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Multivariate statistical regression analysis and relative quantification based on dimensional-reduction method to compare the taste-active components of different chicken breeds

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Abstract

To compare the difference of non-volatile taste-active flavor components of different chicken breeds, four Chinese native yellow feather broilers including Langshan chicken, Chongren chicken, Luyuan chicken and Wenchang chicken were used in the experiment. The contents of free amino acids, 5′-nucleotides and minerals were determined by standard method, and then five principal components were extracted from the multi-index system based on the principal component analysis (PCA). Combined with the Mahalanobis distance analysis method and sensory evaluation results, the advantages and characteristics of each chicken breed were evaluated. The results showed that different kinds of chicken had their own advantages in different evaluation dimensions. The Equivalent umami concentration (ECU) of Wenchang chicken, which had the highest content of amino acids, was 12 g monosodium glutamate (MSG)/100 g, indicating the umami taste of it was very intense. The indexes of Langshan chicken were relatively uniform, with slightly higher mineral content, its overall Mahalanobis distance score was more similar to the "best standard". According to the Mahalanobis distance score, although the difference in amino acid content among each species was the largest, the overall score was more affected by the content of minerals and nucleotides, and there was interaction between each nutrient, which had an impact on the overall Mahalanobis distance score. The sensory evaluation results indicated that Wenchang chicken was the most superior among the taste of the four varieties investigated. Finally, taste compounds affecting the difference of chicken varieties were analyzed by partial least squares regression (PLS), resulting in order of mineral (Ca^{2+}, Mg^{2+}, PO_4^{3-}) > nucleotide (AMP, IMP) > amino acid. This could provide a theoretical basis for quantitative oriented flavor processing and consumer choice of chicken.

Keywords Non-volatile flavor components, Principal component analysis, Mahalanobis distance, sensory evaluation

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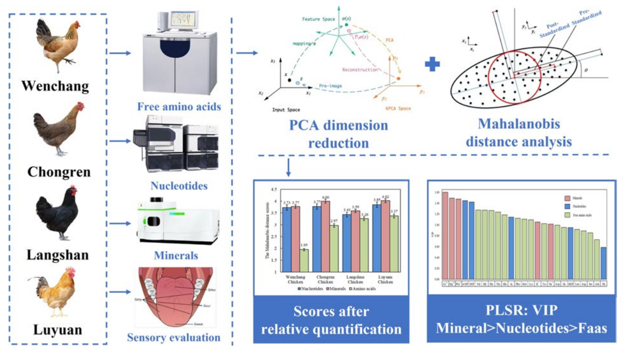
Graphical Abstract



Introduction

Meat flavor, the combination of taste and order, is an important sensory indicator involved in meat purchasing decision of consumers (Zhuang et al., 2016). The taste-related compounds refer to water-soluble compounds with relatively low molecular weight, including 5'-nucleotides, free amino acids (FAA), organic acids, sugars and inorganic ions (Jayasena et al., 2015). Aroma is related to volatile substances such as hydrocarbons, aldehydes, ketones, esters, alcohols, acids, sulfur and nitrogen compounds (Regueiro et al., 2017; Takakura et al., 2014). There are a number of factors that affect the quality and flavor of chicken, which has been reported by many researchers (Dashdorj et al., 2015; Jayasena et al., 2013; H. Li et al., 2016; Qi et al., 2017a, 2017b). From the macro level, the main influencing factors are breed, age, feeding method, diet composition, etc. From the micro level, the diameter, quantity and type of muscle fibers have an important impact on the texture, while the content of intramuscular fat, inosinic acid and thiamine can significantly affect the tenderness, flavor and juiciness of meat (Yue et al., 2016). Generally, the macro level determines the micro level. The flavor precursors of chicken, such as fatty acids, amino acids, reducing sugars, will accumulate with the increase of age and feed. Most of them participate in lipid oxidation reaction, Maillard reaction, amino acid degradation and other biochemical reactions, thereby affecting the flavor of broilers (Jayasena et al., 2013). For example, Takahashi et al. (2012) found that dietary arachidonic acid was significantly positively correlated with the content of volatile flavor substances and taste characteristics of umami and aftertaste in chicken. S. Zhang et al. (2022) explored the difference in nutritional flavor of Lueyang Black-bone chicken muscles at different ages, predicted that hens should be listed between 120 and 150 days of age, and roosters should be listed at 120 days of age. Therefore, it is more convenient to compare the deep differences between different chicken breeds under the same age and breeding conditions. In this study, the age and breeding conditions of different broilers were controlled to make the same starting point for research.

Compared with aroma compounds, taste compounds are low molecular weight water-soluble compounds having no volatility or lower volatility (Dang et al., 2015). Non-volatile taste constituents mainly include free amino acids, small molecular peptides hydrolyzed by muscle protein, 5'-nucleotides degraded by adenosine triphosphate (ATP), inorganic salts and organic acids (Hajeb & Jinap, 2015). The basic taste categories are composed of sweet, sour, bitter, salty and umami, which can be induced by these taste-active compounds substances when dissolved in saliva and bound to receptor proteins on the tongue (Simon et al., 2006). Taste perception is one of the most complex human behaviors. It involves almost all senses and is closely related to olfactory perception. The odor image generated by the olfactory pathway participates in the formation of taste perception (Shepherd, 2006). Although from a physiological point of view, order and taste receptors as well as neural pathways leading to the brain are separated, the flavor of food perceived through oral processing is produced by the joint action of aroma and taste substances that are recognized by the brain (Papies et al., 2022; Serrano-Gonzalez et al., 2021). Therefore, the perception of chicken flavor is the result of the complementary perception of smell and taste.

