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<b>Author</b>	Rong Jia <sup>1,2</sup> , Yucai Yang <sup>1,2</sup> , Guozhou Liao <sup>2</sup> , Yuan Yang <sup>1,2</sup> , Dahai Gu <sup>1,2</sup> , and Guiying Wang <sup>1</sup>
<b>Affiliation</b>	1 College of Food Science and Technology, Yunnan Agricultural University, Kunming, 650201, China 2 Livestock Product Processing and Engineering Technology Research Center of Yunnan Province, Yunnan Agricultural University, Kunming, 650201, China
<b>Special remarks</b> – if authors have additional information to inform the editorial office	
<b>ORCID (All authors must have ORCID) <a href="https://orcid.org">https://orcid.org</a></b>	Rong Jia ( <a href="https://orcid.org/0000-0001-8560-1048">https://orcid.org/0000-0001-8560-1048</a> ) Yucai Yang ( <a href="https://orcid.org/0009-0003-7559-8190">https://orcid.org/0009-0003-7559-8190</a> ) Guozhou Liao( <a href="https://orcid.org/0000-0003-2793-5837">https://orcid.org/0000-0003-2793-5837</a> ) Yuan Yang ( <a href="https://orcid.org/0009-0001-4733-5532">https://orcid.org/0009-0001-4733-5532</a> ) Dahai Gu ( <a href="https://orcid.org/0000-0001-8103-7697">https://orcid.org/0000-0001-8103-7697</a> ) Guiying Wang ( <a href="https://orcid.org/0000-0001-9603-9943">https://orcid.org/0000-0001-9603-9943</a> )
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**CORRESPONDING AUTHOR CONTACT INFORMATION**

For the <b>corresponding author (responsible for correspondence, proofreading, and reprints)</b>	Fill in information in each box below
First name, middle initial, last name	Guozhou Liao
Email address – this is where your proofs will be sent	liaoguzhou@ynau.edu.cn
Secondary Email address	liaoguzhou@ynau.edu.cn
Postal address	Livestock Product Processing and Engineering Technology Research Center of Yunnan Province, Yunnan Agricultural University, Kunming, 650201, China.

Cell phone number	86 087165227700
Office phone number	86 087165227700
Fax number	86 087165227701

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# **Effect of stewing time on the small molecular metabolites, free fatty acids, and volatile flavor compounds in chicken broth**

**Abstract:** Chicken broth has a taste of umami, and the stewing time has an important effect on the quality of chicken broth, but there are fewer studies on the control of the stewing time. Based on this, the study was conducted to analyze the effects of different stewing times on the sensory, small molecular metabolites, free fatty acids, and volatile flavor compounds contents in chicken broths by liquid chromatography-quadrupole/time-of-flight mass spectrometry (LC-Q/TOF-MS), gas chromatography-mass spectrometry (GC-MS), headspace solid-phase microextraction, and gas chromatography-mass spectrometry (HS-SPME-GC-MS). Eighty-nine small molecular metabolites, 15 free fatty acids, and 86 volatile flavor compounds were detected. Palmitic and stearic acids were the more abundant fatty acids, and aldehydes were the main volatile flavor compounds. The study found that chicken broth had the best sensory evaluation, the highest content of taste components, and the richest content of volatile flavor components when the stewing time was 2.5 h. This study investigated the effect of stewing time on the quality of chicken broth to provide scientific and theoretical guidance for developing and utilizing local chicken.

**Keywords:** LC-Q/TOF-MS; GC-MS; HS-SPME-GC-MS; Stewing time; Taste

## **Introduction**

Chicken is unanimously recognized as a nourishing and delicious meat product in China (Zhang et al., 2018), and compared to other meats, chicken has the advantages of low fat, low cholesterol, low calories, and high protein (Wang et al., 2014; Cao et al., 2021). The most

common and nutritious way to cook chicken is to make broth, which has rich nutrients (Qi et al., 2017) and efficacy effects (Rennard et al., 2018). Through some complicated processes that include the Maillard reaction, thiamine breakdown, lipid oxidation, and nutrient leaching by heating, the precursor in chicken meat creates the distinctive taste components of chicken broth during stewing (Sun et al., 2018).

Currently, the research on the nutrition, function, taste, and flavor components of chicken broth mainly involves the stewing of chicken broth from different chicken breeds (Xiao et al., 2021), the stewing technology (Yu et al., 2021; Jia et al., 2023), different cooking methods (Lai et al., 2022), the effect of ultrasonic-assisted stewing on the aroma of chicken broth (Qi et al., 2023), and the analysis and identification of the substances in the stewing process (Xiao et al., 2019; Yu et al., 2021). However, in the process of chicken broth cooking, the influence of chicken broth flavor components, in addition to the selection of chicken breeds and processing conditions that affect the flavor substances of chicken broth, the stewing time of chicken broth will also affect the flavor of chicken broth.

There are many flavor substances in chicken broth, which can be mainly divided into aroma and taste, and some studies have shown that the aroma mainly originates from volatile flavor substances such as aldehydes, alcohols, ketones, carboxylic acids, and sulfur-containing heterocyclic compounds, and the taste mainly originates from free amino acids, nucleotides, and other substances (Brown et al., 2020). Taste is mainly produced by the interaction between water-soluble components of food and the human oral buds, so only water-soluble substances can react to changes in food taste. During the chicken stewing process, a variety of substances in the chicken will dissolve into the chicken broth, mainly including peptides, nucleotides, soluble amino acids, saccharides, inosine, organic acids, and other organic substances.

Lioe et al. (2005) found that bitter amino acids below the threshold of taste presentation enhanced the umami and sweetness of other amino acids. Umami amino acids and their

derivatives contribute the most to the flavor of chicken broth (Li et al., 2018), 5'-nucleotides are also important flavorful compounds in chicken broth, 5'-adenine nucleotides, and 5'-inosine hypoxanthine enhance the flavor of chicken broth (Sabikun et al., 2021), glutamic acid, threonine, tyrosine, and isoleucine all add to the umami flavor of chicken broth and are the major contributors to the flavor of chicken broth (Zhan et al., 2020). Aldehydes are generally produced from precursors through fat oxidative degradation, and it has been shown that aldehydes are the main volatile substances in chicken broth, with allyl aldehydes and dienal aldehydes considered to be the characteristic volatile components of chicken broth (Qi et al., 2017). They have a low threshold value and are the main characteristic flavor substances that maintain the broth. Among the carbonyl compounds, (penta, penta)-2,4-decadienal and (penta)-2-decenal are the most important components in the formation of the "chicken" flavor (Fan et al., 2019).

The stewing time of chicken broth has an important effect on its quality. A reasonable stewing time is conducive to the dissolution of water-soluble substances in chicken broth and the formation of volatile substances in chicken broth, making its taste richer. However, there are currently few research reports on the control of stewing time. Yunnan Province in China has abundant local chicken breed resources, but over the years, the development of Yunnan's local chicken industry has been slow, with fewer deep-processed products. One of the effective ways to promote the development of the local chicken industry in Yunnan is to use Yunnan Wuding chicken and Tegel broiler chicken for hybrid utilization (Liu et al., 2021). The flavor characteristics of its hybrid F1 broilers have not been reported yet. Therefore, in this study, the F1 generation hens of Wuding chicken and Tegel broiler chicken were selected, and liquid chromatography-quadrupole/time of flight-mass spectrometry (LC-Q/TOF-MS), gas chromatography-mass spectrometry (GC-MS), head space solid phase microextraction coupled with gas chromatography mass spectrometry (HS-SPME-GC-MS), and sensory evaluations were used to explore the influence of stewing time on flavor substances in the chicken broth, and the

optimal stewing time was selected to provide a scientific and theoretical basis for the exploitation and utilization of local chickens.

