny and Grosch (1998) demonstrated that it was related to bitter taste when taste-active values of creatine were more than 1 and that the decrease of creatine contributed to the improvement of richness intensities of stewed beef broth. In the present study, taste-active values of creatine did not reach 1, which indicated that it contributed to the improvement of richness of traditional-processed and modern-processed hams.

Previous studies have reported that the content of nucleotides gradually decreased to undetectable levels during the processing of Parma ham (Zhang et al., 2018). In our study, four nucleic acids and their derivatives were identified among three groups; uracil, adenosine and hypoxanthine were the main components of derivations of nucleic acids. Hypoxanthine is usually derived from the degradation of inosine monophosphate (Kuda, Fujita, Goto, & Yano, 2008), and contributes to the bitter attribute of dry-cured ham. There was no obvious difference in the content of uracil, adenosine and hypoxanthine, and these metabolites showed a low level in both traditional-processed ham and modern-processed ham, which further demonstrated that they were not the major taste substances of Jinhua ham.

Glucose and glycerol are the product of glycogen degradation and fat degradation, which both contribute to sweetness of food matrix (Dashdorj et al., 2015). Glucose and glycerol were also identified in our results, and significantly higher content of glycerol was shown in modern-processed ham than in traditional-processed ham. The higher content of glycerol could be attributed to the fact that lipolytic enzymes accelerated the degradation of fat and further enhanced the accumulation of glycerol under modern-processed procedure (Zhang et al., 2011; Zhang, Zhen, Zhang, Zeng, & Zhou, 2009). Furthermore, the analysis of PLS-DA and taste-active values demonstrated that glycerol showed a significant contribution to sweetness, which further indicated that high content of glycerol contributed to the improvement of taste scores of modern-processed ham (Zhang et al., 2018).

4. Conclusions

The modern-processed ham showed higher taste scores and richness intensities compared with the traditional-processed ham. The shift from free water populations to immobilized water populations and the

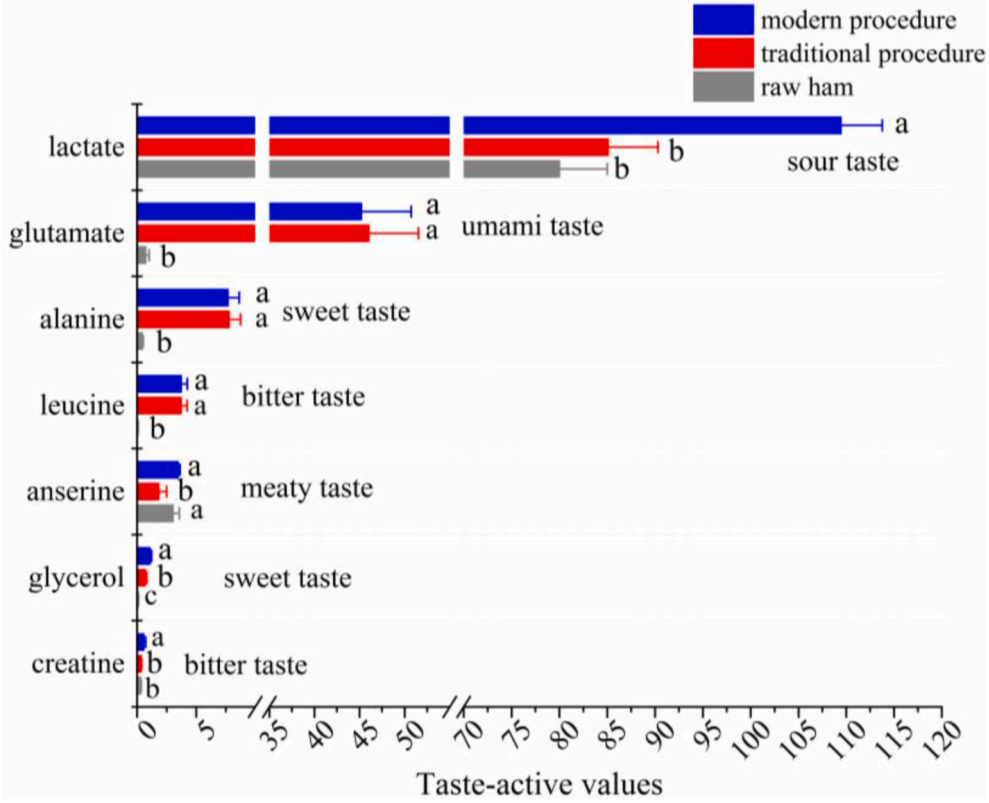

Fig. 6. The changes of taste-active values key taste substances. [a-c] Identical letters indicate that there is no significant difference in different groups ($P > 0.05$).

decrease of moisture content could contribute to the development of texture parameters of Jinhua ham during the modern processing. Free amino acids, small peptides and organic acids were the most intense response in developing the taste and richness of modern-processed ham; glutamic acid, lactate, glycerol, anserine and creatine were responsible for the higher taste scores and richness intensities of modern-processed ham.

CRediT authorship contribution statement

Chang-Yu Zhou: Formal analysis, Validation, Visualization, Data curation, Writing - original draft. **Yun Bai:** Project administration, Investigation. **Chong Wang:** Data curation, Validation. **Chun-Bao Li:** Project administration, Writing - review & editing, Supervision. **Xing-Lian Xu:** Project administration, Supervision. **Dao-Dong Pan:** Methodology, Writing - review & editing, Supervision. **Jin-Xuan Cao:** Conceptualization, Ideas, Methodology, Supervision, Funding acquisition, Writing - review & editing. **Guang-Hong Zhou:** Methodology, Supervision, Funding acquisition, Writing - review & editing.

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.

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Appendix A. Supplementary data

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