Yellow feather broilers are Chinese native chickens which have a longer growth cycle, firmer meat quality and stronger chicken flavor than white-feathered chickens (Qi et al., 2017a, 2017b). They can be divided into three major types: fast growth (50–80 days), medium growth (70–100 days) and slow growth (over 100 days) according to the industry sources. The slower the growth rate, the longer the age, the higher contents of inosine 5′-monophosphate (IMP) (Xu et al., 2021). It is of important significance for the independent development of broiler industry to select high-quality yellow feather broilers for breeding. Mahalanobis distance is a method proposed by Indian statistician Mahalanobis in 1936 (Mahalanobis, 1936) that can effectively calculate the closest distance between a sample and the "center of gravity" of a sample set or the similarity of two unknown sample sets (X. Zhang et al., 2015). Compared with the Euclidean distance, it is not affected by the dimension, which means that the Mahalanobis distance between two points is not related to the measurement unit of the original data (Ramos-Guajardo & Ferraro, 2020). Sensory evaluation is one of the best methods to measure product quality which can apply food theory to practice and simulate consumers liking even towards products (H. Li et al., 2016; Zhao & Chen, 2021). Equivalent umami concentration (EUC) is related to the content of amino acids (glutamic acid and aspartic acid, Glu and Asp) and 5'-nucleotides (AMP and GMP) in the food system (W. Wang et al., 2020). The umami intensity provided by them is converted into the concentration of sodium salt (g/100 g) glutamine with the same freshness, which can reflect the comprehensive influence of different dimensions on the umami taste of chicken (D. Chen & Zhang, 2007).

The volatile compounds of four representative yellow feather broilers have been analyzed from multiple dimensions and published (H. Wang et al., 2021). Both the principal component analysis and the Mahalanobis distance analysis method were used. Combined with the evaluation results of a professionally trained sensory evaluation panels, this article reveals the formation of non-volatile taste-active components of yellow feather broilers.

Material and methods

Reagents

All chemicals and solutions are analytical grade. All standard substances (amino acids, nucleotides, mineral elements) and methanol were purchased from Sigma Aldrich (USA). In addition, perchloric acid, n-hexane, potassium dihydrogen phosphate, hydrogen peroxide, and nitric acid were purchased from Sinopharm (Shanghai, China).

Sample preparation

Whole chickens about four broilers (Langshan chicken, Chongren chicken, Luyuan chicken, Wenchang chicken) whose average weight is about 1.5 kg, were provided by the Yangzhou Poultry Research Institute of the Chinese Academy of Agricultural Sciences, with the same age and feeding conditions. There were still some differences among individuals for yellow feather chicken of the same breed. But if the sample capacity was too large, the experiment cannot proceed smoothly, so the sample size has certain limitations. In order to reduce individual differences, we selected 5 chickens from each breed, mixed them, and each experiment was in triplicate. Totally Twenty hens were removed from the production line after the process of hanging-electric-stunning-slaughtering-draining-defeathering (Jiangsu Lihua Co., Ltd, Changzhou, China). The breast and thigh muscles were quickly picked, vacuum packed in polyethylene vacuum bags and placed in -30 °C freezer for air-cooled quick freezing. After 1 h, the samples were transported to the laboratory in the incubator with ice bags and stored in the freezer at -18 °C.

Chicken cooking procedure: One liter of purified water was added to the pot and heated to 96–99 °C, at which time the water is slightly boiling. The thigh and breast of the same chicken that has been thawed at room temperature of 25 °C for two hours were added to the slightly boiling water bath for 20 min by an induction cooker (C21-SC011, Joyang Co., Ltd., Jinan, China).

Meat sample preparation: The cooked chicken breast and thigh were mixed according to the weight ratio of 1:1, and then crushed and blended at 300 W for 20 s with a meat grinder (J10, Oaks, Guangzhou, China), after which the extraction of free amino acids and 5'-nucleotides components as well as sensory evaluation took place.

Free amino acid (FAA) analysis

Four grams of chicken samples were homogenized and extracted with 20 mL 3% (w/v) 5-sulfosalicylic acid dihydrate in a 50 mL centrifuge tube at 10,000 rpm (three times, 20 s for each) using an Ultra-Turrax macerator (T-25, IKA, Staufen, Germany) in an ice bath. The homogenate was centrifuged at 12,096 g for 15 min at 4 °C in a centrifuge (Avanti J-26XP, Beckman, Shanghai, China) and then the supernatant was shaken with 2 mL of n-hexane (Jo et al., 2018). The aqueous phase was filtered through a 0.45-μm membrane filter after the organic layer was removed, then free amino acids were analyzed on an amino acid automatic analyzer (L-8800, Hitachi, Tokyo, Japan) which was equipped with BioBasic SCX cation exchange column (4.6 mm × 60 mm, 5 μm) and UV detector. Each amino acid was identified by matching its retention time with that of an authentic standard in the L-8800, and quantified using peak areas of the respective amino acid standard.

Nucleotide analysis

LC-16 High Performance Liquid Chromatography (Shimadzu Co., LTD, Japan) with 2998 PDA detector was used to analyze tested nucleotides in chicken. The tested nucleotides including 5'-guanylic acid (GMP), 5'-inosinic acid (IMP), 5'-adenosine acid (AMP), hypoxanthine (Hx) and inosine, were separated by X Bridge C18 column (4.6×250 mm, 5 μm) purchased from Agela Technologies (Shanghai, China). The temperature was controlled at 25 °C. The eluents used were as follows: mobile phase A is 0.05 mol/L potassium dihydrogen phosphate buffer (pH 4.5), while mobile phase B is methanol. To achieve separation of the nucleotides, the flow rate was set to 1.0 mL/min and the following gradient was performed: initial of 98% A for 14 min, linear change to 85% A for at 15 min, hold for 7 min and finely linear change to 98% A for 9 min. The quantification of nucleotides is determined by external standard method (Qi et al., 2017a, 2017b).

Determination of mineral elements

The concentrations of mineral elements (Ca^{2+}, K^+, Mg^{2+}, Na^+, and PO_4^{3-}) were determined by ICP-MS (ICAP RQ, Thermo Fisher, USA). A microwave digestion system (TOPEX$^+$, PreeKem Scientific Instruments Co. Ltd., China) was used for acid digestion of the chicken samples. 5 ml concentrated nitric acid and 0.2 g chicken sample were added into the digestion tube. Digestion is carried out according to the procedure in Table 1 thereafter the tube placed into the microwave digestion apparatus. After the microwave digestion procedure, the samples were digested to a clear solution. The digested solution was transferred to plastic tubes and diluted to 5% of an acid concentration.