## **Materials and methods**

### **Materials**

The experimental procedure and protocol were approved by the Animal Care Committee of the College of Animal Science and Technology, Yunnan Agricultural University. Under the same batch and feeding conditions, thirty 200-day-old hybrid F1 generation hens of Yunnan Wuding chicken and Tegel broiler chicken were selected. After slaughtering, cleaning, and removing the head, neck, and claws, the carcass weight of the chickens was approximately  $1607 \pm 120$  g. All the chickens were from the experimental breeding farm of Yunnan Agricultural University. The chickens were randomly divided into 5 groups with 6 chickens in each group and stewed for 1 h, 1.5 h, 2 h, 2.5 h, and 3 h, respectively.

### **Sample preparation**

The chicken carcasses were divided into two pieces, blanched in boiling water for 3 min, rinsed in cold water, drained, and weighed. Chicken and ultra-pure water were put into a casserole dish according to the ratio of chicken: water = 1:2 (m/m), and then placed on the induction stove to boil with high heat (2100 W). After skimming the upper layer of scum, the chicken was stewed over low heat (300 W) for different times, and the test time was calculated when the water began to boil. Finally, the stewed chicken broth was weighed and supplemented with warm ultra-pure water boiling water to reach the initial weight. And then the chicken broth was divided into two portions, one for sensory evaluation and the other for sampling and determination of other indicators.

### **Analysis method**

#### **Sensory evaluation**

The chicken broth stewed at different times was put into clean disposable paper cups, and 20 sensory evaluators (male to female = 1:1) from the College of Food Science and Technology of Yunnan Agricultural University were invited to carry out the sensory evaluation (Liu et al., 2020). The sensory qualities of the samples were scored separately according to the scoring criteria in Table 1S of the supplementary material, and the total scores were calculated according to the corresponding weights of the five evaluation criteria. The total score  $X=0.15X_1+0.4X_2+0.3X_3+0.15X_4$ , where  $X_1$ ,  $X_2$ ,  $X_3$ , and  $X_4$  indicate the proportion of each weight.

### **Small molecular metabolites analysis**

The technique of LC-Q/TOF-MS (Agilent 1290 Infinity LC System coupled to an Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight) was used to analyze the small molecular metabolites in chicken broth under different stewing times, which were determined according to our previous method (Xun et al., 2020). First, 100  $\mu$ L of chicken broth was pipetted into a 1.5 mL EP tube with a pipette, 800  $\mu$ L of methanol and 10  $\mu$ L of internal standard (3 mg/mL, 2-chlorophenylalanine) were added, then vortexed and mixed for 30 s. Subsequently, the sample solution was placed in a centrifuge at 4°C and 12000 rpm for 15 min, and 200  $\mu$ L of supernatant was collected for testing. The analytical column (C18, 100 mm  $\times$  2.1 mm, 1.8  $\mu$ m, Agilent, USA) was used with a set injection volume of 4  $\mu$ L, autosampler temperature of 4°C, column temperature of 40°C, the flow rate of 0.35 mL/min, and mobile phase elution program (A: water + 0.1% formic acid, B: acetonitrile + 0.1% formic acid) set to 0-5 min, 5% B; 6-8 min, 20%B; 9-12 min, 50%B; 13-15 min, 95%B.

### **Free fatty acids analysis**

A gas chromatography-mass spectrometry (Agilent 7890A-5975C, USA) method was used to analyze free fatty acids in chicken broth according to our previous method (Liu et al., 2019). Firstly, 200  $\mu$ L of chicken broth was placed in a headspace vial and 3 mL of hexane was added.

After vortexing for 1 min, the sample was centrifuged at 4°C and 3500 rpm for 5 min. Subsequently, the extraction was transferred to an SPE column and placed in a constant temperature water bath (90 ~ 95°C) for 1.5 h. Next, 2 mL of saturated saline and 1 mL of hexane were added to the extraction solution, vortexed for 1 min, and centrifuged at 4°C and 3500 rpm for 5 min. Finally, 195 µL of supernatant was taken and 5 µL of nineteen methyl carbonate was added, vortexed for 1 min, and 60 µL sample solution was taken into the injection vial for detection. The injection volume was 1 µL, the column flow rate was 0.3 mL/min, and the analysis was performed with a capillary column Agilent DB-225 (10 m × 0.1 mm × 0.1 µm) and FID (250°C).

#### **Analysis of volatile compounds**

The HS-SPME-GC-MS (57330U, Supelco, USA; 7890A-5975C, Agilent, USA) technique was used to analyze the volatile flavor compounds of chicken broth at different stewing times, according to the method of our previous study and with slight modifications (Wu et al., 2020). A 5 mL sample of chicken broth was shaken at 250 rpm for 15 min at 60°C and extracted for 30 min. The GC cycle time was 57 min, with an internal standard of 200 ng 2-methyl-3-heptanone (100 µg/mL × 2 µL). The injection volume was set to 5 µL, the injection temperature was 260°C, the carrier gas was helium (99.999%), and the flow rate was 1 mL/min. The temperature increase program was set to hold at 40°C for 5 min, increase to 250°C at a rate of 5 °C/min, and hold at this temperature for 5 min, for a total of 52 min of the detection process. The relative content of each component was determined by the internal standard method.

#### **Data statistics and analysis**

The test data were initially collected using Excel 2010, and all samples were run six times in parallel. SPSS 19.0 software was used to perform an ANOVA analysis of variance (ANOVA) on the resulting data. SIMCA 14.1 software was used to analyze the partial orthogonal partial

least squares discriminant analysis (OPLS-DA). Duncan's complex polarization method (Duncan's) was used for the analysis of multiple significant differences ( $P < 0.05$ ).

## **Results and discussion**

### **Sensory quality**

After prolonged heating and stewing of chicken meat, the water-soluble substances in the meat continuously decreased, with some dissolved in the chicken broth and some transformed into other flavor substances (Qi et al., 2019). The shorter the stewing time of chicken broth, the less flavor and taste it may have, while the longer the stewing time, the more volatile flavor is lost, which affects the overall sensory quality of chicken broth. Therefore, it is necessary to study the appropriate stewing time for specific broiler breeds to maximize the retention of the taste and flavor substances of the chicken broth. As shown in Fig 1, Table 2S of the supplementary material, the taste score of chicken broth was the highest when stewed for 3 h, which was 28.35% ( $P < 0.05$ ), 21.26% ( $P < 0.05$ ), 10.1% ( $P < 0.05$ ), and 7.22% ( $P > 0.05$ ) higher than that of chicken broth stewed for 1 h, 1.5 h, 2 h, and 2.5 h, respectively. The aroma score of chicken broth was the highest when stewed for 2.5 h, which was 31.77% ( $P < 0.05$ ), 28.45% ( $P < 0.05$ ), 22.04% ( $P < 0.05$ ), and 22.41% ( $P < 0.05$ ) higher than that of chicken broth stewed for 1 h, 1.5 h, 2 h, and 3 h, respectively. From the total score results, it could be seen that the total score of stewing chicken soup for 2.5 h was the highest, 4.4% higher than that of stewing chicken soup for 3 h ( $P > 0.05$ ). According to the sensory evaluation results of chicken soup, the taste, and flavor of chicken soup were the best after stewing for 2.5 h.