Equivalent umami concentration (EUC)

The equivalent umami concentration (EUC, g MSG/100 g) can be calculated by the following addition equation (D. Chen & Zhang, 2007):

Table 1 Microwave digestion program

Stage	Power/W	Heating time/min	Temperature/℃	Duration/min
The first stage	1600	10	120	10
The second stage	1600	5	150	5
The third stage	1600	5	170	5
The fourth stage	1600	5	190	20

$$EUC = \sum a_i\beta_i + 1218\left(\sum a_i\beta_i\right)\left(\sum a_j\beta_j\right)$$

where: α_i is the concentration of umami amino acids (glutamic acid and aspartic acid) (g/100 g); β_i is the relative umami concentration(RUC) of umami amino acids relative to MSG (Glu,1; Asp,0.077); α_j is the concentration(g/100 g) of each umami 5'-nucleotides including 5'-AMP, 5'-IMP and 5'-GMP; β_j is the RUC of umami 5'-nucleotides to IMP (AMP, 0.18; IMP,1; GMP,2.3); 1218 is the synergy constant used in the concentration (Khan et al., 2015).

Taste activity value (TAV)

TAV was defined as the ratio between the concentration of a substance determined in the samples to the taste threshold value of it (J. Zheng et al., 2015). TAV can reflect the contribution of a single compound to the overall taste: when TAV is less than 1, the substance does not contribute or contributes little to the taste; when TAV is more than 1, the substance contributes significantly to the taste, the larger the value, the greater the contribution (Khan et al., 2015). The calculation formula of TAV is as follows:

$$TAV = C_m/C_n$$

C_m is the concentration of the flavor compound (mg/L), and C_n is the taste threshold (mg/L).

The principal component analysis (PCA) and Mahalanobis distance

The principal component analysis (PCA) process can map high-dimensional features to low-dimensional feature spaces, thereby constructing brand-new orthogonal features to eliminate many overlapping information. The principal components and scores of multiple variables which were compressed can be obtained. Using score data instead of the original nutritional index data to calculate Mahalanobis distance can not only reflect all data information, but also compress the number of variables participating in the calculation of Mahalanobis distance, and ensure that there is no collinear problem in the M matrix (T. Chen et al., 2015).

The calculation of Mahalanobis distance requires a data "center of gravity" as the "origin" in the calculation process which can reflect the difference between samples. In order to reflect the accuracy of the Mahalanobis distance, the "best standard" sample was simulated and formulated with references (T. Chen et al., 2003; Jayasena et al., 2015; Jo et al., 2018; Y. Li et al., 2019; Rikimaru & Takahashi, 2010; Shi et al., 2018; Shu et al., 2001; Tang et al., 2009; K. Wang et al., 1999; W. Wang et al., 2018a, 2018b; X. Wang et al., 2018a, 2018b; Y. Wang et al., 2012; Xi et al., 2020;

Table 2 Standard preparation of different concentrations

Taste	Standard	The concentration and score of the standard
Sweet	Sucrose	1%, 2%, 5% Sucrose aqueous solution = 1,2,5
Salty	Sodium chloride	0.05%, 0.2%, 0.35% Sodium chloride aqueous solution = 1,2,5
Umami	Sodium glutamate	0.1%, 0.2%, 0.5% Sodium glutamate aqueous solution = 1,2,5

Xu et al., 2023; Yang et al., 2007; J. Zhang et al., 2018; X. Zheng et al., 2010). All the studies were related to taste compounds including nucleotides and free amino acids in chicken, most of which were for Chinese yellow feather broilers, can be found on China National Knowledge Infrastructure (CNKI). It has the characteristics of being virtual, based on reality, and approaching the best state in each evaluation dimension. The four chicken breeds in this study were compared with it to get the difference.

Calculate the respective score T of different samples given by multiple principal component variables (Eq. (1)).

$$T_{n\times f} = X_{n\times m}\, P_{m\times f} \quad (1)$$

where: X is the index variable matrix; P is the load matrix; n is the number of samples; m is the number of variables; f is the number of principal components.

Calculate the Mahalanobis distance matrix from the sample data to the "best standard" (Eq. (2)).

$$D^2 = (T_i - T)M^{-1}(T_i - T)' \quad (2)$$

where: D is the Mahalanobis distance; M is the covariance matrix of the score matrix (Score) in the standard index set factor analysis; T_i is the score vector of the sample; T is the reference standard value of n samples; i is different samples.

The Mahalanobis distance represents the degree of agreement between the nutritional index of the sample and the "best standard". The larger the value, the longer the distance from the sample, and the lower the degree of agreement with the "best standard", which means negatively correlated. Relatively, the greater the value, the smaller the distance, and the more similar nutrient content of the sample, which is a positive correlation.

Sensory evaluation

The total 40 taste panels (recruited among students at the University of Nanjing Agriculture) for the sensory evaluations were trained in weekly for three time according with the "General Guidelines for Selection, Training and Management of Evaluators" (GB/T 1629.2–2012. General guidelines for selection, training and management of evaluators, 2012) in order to become familiar with the taste language and methodologies, all of them voluntarily joined the sensory sessions and signed an informed consent. After three training sessions, 12 preferred evaluators (age 18–22, male 4, female 8) were finally selected. They have a relatively high sensitivity to taste solutions such as sodium glutamate solution and sucrose solution, and can accurately distinguish and sort them. Quantitative description analysis (QDA) was a comprehensive analysis method in sensory evaluation (Marvell & Ervina, 2022), where evaluators score the status of samples numerically, including aroma, texture, taste, and other aspects. The data was not generated through consistency discussions, it was continuous. Therefore, sensory practitioners can perform various processing on the data, and consistency can be obtained through the mean or other mathematical calculation methods. When analyzing the sensory attributes of different samples, many statistical analysis methods based on continuous data can be used, such as analysis of variance, principal component analysis, cluster analysis, etc. (Moelich et al., 2023). Scores for different taste attributes including umami, sweet and salty with reference to different concentrations of standard in Table 2 were given by each panel. The results were analyzed and statistically processed by two-way ANOVA models to determine the mean scores and significance between the samples.

Statistical analysis

Each experiment regarding four yellow feather chickens was conducted in Triplicate. The data processing, graphing of this experiment was carried out by Excel (Microsoft Office 2016, USA) and Minitab (version16.2.3, USA). The significance analysis was assessed by a two-way analysis of variance (ANOVA) with Duncan's multiple range test in a *P* value of 0.05. The result was presented using the mean standard deviation format. Mahalanobis distance were performed using Matlab (2014 software, USA). Principal component analysis and Mahalanobis distance were performed using Matlab (2014 software, USA). The analysis of Particle least square regression analysis was performed using Python software (Pycharm 3.6.5, USA).

Results and discussion

Free amino acids

As indicated in Table 3, fifteen amino acids in the four broilers were identified. The TAV was calculated by the

Table 3 The concentrations of free amino acids in different chicken species

FAA	Abbr	Threshold	Concentration (mg/100 g)				TAV			
		(mg/100 g)	Wenchang	Chongren	Langshan	Luyuan	Wenchang	Chongren	Langshan	Luyuan
Aspartic acid	Asp	100	14.10 ± 3.05^{a}	15.10 ± 2.67^{a}	12.53 ± 2.55^{a}	10.2 ± 1.29^{a}	0.14	0.15	0.13	0.10
Threonine	Thr	260	11.53 ± 0.85^{a}	6.60 ± 1.27^{b}	9.72 ± 1.07^{a}	5.43 ± 1.59^{b}	0.04	0.03	0.04	0.02
Serine	Ser	150	7.38 ± 0.74^{ab}	7.63 ± 1.74^{a}	6.60 ± 0.91^{ab}	5.40 ± 0.66^{b}	0.05	0.05	0.04	0.04
Glutamic acid	Glu	30	33.73 ± 1.56^{a}	23.92 ± 1.73^{bc}	25.10 ± 3.14^{b}	20.93 ± 1.44^{c}	1.12	0.80	0.84	0.70
Glycine	Gly	130	8.28 ± 0.63^{a}	7.76 ± 1.51a	5.23 ± 0.75^{b}	4.36 ± 1.31^{b}	0.06	0.06	0.04	0.03
Alanine	Ala	60	11.25 ± 0.77^{a}	10.90 ± 1.25^{a}	11.29 ± 1.13^{a}	7.93 ± 1.97^{b}	0.19	0.18	0.19	0.13
Valine	Val	40	10.52 ± 1.93^{a}	6.50 ± 0.83^{b}	5.69 ± 0.5b^{c}	4.08 ± 0.44^{c}	0.23	0.12	0.13	0.14
Methionine	Met	30	11.50 ± 0.96^{a}	8.30 ± 2.25^{b}	8.81 ± 0.51^{b}	5.59 ± 0.64^{c}	0.35	0.22	0.19	0.14
Isoleucine	Ile	90	19.9 ± 3.97^{a}	15.80 ± 0.55^{bc}	15.05 ± 0.34^{c}	14.01 ± 1.77^{c}	0.13	0.09	0.10	0.06
Leucine	Leu	190	7.09 ± 1.43^{ab}	6.07 ± 1.01^{bc}	8.38 ± 0.54^{a}	5.03 ± 0.49^{c}	0.10	0.08	0.08	0.07
Tyrosine	Tyr	—	9.21 ± 0.57^{a}	6.37 ± 1.4b	5.96 ± 0.29^{b}	6.43 ± 0.38^{b}	—	—	—	—
Phenylalanine	Phe	90	24.80 ± 1.14^{a}	19.22 ± 1.11^{b}	21.23 ± 1.73^{b}	15.75 ± 1.46^{c}	0.10	0.07	0.07	0.07
Lysine	Lys	50	13.69 ± 1.49^{a}	8.64 ± 0.57^{b}	6.84 ± 1.08^{b}	7.37 ± 1.49^{b}	0.50	0.38	0.42	0.32
Histidine	His	50	13.89 ± 1.44^{a}	11.87 ± 1.47^{ab}	11.50 ± 1.12^{ab}	10.66 ± 0.98^{b}	0.27	0.17	0.14	0.15
Arginine	Arg	300	14.10 ± 3.05^{a}	15.10 ± 2.67^{a}	12.53 ± 2.55^{a}	10.20 ± 1.29^{a}	0.05	0.04	0.04	0.04

The contents of the free amino acids are in mg/100 g, expressed as the means ± SD, n =3. Means with different superscript letters in the same row are significantly different ($P < 0.05$)

Taste activity value (TAV)

threshold of free amino acids referring to Zhuang et al. (2016). There are significant differences in the ratio of free amino acids between samples ($P < 0.05$). Phenylalanine and glutamic acid were the major flavor amino acids, which were amounted to approximately 25% of total amino acids commonly found in Chinese yellow feather chicken meat. The content of glutamic acid in Wenchang chicken was the highest at 33.73 mg/g among four breeds, the only data with a TAV value greater than 1. In addition, the content of lysine, methionine, valine, glycine, threonine in Wenchang chicken was also the highest, almost twice of Luyuan chicken in the order of TAV from high to low. The TAV values of threonine, serine and arginine were extremely low, which were all lower than 0.1 in the four breeds. Figure 1 shows the proportions of different types of amino acids of including umami, sweet and bitter (UAA, SAA, BAA), as well as the contents of total amino acids (TAA) and total essential amino acids (TEAA) of the four chicken breeds.

Aspartic acid and Glutamic acid are important contributors to the umami flavor, arginine can work synergistically with umami amino acids and sodium chloride to provide a pleasant overall taste (J. Zheng et al., 2015), which was the highest in Chongren chicken, and the lowest in Luyuan chicken. The levels of isoleucine, aspartic acid and histidine were relatively high, which were all over 10 mg/g and similar to previous reports (Rikimaru & Takahashi, 2010). The TAA and TEAA value (210.97 mg/100 g, 99.03 mg/100 g) of Wenchang chickens were significantly higher than that of the other three breeds indicating an excellent nutritional characteristic. The contents of various amino acids in Luyuan chicken were the minimum, and the bitter amino acids accounted for the highest proportion, so it was relatively inferior in amino acid composition. Chongren and Langshan chicken were similar, although their total amino acid content was lower than that of Wenchang chicken, their proportion of sweet and umami amino acids was considerable, especially the proportion of sweet amino acids was significantly higher than that of Wenchang chicken, and the proportion of bitter amino acids was significantly lower than that of the other two breeds ($P < 0.05$).