### **Analysis of small molecular metabolites**

Eighty-nine small molecular metabolites were screened from chicken broth with different stewing times, which are important flavor precursors of chicken broth. The results are shown in Table 3S of the supplementary material, Fig. 2 (a), (b), (c), and (d). Fig. 1 (a) and (b) show the

plots of partial least squares analysis results (OPLS-DA) and calculated variable projection importance plots (VIP) of small molecular metabolites in chicken broth at different stewing times. Modeling the relationship between metabolite expression and sample category can achieve the prediction of sample category and screening of marker metabolites (Chong et al., 2018). The chicken broth samples with different stewing times had a significant trend to separate, indicating that the small molecular metabolites profiles were differentiated in different stewing times, and the cross-validation results were  $R^2Y = 0.963$  and  $Q^2 = 0.832$ , indicating that the extracted information could reflect most of the information in the original data. It can be seen from the VIP chart that 48 substances had made important contributions to the intergroup differences of small molecular metabolites in chicken broth samples at different stewing times, mainly taurine, adenosine, xanthine nucleoside, nicotinamide, IMP, hypoxanthine, inosine, and amino acids, among which the contents of inosine and IMP were significantly higher than other substances, and they occupied an important role in the contribution of the flavor.

Fig. 2 (c) and (d) show the OPLS-DA score and VIP plots of the main nucleic acid substances in chicken broth at different stewing times, with cross-validation results of  $R^2Y = 0.843$  and  $Q^2 = 0.746$ . There was a significant tendency to separate nucleic acid substances in chicken broth samples at different stewing times, indicating that there were differences in nucleic acid substances in chicken broth at different stewing times. Among them, eight substances made significant contributions to the differences between groups, mainly including DIDP, xanthine nucleoside, DUDP, adenosine, and others. Studies have shown that adenosine (AMP), guanosine (GMP), inosine (I), IMP, and hypoxanthine (Hx) among 5'-nucleotides contributed significantly to the flavor of chicken broth, and the relative content of 5'-nucleotides increased with the increase of stewing time in the present study (Qi et al., 2018), with the greater changes in Hx and I. AMP, GMP, and IMP are also important nucleotides that are important flavorful substances for chicken meat (Dashdorj et al., 2015; Madruga et al., 2010), which were also detected in the

present study (Sabikun et al., 2021). Overall, the 5`-nucleotides produced in chicken broth increased with increasing stewing time and contributed significantly to the flavor of chicken broth.

### **Analysis of free fatty acids**

As can be seen in Table 1, a total of 15 free fatty acids were detected during the stewing process, with high levels of palmitic (C16:0) and stearic (C18:0) acids, which conformed with previous studies (Yu et al., 2021; Nkukwana et al., 2014), and there was no significant difference between C16:00 and C18. Xiao et al. (2019) found palmitic (C16:0), stearic (C18:0), oleic acid (C18:1n-9), and linoleic (C18:2n-6) to be the major fatty acids in Wuding chicken. Palmitic and stearic acids are excellent sources of biologically active lipids that are essential for human development (Salazar et al., 2020). It has been shown that medium-and long-chain free fatty acids ( $C > 6$ ), which are aroma precursors, can be further degraded as substrates to produce small-molecule volatile flavor substances, such as aldehydes and acids (Huang et al., 2020). Different fatty acid compositions resulted in different flavors in various types of meat products. For example, the main fatty acids in cured duck were made up of palmitic and stearic acids, and the main fatty acids in pork were palmitic, stearic, oleic, and linolenic acids (Barola et al., 2020), but the fatty acid with the lowest concentration in dry-cured hams was palmitoleic acid (Li et al., 2018). The most abundant free fatty acids found in dairy products such as milk were 7-hydroxystearic acid and 10-hydroxystearic acid (Sun et al., 2018).

With the prolongation of the stewing time, the concentration of various fatty acids in the chicken broth had an increasing trend. Among them, the concentration of heptadecanedioic acid (C17:0) in the chicken broth stewed for 1 h was 21.26% lower than that stewed for 3 h ( $P < 0.05$ ), and the concentration of oleic acid (C18:1n9c) in chicken soup stewed for 2.5 h was 27.22% higher than that in the chicken broth stewed for 3 h ( $P < 0.05$ ). The highest concentration of polyunsaturated fatty acids (MUFA), monounsaturated fatty acids (PUFA), and saturated fatty

acids (SFA) was found in the chicken broth stewed for 2.5 h, 2 h and 2.5 h, respectively. There was no significant difference in the concentration of total unsaturated fatty acids (UFA) between chicken broth stewed for 2 h and 2.5 h. The free fatty acids in chicken broth were mainly saturated fatty acids, which were about 7 times the concentration of unsaturated fatty acids. As the stewing time was prolonged, the total fatty acid concentration in chicken broth increased and then decreased, reaching its highest value at 2.5 h of stewing. This may be because as the stewing time increases, the fatty acids in the chicken broth are thermally decomposed into other substances, resulting in a decrease in total fatty acid concentration (Almela et al., 2010).

### **Analysis of volatile flavor compounds**

Eighty-six volatile compounds were detected from the chicken broth at different stewing times, and these compounds mainly consisted of 20 aldehydes, 18 alcohols, 2 furans, 16 alkene hydrocarbons, 4 esters, 8 ketones, 4 aromatic hydrocarbons, and 14 other compounds, as can be seen in Table 3S of the supplementary material. These compounds collectively affect the flavor quality of chicken broth (Lorenzo & Franco et al., 2012). In a range of time, the concentration of aldehydes, alcohols, furans, alkenes, esters, ketones, and aromatic hydrocarbons increased significantly ( $P < 0.05$ ), but some of these substances reduced significantly when stewed for 2.5 h. This may be because as the stewing time increases, lipid oxidative degradation produces a large number of volatile compounds, but it also leads to an increase in cooking losses and a decrease in volatile compound concentration (Sun et al., 2018; Fan et al., 2018; Feng et al., 2018).

As can be seen in Fig 3, the total amount of various volatile substances, such as aldehydes, alcohols, furans, alkenes, and ketones, was the highest in chicken broth stewed for 2.5 h, with values of 8840.82 ng/100 mL, 1012.98 ng/100 mL, 148.15 ng/100 mL, 643.77 ng/100 mL, and 297.28 ng/100 mL, respectively. This indicated that the volatile compounds in chicken broth were mainly composed of aldehydes, which were important for the formation of the unique meat

flavor of chicken broth (Qi et al., 2017; Barola et al., 2020). Among the aldehydes, hexanal had the largest concentration rise, reaching 5393.02 ng/100 mL, showing that hexanal had a substantial part in the flavor of chicken broth, which is consistent with earlier research (Xu et al., 2020). Among the alcohols, 1-hexanol, 1-octen-3-ol, 1-heptanol, and 1-octanol had higher concentrations, among which 1-octen-3-ol was the compound with the highest concentration increase, reaching 406.5 ng/100 mL, indicating that it had an impact on the volatile flavor substances of chicken broth. Furans are heterocyclic compounds that contribute significantly to meat flavor. In this study, 2-pentylfuran had the highest concentration increase, reaching 137.85 ng/100 mL, which may be likely to be the main furan that affected the volatile flavor of chicken broth. Jia et al. (2023) found hexanal, (E)-2-heptenal, octanal, nonanal, and alkenal were the chief aldehyde volatile flavor substances in chicken broth, which may be due to different types of chicken and the way chicken soup was handled. Furans are produced by lipid oxidation and impart good flavor to meat products. Furan 2-pentylfuran has been detected in volatile matter, which is considered to have a sweet aroma (Chen et al., 2020). Yu et al. (2021) and Shen et al. (2016) found that 2-ethylfuran, produced by the oxidation of linoleic acid, played an important role in harmonizing the flavor of chicken meat.