Mineral elements

The concentrations and TAVs of mineral elements including sodium, potassium, calcium magnesium and phosphate ions were shown in Table 4. Phosphate was the mineral with the highest content, especially in Wenchang chicken, which is up to 580.20 mg/100 g, and its TAV value in all breeds was higher than 3.6. Potassium ion is another major mineral in chicken except phosphoric acid, and its TAV value in Chongren and Langshan chickens was greater than 1, which had a synergistic effect with sodium ion. Phosphate ions play a vital role in the taste, which can reduce bitterness and increase the taste intensity provided by MSG (J. Zheng et al., 2015), it was regarded as the most important mineral element in chicken. It was found that reducing the content of Mg^{2+} and Ca^{2+} in beef soup could reduce the saltiness of the broth through the deletion test (Schlichtherle-Cerny & Grosch, 1998), both of them were the highest in Langshan chicken. Sodium ions can enhance the umami taste of chicken by combining 5'-nucleotide and FAA (W. Wang et al., 2020). Generally, Langshan chicken

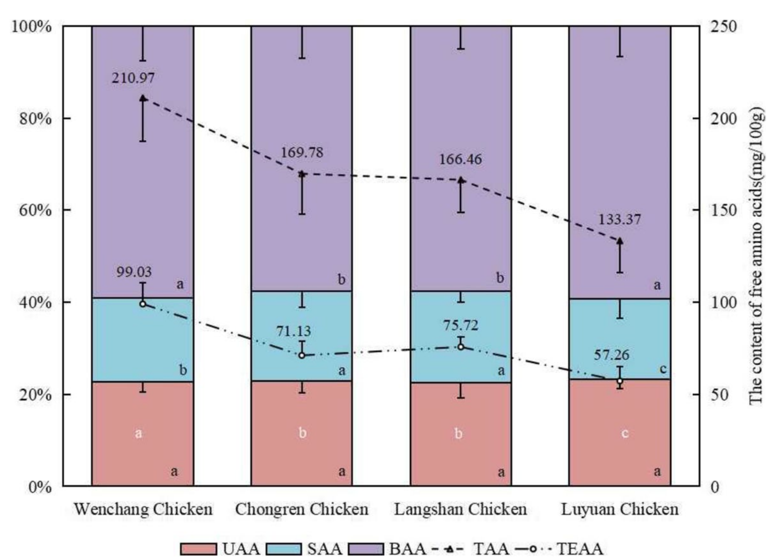


Fig. 1 The classification analysis of free amino acids in four breeds of chickens

Table 4 The concentrations of minerals in different chicken species

Minerals	Threshold (mg/100 g)	Concentration (mg/100 g)				TAV			
		Wenchang	Chongren	Langshan	Luyuan	Wenchang	Chongren	Langshan	Luyuan
PO_4^{3-}	130	580.20 ± 4.32[a]	532.43 ± 10.00[b]	477.29 ± 23.47[c]	547.89 ± 15.39[d]	4.46	4.10	3.67	4.21
Mg^{2+}	150	23.03 ± 2.45[c]	23.22 ± 2.18[c]	32.83 ± 2.69[b]	30.11 ± 2.87[a]	0.15	0.15	0.22	0.33
Ca^{2+}	150	6.18 ± 0.67[b]	5.78 ± 1.09[b]	9.48 ± 1.24[a]	6.26 ± 0.59[b]	0.04	0.04	0.06	0.04
Na^{+}	180	48.89 ± 2.67[b]	55.87 ± 2.52[a]	58.85 ± 2.80[a]	40.57 ± 0.95[c]	0.27	0.31	0.33	0.23
K^{+}	130	118.29 ± 4.4[b]	148.57 ± 2.64[a]	150.36 ± 3.22[a]	93.28 ± 2.51[c]	0.91	1.14	1.16	0.72

The content of minerals in the same row with different superscript letters (a, b) are significant differences ($P < 0.05$) between four broilers, Taste activity value (TAV)

Table 5 The Concentrations of 5′-nucleotides in different chicken species

Nucleotides	Threshold (mg/100 g)	Concentration (mg/100 g)				TAV			
		Wenchang	Chongren	Langshan	Luyuan	Wenchang	Chongren	Langshan	Luyuan
5′-GMP	12.5	15.37 ± 0.72[a]	14.62 ± 1.21[a]	12.71 ± 2.50[a]	9.61 ± 1.18[b]	1.23	1.17	1.02	0.77
5′-IMP	25	254.87 ± 8.30[a]	218.86 ± 7.37[b]	142.13 ± 10.53[c]	120.46 ± 7.74[d]	10.19	8.75	5.69	4.82
5′-AMP	50	14.79 ± 0.96a	15.31 ± 1.98a	10.51 ± 1.95b	9.38 ± 0.78b	0.30	0.31	0.21	0.19
Inosine	—	7.21 ± 1.37[a]	5.79 ± 0.49[a]	6.74 ± 1.17[a]	5.92 ± 0.54a	—	—	—	—
Hx	—	30.39 ± 1.32[b]	33.12 ± 0.49[a]	30.61 ± 0.63[b]	26.46 ± 0.99[c]	—	—	—	—

The content of nucleotides was in mg/100 g. Means in the same row with different superscript letters (a, b) are significant differences ($P < 0.05$) between four broilers
5′- GMP: 5′-guanosine monophosphate; 5′-IMP: 5′-inosinic acid; 5′-AMP: adenosine 5′-monophosphate; Hx: hypoxanthine; Taste activity value (TAV)

had a significant advantage in mineral elements with the highest value of sodium and potassium among the four chicken breeds. Combined with flavor nucleotide, Wenchang chicken had the highest EUC value, followed by Chongren chicken and Langshan chicken, and Luyuan chicken was the worst, which was also in consistent with flavor nucleotides.

Nucleotides

The concentrations of nucleotides were shown in Table 5. The content of inosinic acid (IMP) in Wenchang chicken was 254.87 mg/100 g, which was similar to previous studies (Tang et al., 2009). It reported that IMP contents in breast and thigh were 3.59 and 1.52 mg/g, respectively, with a mean of 2.55 mg/g. Flavor nucleotides including IMP, adenylic acid (AMP) and guanylic acid (GMP) normally contribute to umami taste (Dashdorj et al., 2015). The concentrations of IMP and GMP were well above its taste threshold, IMP might be the main contributor to the umami taste to generate a better taste for chicken (Yue et al., 2016). IMP was the most abundant nucleotide, and its content in Wenchang chicken was more than twice that in Luyuan chicken. The GMP content of Luyuan chicken was significantly lower than that of the other three breeds. The concentrations of AMP were below the threshold, which can make auxiliary contribution to umami taste. Chongren chicken had the highest content of AMP. Inosine and hypoxanthine were considered as undesirable flavor nucleotides. Although there was no significant difference in inosine content among the four breeds ($P > 0.05$), there was a significant difference in hypoxanthine ($P < 0.05$), which was the highest in Chongren chickens at 33.12 mg/100 g. On the whole, Wenchang chicken had the highest flavor nucleotide content, followed by Chongren chicken and Langshan chicken, while Luyuan chicken was the least.

The equivalent umami concentration (EUC) analysis

Figure 2 shows the EUC value of four yellow feather broilers. The results showed that the EUC value of Wenchang chicken was 12.42 g MSG/100 g, almost three times of Luyuan chicken. Langshan chicken and Chongren chicken are in the middle level, and the EUC value was 5.5 and 7.8 g MSG/100 g respectively. The individual and mutual interactions of various nutrients can affect the umami taste of chicken (Dang et al., 2015).The umami taste receptor including T1R1 and T1R3, which belong to class C G-protein-coupled receptors can be activated by umami molecules (Liu et al., 2017, 页 3; Yu et al., 2017). The free aspartic acid and glutamic acid taste sour, mostly, and always have umami characteristics in the presence of sodium salt (Kurihara, 2009). Monosodium salts were MSG-like components having a strong umami taste that can be

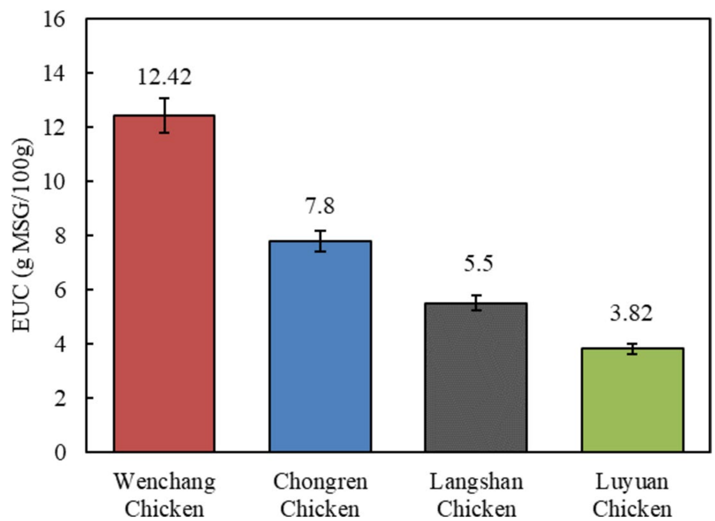


Fig. 2 The EUC values of four breeds of chicken

Table 6 The relevant index content of the "best standard" sample

	Free amino acids			Nucleotides	Minerals
Content (mg/100 g)	Asp 18.26	Ala 11.83	Tyr 7.3	5'-GMP 16.99	PO4 3- 583.81
	Thr 12.33	Val 9.8	Phe 13.2	5'-IMP 176.52	Mg2 + 40.13
	Ser 8.34	Met 12	Lys 25.4	5'-AMP 20.59	Ca2 + 10.85
	Glu 39.5	Ile 14	His 15.78	Inosine 16.89	Na + 68.3
	Gly 8.84	Leu 24	Arg 17	Hx 33.76	K + 198.5

drastically enhanced by umami 5'-nucleotides and free amino acids. In addition, the addition of glycine can also improve umami taste (Ying et al., 2016). The high EUC of Wenchang chicken was corresponding to the content of umami nucleotides and amino acids, and its umami characteristics were closely related to the synergistic effect between umami molecules.

The analysis of principal component and Mahalanobis distance

In order to reflect the accuracy of the Mahalanobis distance, the "best standard" sample was formulated as shown in 2.8. The related indicators are shown in Table 6. The sample contains 25 dimensions in three aspects: free amino acids, nucleotides and minerals. Matlab software was used to standardize the amino acid, nucleotide and mineral content of the four broilers. The principal component analysis was performed after eliminating data differences in different dimensions. According to the principle that the cumulative contribution rate is greater than 90%, five principal components were extracted. As shown in Table 7, the cumulative contribution rate reached 91.68%, indicating that the five principal components basically contain all variable information. The results of comprehensive Mahalanobis distance analysis (MDs) on the five principal components are shown in Fig. 3. There was little difference between four broilers ($P < 0.05$). Langshan chicken had the smallest comprehensive Mahalanobis distance, which was 3.84, Wenchang chicken and Luyuan chicken followed by 4.02 and 4.05, respectively. The broiler with the farthest distance is Chongren Chicken with a score of 4.28. The lower the comprehensive Mahalanobis distance score of

Table 7 Eigenvalue and variance contribution rate

Principal component	Eigenvalue	Contribute/%	Cumulative contribute /%
F1	12.49	49.95	49.95
F2	5.05	20.18	70.13
F3	2.85	11.42	81.55
F4	1.45	5.81	87.36
F5	1.08	4.33	91.68

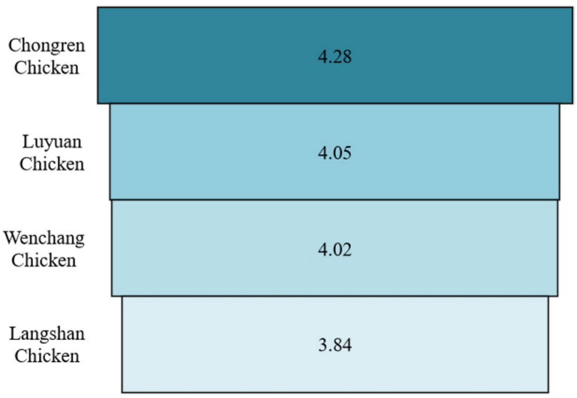


Fig. 3 The result of comprehensive Mahalanobis distance

the broiler, the smaller the distance and the higher similarity with the "best standard" sample.