## **Conclusion**

Small molecular metabolites, free fatty acids, and volatile flavor compounds were analyzed by LC-Q/TOF-MS, GC-MS, and HS-SPME-GC-MS in chicken broths with different stewing times, and sensory evaluations were conducted. It was found that the 5'-nucleotides produced in chicken broth increased with stewing time, with AMP and IMP making a significant contribution to the flavor of the broth. The content of palmitic acid and stearic acid was high, and the fatty acids in chicken soup were mainly saturated fatty acids, which were about 7 times higher than unsaturated fatty acids. Volatile flavor compounds consistently increased with stewing time, and

some significantly decreased after 2.5 h of stewing ( $P < 0.05$ ). The concentrations of hexanal, 2-pentylfuran, and 2-pentylfuran increased the most, reaching 5393.02 ng/100 mL, 406.5 ng/100 mL, and 137.85 ng/100 mL, respectively. The stewing time had a certain effect on the taste and flavor substances of the chicken broth, and the best sensory quality of the chicken broth was achieved when the stewing time was 2.5 h. The results of this study provide a Scientific theory basis for the deep processing of local chicken. Further work is necessary to explore the chemical formation mechanism of chicken broth based on its characteristic flavor components.

### **Conflicts of Interest**

none.

### **Acknowledgment**

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### **Author Contributions**

Conceptualization: Jia R, Yang YC, Liao GZ. Data curation: Jia R, Yang YC, Liao GZ, Gu DH. Formal analysis: Jia R, Yang YC, Yang Y, Wang GY. Methodology: Jia R, Yang YC, Liao GZ, Gu DH, Wang GY. Software: Jia R, Yang YC, Yang Y, Gu DH. Validation: Jia R, Yang YC, Liao GZ. Investigation: Jia R, Yang YC, Gu DH. Writing-original draft: Jia R, Yang YC, Liao GZ. Writing-review & editing: Jia R, Yang YC, Liao GZ, Yang Y, Gu DH, Wang GY.

## Ethics Approval

There are no human or animal participants.

## References

- Almela E, Jordan MJ, Martinez C, Sotomayor JA, Bedia M, & Banon S. 2010. Ewe's diet (pasture vs grain-based feed) affects volatile profile of cooked meat from light Lamb. *Journal of Agricultural and Food Chemistry*. 58(17), 9641-9646.
- Barola C., Moretti S, Giusepponi D, Paoletti F, Saluti G, Cruciani G, ... Galarini R. 2020. A liquid chromatography-high resolution mass spectrometry method for the determination of thirty-three per-and polyfluoroalkyl substances in animal liver. *Journal of Chromatography A*. 1628, 461442.
- Brown LK, Miller A, & Miller E. 2020. Chicken Soup for the Treatment of Respiratory Infections. *Chest*.158(5), 2231-2232.
- Cao CW, Xiao ZC, Tong HQ, Tao XS, Gu DH, Wu YL, ... Ge CR. (2021). Effect of ultrasound assisted enzyme treatment on the quality of chicken breast meat. *Food Bioprod. Process*. 125, 193-203.
- Chen X, Zou Y, Wang DY, Xiong GY, & Xu WM. 2020. Effects of ultrasound pretreatment on the extent of Maillard reaction and the structure, taste and volatile compounds of chicken liver protein. *Food Chemistry*. 331, 127369.
- Chong J, & Xia JG. 2018. MetaboAnalystR: An R package for flexible and reproducible analysis of metabolomics data. *Bioinformatics*. 34(24), 4313-4314.

- Dashdorj D, Amna T, & Hwang I. 2015. Influence of specific taste-active components on meat flavor as affected by intrinsic and extrinsic factors: an overview. *European Food Research and Technology*. 241, 157-171.
- Fan M, Xiao Q, Xie J, Cheng J, Sun B, Du W, ... Wang T. 2018. Aroma compounds in chicken broths of Beijing Youji and commercial broilers. *Journal of Agricultural and Food Chemistry*. 66(39), 10242-10251.
- Fan XY, Li XP, Tao NP, Zhang J, Wang MF, Qian XL, ... Zhong J. 2019. Effect of Salt Addition Time on the Nutritional Profile of *Thunnus obesus* Head Soup and the Formation of Micro/Nano-Sized Particle Structure. *Molecules*. 24(24), 4447.
- Feng Y, Cai Y, Fu X, Zheng L, Xiao Z, & Zhao M. 2018. Comparison of aromaactive compounds in broiler broth and native chicken broth by aroma extract dilution analysis (AEDA), odor activity value (OAV) and omission experiment. *Food Chemistry*. 265, 274-280.
- Jia R, Yin XY, Liao GZ, Yang ZJ, Gu DH, Pu YH, ... Wang GY. 2023. Effects of pre-ultrasonication on small molecular metabolites and flavour compounds in chicken broth. *International Journal of Food Science and Technology*. 58(2), 1365-2621.
- Huang JJ, Zhao QY, Bu W, Zhang CM, Yang Z, Zhang X, Zhang KN. 2020. Ultrasound-assisted hydrolysis of lard for free fatty acids catalyzed by combined two lipases in aqueous medium. *Bioengineered*. 11(1), 241-250.
- Lai J, Wu RY, Wang J, Wang Y, Zhang, X., Zhou, L. Y., & Zhu, Y. C. 2022. Effect of cooking modes on quality and flavor characteristic in *Clitocybe squamulose* chicken soup. *Frontiers in Nutrition*. 9, 1048352.
- Lioe HN, Apriyantono A, Takara K, Wada K, & Yasuda M. 2005. Umami taste enhancement of MSG/NaCl mixtures by subthreshold L- $\alpha$ -aromatic amino acids. *Journal of Food Science*. 70(7), S401-S405.

- Li X, Zhu J, Qi J, Wang P, Xu XL, & Zhou GH. 2018. Superchilled storage ( $-2.5 \pm 1^{\circ}\text{C}$ ) extends the retention of taste-active and volatile compounds of yellow-feather chicken soup. *Animal Science Journal*. 89(6), 906-918.
- Liu SY., Wang GY, Xiao ZC, Pu YH, Ge CR, & Liao GZ. 2019.  $^1\text{H-NMR}$ -based water-soluble low molecular weight compound characterization and free fatty acid composition of five kinds of Yunnan dry-cured hams. *LWT*. 108, 174-182.
- Liu ZY, Zhu YW, Wang WL, Zhou XR, Chen GL, & Liu Y. 2020. Seven novel umami peptides from Takifugu rubripes and their taste characteristics. *Food Chemistry*. 330, 127204.
- Liu MZ, Dou TF, Wang K, Li FY, Zhang R., ... Ge CR. 2021. Analysis of body size Traits and Carcass Performance in F1 hybrids of Wuding and Digao chickens. *Animal Husbandry and Veterinary Medicine*. 53(01), 1-6. (In Chinese)
- Lorenzo JM, & Franco D. 2012. Fat effect on physico-chemical, microbial and textural changes through the manufactured of dry-cured foal sausage Lipolysis, proteolysis and sensory properties. *Meat Science*. 92(4), 704-714.
- Madruga MS, Elmore JS, Oruna-Concha MJ, Balagiannis D, & Mottram DS. 2010. Determination of some water-soluble aroma precursors in goat meat and their enrolment on flavor profile of goat meat. *Food Chemistry*. 123(2), 513-520.
- Nkukwana TT, Muchenje V, Masika PJ, Hofman LC, Dzama K, & Descalzo AM. 2014. Fatty acid composition and oxidative stability of breast meat from broiler chickens supplemented with moringa oleifera leaf meal over a period of refrigeration. *Food Chemistry*. 142, 255-261.
- Qi J, Jia CK, Zhang WW, Yan HM, Cai QY, Yao XM, .... Li MQ. 2023. Ultrasonic-assisted stewing enhances the aroma intensity of chicken broth: A perspective of the aroma-binding behavior of fat. *Food Chemistry*. 398, 133913.
- Qi J, Liu D Y, Zhou GH, & Xu XL. 2017. Characteristic Flavor of Traditional Soup Made by Stewing Chinese Yellow-Feather Chickens. *Journal of Food Science*. 82(9), 2031-2040.

- Qi J, Li X, Zhang WW, Wang HH, Zhou GH, & Xu XL. 2018. Influence of stewing time on the texture, ultrastructure and in vitro digestibility of meat from the yellow-feathered chicken breed. *Animal Science Journal*. 89(2), 474-482.
- Rennard SI, Kalil AC, & Casaburi R. 2020. Chicken Soup in the Time of COVID. *Chest*. 158(3), 864-865.
- Sabikun N, Bakhsh A, Rahman MS, Hwang YH, & Joo ST. 2021. Volatile and nonvolatile taste compounds and their correlation with umami and flavor characteristics of chicken nuggets added with milkfat and potato mash. *Food Chemistry*. 343, 128499.
- Salazar E, Cayuela JM, Abellán A, Bueno-Gavilá E, & Tejada L. 2020. Fatty Acids and Free Amino Acids Changes during Processing of a Mediterranean Native Pig Breed Dry-Cured Ham. *Foods*. 9(9), 1170.
- Shen MY, Liu Q, Jia HB, Jiang YJ, Nie SP, Xie JH, ... Xie MY. 2016. Simultaneous determination of furan and 2-alkylfurans in heat-processed foods by automated static headspace gas chromatography-mass spectrometry. *LWT - Food Science and Technology*. 72, 44-54.
- Sun J, Wang Y, Li N, Zhong H, Xu H, Zhu Q, Liu Y. 2018. Comparative Analysis of the Gut Microbial Composition and Meat Flavor of Two Chicken Breeds in Different Rearing Patterns. *Biomed Research International*. 4343196.
- Wang XM, Zhang J, Wu LH, Zhao YL, Li T, Li JQ, ... Liu HG. 2014. A mini-review of chemical composition and nutritional value of edible wild-grown mushroom from China. *Food Chemistry*. 151, 279-285.
- Wu WH, Zhou Y, Wang GY, Zhu RJ, Ge CR, & Liao GZ. 2020. Changes in the physicochemical properties and volatile flavor compounds of dry-cured Chinese Laowo ham during processing. *Journal of Food Processing and Preservation*. 44(8): 14593.

- Xiao ZC, Luo YT, Wang GY, Ge CR, Zhou GH, Zhang WG, & Liao GZ. 2019. <sup>1</sup>H NMR-based water-soluble lower molecule characterization and fatty acid composition of boiled Wuding chicken during processing. *Journal of the Science of Food and Agriculture*. 99(1), 429-435.
- Xiao ZC, Zhang WA, Yang HT, Yan ZY, Ge CR, Liao GZ, & Su HW. 2021. <sup>1</sup>H NMR-based water-soluble lower molecule characterization and fatty acid composition of Chinese native chickens and commercial broiler. *Food Research International*. 140, 110008.
- Xun W, Wang GY, Zhang YJ, Liao GZ, & Ge CR. 2020. Analysis of flavor-related compounds in four edible wild mushroom soups. *Microchemical Journal*. 159, 105548,
- Xu Y, Chen YP, Deng SL, Li CB, Xu XL, Zhou GH, & Liu Y. 2020. Application of sensory evaluation, GC-ToF-MS, and E-nose to discriminate the flavor differences among five distinct parts of the Chinese blanched chicken. *Food Research International*. 137, 109669,
- Yu YR, Wang GY, Yin XY, Ge CR, & Liao GZ. 2021. Effects of different cooking methods on free fatty acid profile, water-soluble compounds and flavor compounds in Chinese Piao chicken meat. *Food Research International*. 149, 110696.
- Zhan H, Hayat K, Cui H P, Hussain S, Ho CT, & Zhang XM. 2020. Characterization of flavor active non-volatile compounds in chicken broth and correlated contributing constituent compounds in muscle through sensory evaluation and partial least square regression analysis. *LWT*. 118, 108786.
- Zhang M, Chen X, Hayat K, Duhoranimana E, Zhang XM, Xia SQ, ... Xing FL. 2018. Characterization of odor-active compounds of chicken broth and improved flavor by thermal modulation in electrical stewpots. *Food Research International*. 109, 72-81.

Table 1 Composition of free fatty acids in chicken soup of different treatment groups ( $\mu\text{g/mL}$ )