Sensory evaluation test

The sensory evaluation results are shown in Table 8. There was no obvious difference in sweetness between four chicken breeds ($P > 0.05$), with Wenchang chicken relatively high and Langshan chicken relatively low. A variety of free amino acids such as glycine and alanine can contribute to sweet taste (Takakura et al., 2014), which can interact with IMP to enhance sweetness (Kawai, 2002). In terms of umami, the umami sensory score of Wenchang chickens was the highest, early twice that of Luyuan chicken, which could correspond to the relatively high results of IMP and GMP in the nucleotide test and the results of free amino acids of Wenchang chicken. The origin of salty taste is mainly due to the minerals in muscles (Qi et al., 2017a, 2017b). The saltiness sensory score of Langshan chicken was relatively high among the four broilers, which corresponds to the highest content of Na^{+} and Mg^{2+} in these two breeds. On the overall sensory level, sensory evaluators had a higher preference for Wenchang chicken.

To further explore the influence of each nutrient on the overall score, the data of nucleotides, minerals and free amino acids were respectively pre-processed by standardization and principal component analysis. According to the principle that the cumulative contribution rate was greater than 90%, the Mahalanobis distance scores of nucleotides, minerals and free amino acids were calculated respectively as shown in Fig. 4. Four broilers had little difference in nucleotides with the lowest score of Langshan chicken at 3.43 and the highest score of Luyuan chicken at 3.85 ($P < 0.05$). The mineral content between Wenchang chicken and Langshan chicken were similar, the same change with Chongren and Luyuan chicken.

Table 8 Sensory rating scores for different species

Evaluation dimension	Chicken breed			
	Wenchang	Chongren	Langshan	Luyuan
sweetness	1.93 ± 0.18^{a}	1.38 ± 0.17^{b}	1.36 ± 0.11^{b}	1.57 ± 0.12^{a}
saltiness	2.00 ± 0.12^{bc}	1.63 ± 0.13^{c}	2.86 ± 0.37^{a}	2.33 ± 0.48^{ab}
umami	4.05 ± 0.38^{a}	3.62 ± 0.33^{a}	3.00 ± 0.11^{c}	2.16 ± 0.28^{b}

Different superscript letters in the same row represent significant differences ($P < 0.05$)

There are significant differences in amino acid content between four broilers ($P < 0.05$). Wenchang chicken has obvious advantages over other chicken breeds, while Luyuan chicken has a relatively poor score. Wenchang chicken performed best in the comparison of free amino acids, but ranked second in the overall comparison, indicating that free amino acids had less influence on the overall comparison. In the comparison of nucleotides and minerals, the distance between Wenchang chicken and Langshan chicken was smaller than that of Chongren chicken and Luyuan chicken, which was consistent with the overall distance distinction, indicating that nucleotides and minerals had a greater impact on the overall distance.

Partial least square regression analysis of sensory evaluation and taste compounds

Partial Least Squares (PLS), a new multivariate statistical data analysis method, can solve the multicollinearity problem caused by the high correlation between independent variables in the regression modeling process by extracting components containing the variation information of the original data from the data tables of independent variables and dependent variables to establish the regression model (Song et al., 2014). It was widely used in food composition analysis (Xiao et al., 2018; M. Zhang et al., 2017). Nguyen et al. (2020) used the partial least squares regression model to study the relationship between product quantity and consumption characteristics, which indicated that the partial least squares regression model was suitable for clarifying the relationship between sensory evaluation of consumer attitude, characteristics and expectation of food product.

In order to explore the relationship between the concentration of each taste-active compound and the evaluation conclusions obtained by the preferred evaluators, partial least squares regression analysis was carried out with the concentration of non-volatile flavor substance as the independent variable and the sensory evaluation results as the dependent variable. R^2X_{cum}, R^2Y_{cum} and Q^2_{cum} were used to evaluate the fitting effect of PLS model. R^2X_{cum} and R^2Y_{cum} represented the proportion