Name	Stewing times / h				
	1.0	1.5	2.0	2.5	3.0
C10:0	1.69±0.07 <sup>a</sup>	1.72±0.17 <sup>a</sup>	1.92±0.08 <sup>b</sup>	1.93±0.01 <sup>b</sup>	2.03±0.10 <sup>b</sup>
C12:0	3.54±0.49 <sup>a</sup>	3.23±0.31 <sup>a</sup>	3.52±0.06 <sup>a</sup>	3.27±0.12 <sup>a</sup>	3.49±0.05 <sup>a</sup>
C14:0	9.32±1.22 <sup>a</sup>	9.82±0.96 <sup>a</sup>	10.04±0.74 <sup>a</sup>	10.12±1.17 <sup>a</sup>	9.31±0.56 <sup>a</sup>
C14:1n5	2.35±0.38 <sup>a</sup>	2.42±0.02 <sup>a</sup>	2.61±0.13 <sup>a</sup>	2.44±0.09 <sup>a</sup>	2.32±0.29 <sup>a</sup>
C15:0	3.12±0.20 <sup>a</sup>	3.16±0.32 <sup>a</sup>	3.4±0.42 <sup>a</sup>	3.34±0.10 <sup>a</sup>	3.31±0.20 <sup>a</sup>
C16:0	447.73±21.59 <sup>a</sup>	459.08±35.39 <sup>a</sup>	499.45±75.26 <sup>a</sup>	511.63±52.53 <sup>a</sup>	503.92±67.97 <sup>a</sup>
C17:0	4.14±0.19 <sup>a</sup>	4.4±0.53 <sup>ab</sup>	4.56±0.55 <sup>ab</sup>	4.9±0.34 <sup>ab</sup>	5.02±0.51 <sup>b</sup>
C18:0	300.59±50.31 <sup>a</sup>	303.46±117.65 <sup>a</sup>	360.05±61.81 <sup>a</sup>	362.86±54.41 <sup>a</sup>	357.32±61.32 <sup>a</sup>
C18:1n9c	17.13±1.98 <sup>ab</sup>	17.13±1.63 <sup>ab</sup>	17.43±1.13 <sup>ab</sup>	19.49±0.40 <sup>b</sup>	15.32±2.56 <sup>a</sup>
C18:2n6c	51.46±2.11 <sup>a</sup>	52.4±0.28 <sup>a</sup>	55.37±2.28 <sup>a</sup>	52.95±0.98 <sup>a</sup>	51.46±3.04 <sup>a</sup>
C20:0	1.61±0.30 <sup>a</sup>	1.64±0.53 <sup>a</sup>	1.99±0.15 <sup>a</sup>	1.98±0.26 <sup>a</sup>	1.86±0.22 <sup>a</sup>
C20:1	1.23±0.05 <sup>a</sup>	1.25±0.02 <sup>a</sup>	1.32±0.27 <sup>a</sup>	1.17±0.09 <sup>a</sup>	1.15±0.07 <sup>a</sup>
C22:1n9	32.25±1.32 <sup>a</sup>	32.49±0.89 <sup>a</sup>	32.56±0.99 <sup>a</sup>	33.06±1.96 <sup>a</sup>	32.63±1.88 <sup>a</sup>
C22:2	7.28±0.23 <sup>a</sup>	7.33±0.85 <sup>a</sup>	7.36±0.34 <sup>a</sup>	7.37±0.50 <sup>a</sup>	6.4±0.31 <sup>a</sup>
C24:0	1.72±0.31 <sup>a</sup>	1.72±0.09 <sup>a</sup>	1.74±0.25 <sup>a</sup>	1.82±0.20 <sup>a</sup>	1.69±0.15 <sup>a</sup>
TF	885.16±66.02 <sup>a</sup>	901.26±157.23 <sup>a</sup>	1003.32±139.75 <sup>a</sup>	1018.32±111.36 <sup>a</sup>	997.25±126.92 <sup>a</sup>
SFA	773.46±67.56 <sup>a</sup>	788.24±155.15 <sup>a</sup>	886.67±137.90 <sup>a</sup>	901.85±108.37 <sup>a</sup>	887.96±129.65 <sup>a</sup>
MUFA	52.96±2.11 <sup>ab</sup>	53.29±1.69 <sup>ab</sup>	53.93±1.68 <sup>ab</sup>	56.15±2.25 <sup>b</sup>	51.42±1.61 <sup>a</sup>
PUFA	58.74±1.89 <sup>ab</sup>	59.73±0.66 <sup>ab</sup>	62.73±2.57 <sup>ab</sup>	60.32±1.45 <sup>b</sup>	57.86±3.08 <sup>a</sup>

Note: Different letters in the same row indicate significant differences ( $P < 0.05$ ).

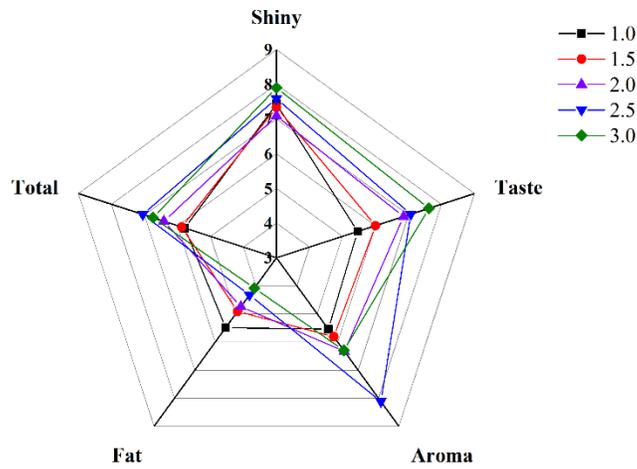
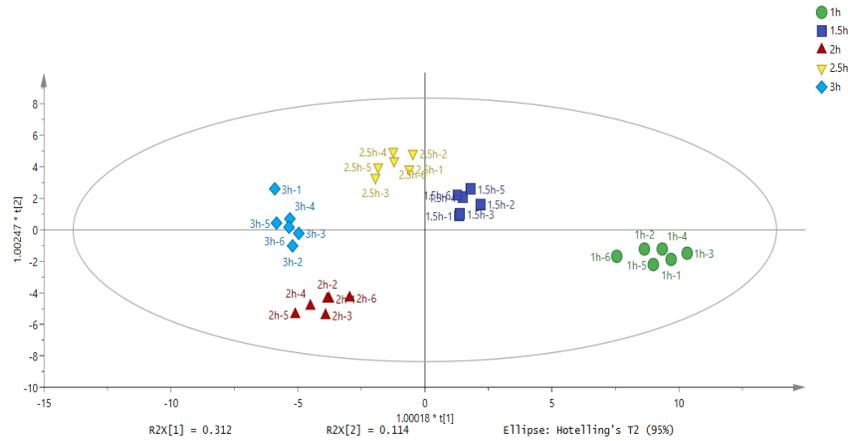
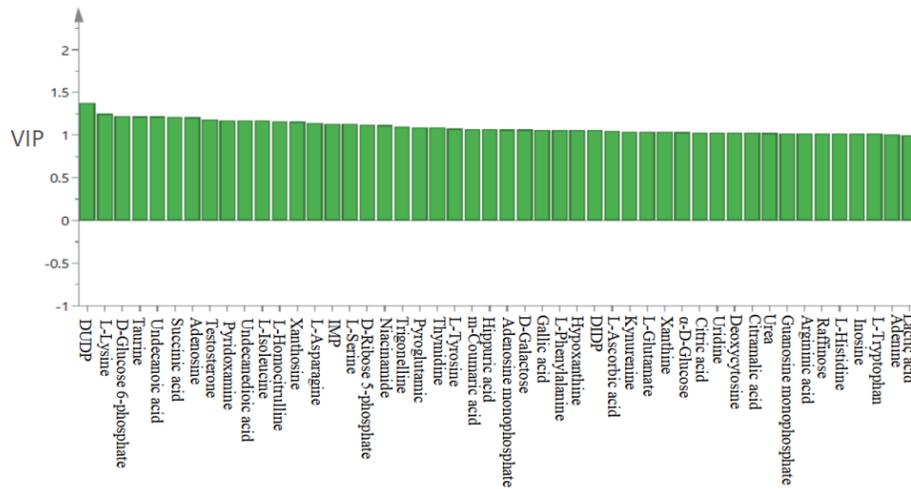


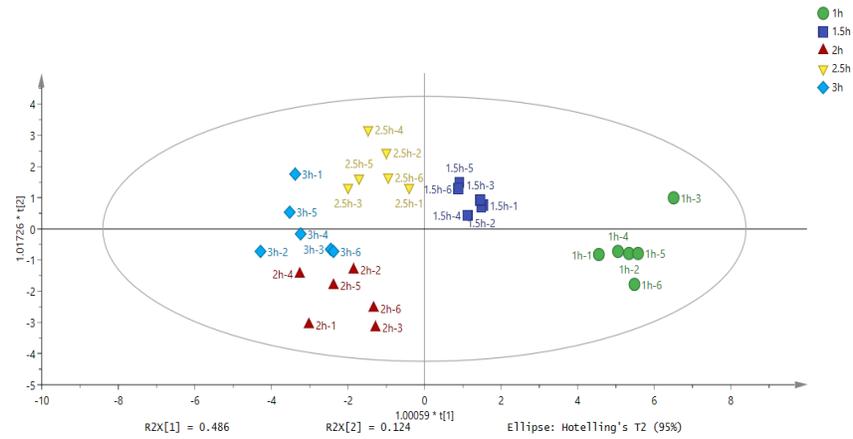
Fig. 1. Sensory evaluation score of chicken broth in different treatment groups.



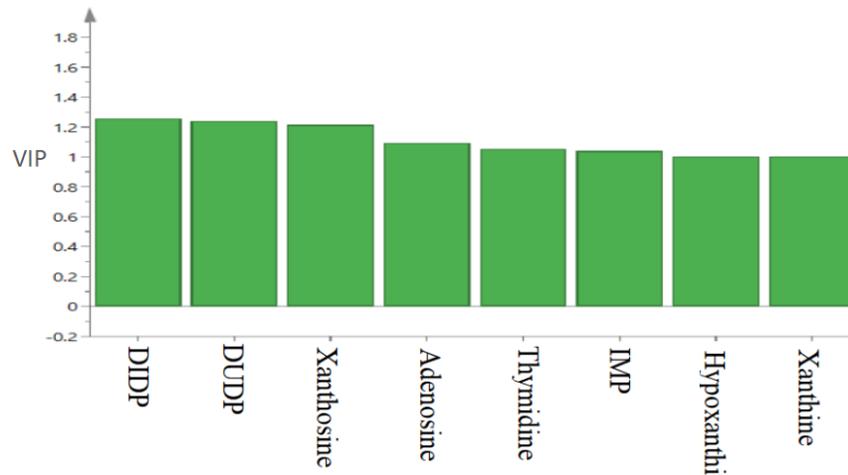
( a )



(b)



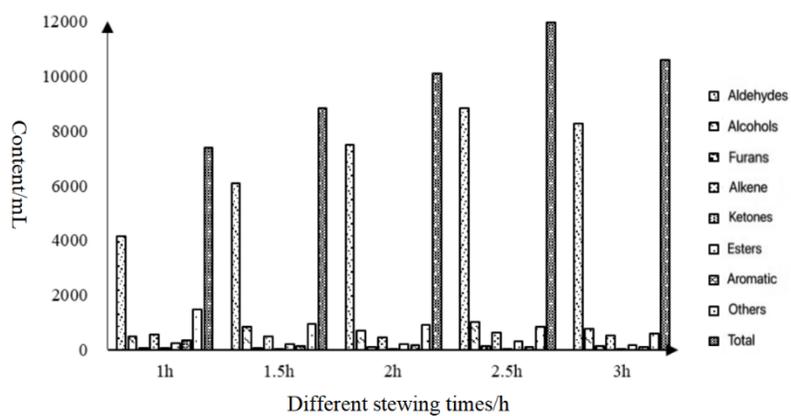
(c)



(d)

**Fig. 2.** OPLS-DA scores plot (a) and VIP scores (b) of small molecular metabolites in chicken broth at different stewing times. OPLS-DA score plot (c) and VIP plot (d) of nucleic acids in chicken broth at different stewing times.

Note: 1 h, 1.5 h, 2 h, 2.5 h, 3 h in the figure represent the chicken broth treatment group with different stewing times, the cooking time is 1 h, 1.5 h, 2 h, 2.5 h, 3 h.



**Fig. 3.** The change of volatile compounds in chicken broth at different stewing times.

ACCEPTED

Table 1S Criteria for sensory evaluation of chicken soup

Evaluation criteria	Score			
	9~10	6~8	3~5	0~2
Shiny ( 15% )	Pale yellow or milky white	beige	Light yellow	colorless
Taste ( 40% )	rich with a clear umami	Lack of umami, pure taste	Taste light, no aftertaste, no special odor	no umami, bad smell
Aroma ( 30% )	fragrant and rich meat smell	strong meat flavor, light aroma	Less meat smell, no peculiar smell	No meat smell, bad smell
Fat ( 15% )	no obvious oil slick on the surface of the soup	a small amount of particle precipitation	a lot of grease on the surface of the soup	The surface is covered with oil, and the grease layer is thicker

Table 2S Sensory evaluation results of different treatment groups of chicken broth

Standard/weight	Stewing times /h				
	1.0	1.5	2.0	2.5	3.0
Shiny (15%)	7.43±0.74 <sup>a</sup>	7.34±0.34 <sup>a</sup>	7.09±0.16 <sup>a</sup>	7.59±0.46 <sup>a</sup>	7.90±0.42 <sup>a</sup>
Taste (40%)	5.46±0.32 <sup>c</sup>	6.00±0.15 <sup>c</sup>	6.85±0.23 <sup>b</sup>	7.07±0.28 <sup>ab</sup>	7.62±0.59 <sup>a</sup>
Aroma (30%)	5.54±0.41 <sup>c</sup>	5.81±0.41 <sup>bc</sup>	6.33±0.05 <sup>b</sup>	8.12±0.18 <sup>a</sup>	6.30±0.45 <sup>b</sup>
Fat (15%)	5.49±0.51 <sup>a</sup>	4.91±0.19 <sup>b</sup>	4.74±0.18 <sup>bc</sup>	4.32±0.26 <sup>cd</sup>	4.08±2.24 <sup>d</sup>
Total	5.78±0.15 <sup>c</sup>	5.87±0.28 <sup>c</sup>	6.41±0.15 <sup>b</sup>	7.05±0.15 <sup>a</sup>	6.74±0.29 <sup>ab</sup>

Note: Different letters in the same row indicate significant differences ( $P < 0.05$ ).

Table 3S List of small molecular metabolites in chicken soup of different treatment groups

NO.	Metabolite	NO.	Metabolite	NO.	Metabolite
71	Hypoxanthine	96	L-Methionine	184	Salicylic acid
223	Inosine	107	L-Histidine	214	Undecanoic acid
326	Adenosine monophosphate	156	L-Tyrosine	231	Undecanedioic acid
327	IMPIMP	187	Argininic acid	255	Sebacic acid
352	Guanosine monophosphate	206	L-Tryptophan	314	Cis-9-palmitoleic acid
70	Adenine	8	$\beta$ -Alanine	325	Tetradecanedioic acid
168	Deoxycytosine	32	Taurine	424	Arachidonic Acid
193	Thymidine	35	Pyroglutamic acid	608	Nutriacholic acid
243	Guanosine	38	Creatine	51	Niacinamide
346	Uridine	62	L-Glutamate	133	Pyridoxamine
236	Cytidine	144	Citrulline	145	L-Ascorbic acid
426	Xanthosine	195	Hippuric acid	154	Pantothenic Acid
437	DIDP	215	Kynurenine	199	Pyridoxamine-5'-Phosphate
470	UTP	220	L-Homocitrulline	269	Thiamine
509	Adenosine triphosphate	433	L-Octanoylcarnitine	275	Glutathione
65	Xanthine	1	Acetic acid	599	Vitamin D3
101	Guanine	168	Ribonic acid	97	$\alpha$ -D-Glucose
348	Adenosine	18	D-2-Aminobutyric acid	127	L-Rhamnulose
385	DUDP	20	Lactic acid	150	D-Galactose
395	XMP	28	Succinic acid	172	D-Ribose 5-phosphate
420	ADP	44	Malic acid	213	D-Glucose 6-phosphate
596	DTDPDTDP	52	Malonic acid	795	Raffinose
42	L-Aspartic Acid	63	Citraconic acid	493	Taurocholic acid
78	L-Phenylalanine	77	Guanidineacetic acid	594	Campesterol
84	L-Isoleucine	87	Gallic acid	3	Urea
85	L-Asparagine	94	Citramalic acid	24	Hydantoin
105	Ornithine	116	Citric acid	436	Testosterone
134	L-Lysine	127	m-Coumaric acid	539	B2TXB2
54	L-Serine	142	Aconitic acid	91	Trigonelline
65	L-Leucine	155	Nonanoic acid		