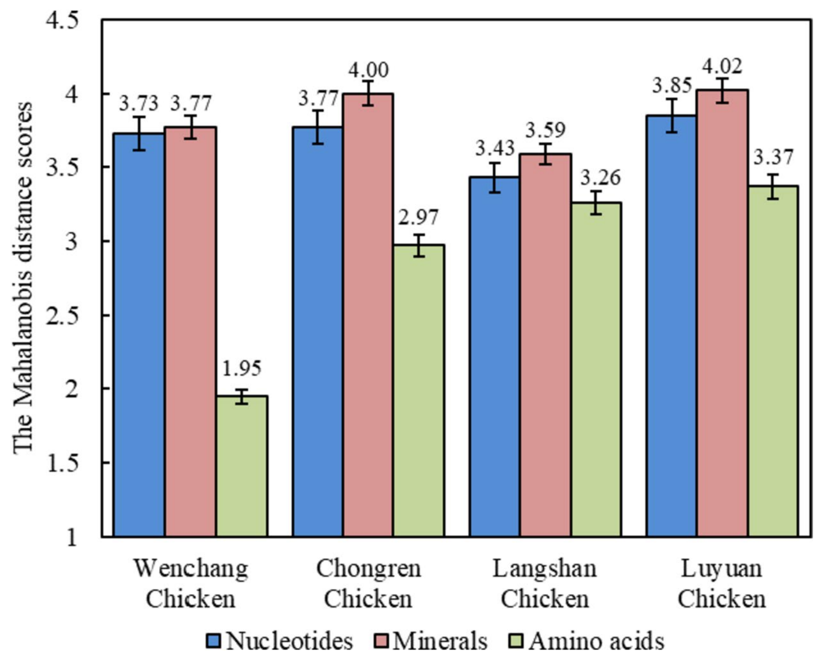


Fig. 4 The Mahalanobis distance scores of nucleotides, minerals and amino acids

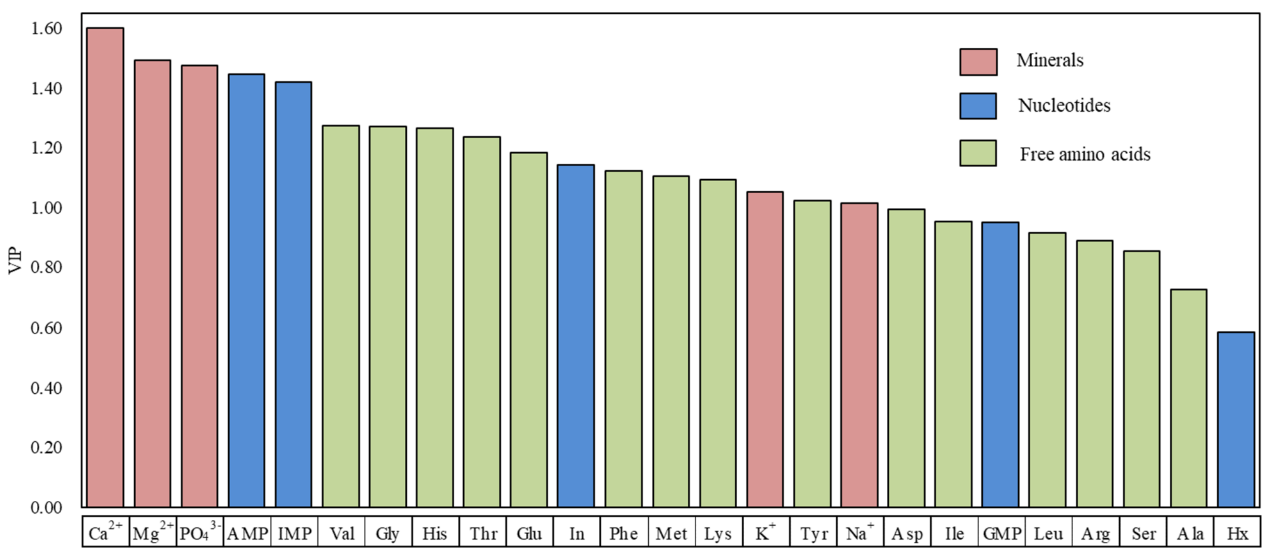


Fig. 5 The VIP results of sensory analysis and taste-active compounds

of the total variance of the independent and dependent variables that can be explained by the principal components of all extracted partial least squares models respectively. Q^2_{cum} was the cross-validation square coefficient, which represented the prediction ability of the model. If $Q^2_{cum} > 0.5$, the model had good prediction ability. In this model, six principal components were extracted, $R^2X_{cum} = 0.821$, $R^2Y_{cum} = 0.973$, $Q^2_{cum} = 0.993$, indicating that the model had strong reliability and good prediction ability.

In partial least squares regression analysis, Variable Importance for Projection (VIP) was commonly used to measure the explanatory power of each independent variable to the dependent variable. It was generally believed that independent variables with VIP greater than 1 are particularly important, those with VIP between 0.5 and

1 are important, and those with VIP less than 0.5 are not important. With the concentration of taste as the independent variable and sensory evaluation as the dependent variable, the partial least square regression variable projection importance technique was used for the analysis, which was shown in Fig. 5. The ranking of indexes with significant influence on sensory evaluation results was $Ca^{2+} > Mg^{2+} > PO_4^{3-}$ > AMP > IMP > valine > glycine > histidine > threonine > glutamate > inosine > phenylalanine > methionine > lysine > K^+ > tyrosine > Na^+, which were all greater than 1. The top three VIPs factors, Ca^{2+}, Mg^{2+}, PO_4^{3-}, were all minerals, which indicated that minerals were the most important independent variables. Followed by AMP and IMP, nucleotides were the second important influencing factors, while free amino acids were the least important. This was consistent with comprehensive Mahalanobis distance analysis. Although Wenchang chicken had an advantage in free amino acids, its comprehensive Mahalanobis distance score was worse than Langshan chicken whose mineral content was the highest.

In addition to flavor substances, there were also some differences between chicken breeds of the same feeding environment and age. Luyuan chicken was large with an eviscerated weight of about 1.8 kg, while Chongren and Langshan chicken were obviously smaller with 1.2 kg. This phenomenon may be related to the different growth genes of the chicken breeds. The growth rate, feed conversion rates, and feeding costs of different chicken breeds will affect the enthusiasm of farmers and the choice of consumers. The establishment of a complete yellow feather broiler quality evaluation system from multiple perspectives will be of great significance to the development of yellow feather broiler industry.

Conclusions

The taste of Wenchang chicken was the most preferred by sensory panels, which was consistent with the absolutely advantage in EUC value. In contrast, Luyuan chicken had a lower content of umami molecules which was also reflected in the result of sensory evaluation. The Mahalanobis distance score showed that the taste differences between chicken breeds were mainly relevant to the mineral content and amino acid content. The mineral content directly affected the salty taste, while the amino acid content affected the umami and sweet taste of chicken. The results of the various physical and chemical data were similar to the sensory evaluation results, which were more suitable for consumers' choices of chicken species in daily life. The relationship between taste active substances and sensory evaluation was established by partial least square method, and the most important substances affecting taste difference of four different chicken varieties were minerals, which was consistent with Mahalanobis distance, further indicating the materiality of saltiness. This could provide a theoretical basis for quantitative oriented flavor processing and consumer choice of chicken.

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Authors' contributions
Na Xu: Conceptualization, Methodology, Investigation, Data Curation, Writing-Original Draft; Hao Wang: Conceptualization, Methodology, Investigation, Data Curation, Writing-Original Draft; Lei Liu: Resources, Formal analysis; Xinglian Xu: Writing—Review & Editing; Verification; Resources; Supervision; Funding acquisition; Peng Wang: Writing—Review & Editing; Verification; Resources; Supervision; Funding acquisition. All the authors listed have approved the manuscript that is enclosed.

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Availability of data and materials
The datasets generated and/or analyzed during the current study are not publicly available due to privacy and confidentiality agreements. The authors confirm that the data supporting the findings of this study were available within the article.

Declarations

Ethics approval and consent to participate
There was no human ethics committee in Nanjing Agriculture University. The sensory evaluation was implemented according to formal documentation process, which had been described in the manuscript. We confirm that the appropriate protocols for protecting the rights and privacy of all participants were utilized during the sensory evaluation. The study requirements and risks had been stated fully. The sensory evaluation has obtained informed consent of all sensory evaluators, who participate voluntarily. The personal information was kept confidential and will not appear in any journal articles in any form.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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