Table 4S Changes in volatile substances in chicken broth of different treatment groups (ng/100 mL)

Name	Stewing times /h				
	1.0	1.5	2.0	2.5	3.0
<b>Aldehyde</b>					
Acetaldehyde	30.71±3.67 <sup>a</sup>	31.1±3.46 <sup>a</sup>	26.32±4.57 <sup>a</sup>	42.93±5.81 <sup>b</sup>	27.31±3.58 <sup>a</sup>
Propanal	5.73±0.73 <sup>a</sup>	8.87±0.66 <sup>c</sup>	7.7±0.26 <sup>bc</sup>	8.9±1.08 <sup>c</sup>	7.18±0.28 <sup>b</sup>
2-methyl- Butanal	4.56±0.60 <sup>d</sup>	2.69±0.26 <sup>c</sup>	1.87±0.21 <sup>b</sup>	2.03±0.15 <sup>b</sup>	1.22±0.15 <sup>a</sup>
3-methyl- Butanal	7.17±0.62 <sup>d</sup>	3.93±0.40 <sup>c</sup>	2.67±0.38 <sup>b</sup>	2.58±0.38 <sup>ab</sup>	1.89±0.14 <sup>a</sup>
Pentanal	145.44±5.62 <sup>a</sup>	187.12±11.38 <sup>b</sup>	245.58±8.72 <sup>c</sup>	278.87±29.89 <sup>d</sup>	238.2±15.01 <sup>c</sup>
(E)-2-Butenal	4.13±0.49 <sup>a</sup>	7.8±1.39 <sup>b</sup>	5.08±0.62 <sup>a</sup>	7.93±0.28 <sup>b</sup>	7.34±0.91 <sup>b</sup>
Hexanal	2673.97±168.71 <sup>a</sup>	3981.09±151.19 <sup>b</sup>	4676.16±214.37 <sup>c</sup>	5393.02±286.98 <sup>d</sup>	4880.09±230.10 <sup>c</sup>
Heptanal	101.34±9.70 <sup>a</sup>	166.66±9.09 <sup>b</sup>	212.9±15.24 <sup>c</sup>	241.44±8.38 <sup>d</sup>	231.88±18.27 <sup>cd</sup>
(E)-2-Hexenal,	3.31±1.67 <sup>a</sup>	8.72±1.19 <sup>b</sup>	8.36±0.14 <sup>b</sup>	10.88±0.20 <sup>c</sup>	10.73±0.49 <sup>c</sup>
Octanal	113.97±16.48 <sup>a</sup>	154.69±102.15 <sup>a</sup>	203.98±135.11 <sup>ab</sup>	301.79±21.07 <sup>b</sup>	330.17±20.08 <sup>b</sup>
(Z)-2-Heptenal	62.42±4.35 <sup>a</sup>	145.51±18.56 <sup>b</sup>	151.11±8.36 <sup>b</sup>	206.68±7.18 <sup>c</sup>	213.22±3.11 <sup>c</sup>
Nonanal	443.63±47.44 <sup>a</sup>	609.54±53.71 <sup>b</sup>	873.24±66 <sup>b</sup>	1003.38±61.28 <sup>c</sup>	1096.66±82.29 <sup>c</sup>
(E)-2-Octenal	4.27±0.40 <sup>a</sup>	6.79±1.05 <sup>ab</sup>	9.39±1.85 <sup>bc</sup>	14.41±3.76 <sup>d</sup>	11.45±0.94 <sup>cd</sup>
Benzaldehyde	126.39±10.50 <sup>c</sup>	109.36±7.89 <sup>b</sup>	81.15±3.45 <sup>a</sup>	143.31±11.73 <sup>d</sup>	86.43±4.71 <sup>a</sup>
(E)-2-Nonenal	36.22±5.73 <sup>a</sup>	63.13±9.77 <sup>b</sup>	90.56±7.16 <sup>c</sup>	106.17±4.47 <sup>d</sup>	102.83±2.34 <sup>d</sup>
(E)-2-Decenal	170.05±28.43 <sup>a</sup>	304.74±49.16 <sup>b</sup>	450.64±39.06 <sup>c</sup>	533.82±29.26 <sup>c</sup>	499.27±63 <sup>c</sup>
(E,E)-2,4-Nonadienal	29.61±3.19 <sup>a</sup>	37.22±6.80 <sup>a</sup>	59.34±6.82 <sup>b</sup>	78.39±6.67 <sup>c</sup>	73.46±10.15 <sup>c</sup>
3-ethyl-Benzaldehyde	3.08±0.39 <sup>a</sup>	7.37±0.32 <sup>c</sup>	6.11±0.47 <sup>b</sup>	8.17±0.26 <sup>d</sup>	8.49±0.35 <sup>d</sup>
2-Undecenal	129.45±21.19 <sup>a</sup>	212.58±38.48 <sup>b</sup>	319.6±20.16 <sup>c</sup>	356.34±26 <sup>c</sup>	339.27±27.84 <sup>c</sup>
(E,E)-2,4-Decadienal	40.86±11.95 <sup>a</sup>	48.17±12.97 <sup>a</sup>	67.11±6.07 <sup>b</sup>	99.79±5.88 <sup>c</sup>	92.85±4.27 <sup>c</sup>
Total	4136.35±311.89 <sup>a</sup>	6097.06±413.94 <sup>b</sup>	7498.86±205.82 <sup>c</sup>	8840.82±444.27 <sup>d</sup>	8259.97±349.74 <sup>d</sup>
<b>Alcohols</b>					
2-Hexanol	1.85±0.09 <sup>a</sup>	2.19±0.61 <sup>a</sup>	2.03±0.68 <sup>a</sup>	3.44±0.86 <sup>b</sup>	2.9±0.21 <sup>ab</sup>
Ethanol	29.61±2.46 <sup>d</sup>	17.33±2.09 <sup>c</sup>	7.75±1.24 <sup>b</sup>	5.35±0.51 <sup>ab</sup>	3.69±0.61 <sup>a</sup>
3,5,5-Trimethyl-1-hexanol	ND	4.09±1.46 <sup>b</sup>	8.96±4.20 <sup>c</sup>	11.79±0.67 <sup>c</sup>	19.08±0.94 <sup>d</sup>
(S)-(-)-1,2,4-Butanetriol	2.95±1.00 <sup>c</sup>	2.15±0.39 <sup>bc</sup>	1.68±0.56 <sup>b</sup>	ND	ND
<b>Butanetriol</b>					
1-Pentanol	12.46±0.49 <sup>a</sup>	35.06±9.10 <sup>bc</sup>	25.78±9.46 <sup>b</sup>	38.42±3.72 <sup>c</sup>	30.55±2.52 <sup>bc</sup>
2-ethoxy- Ethanol	2.5±0.18 <sup>c</sup>	2.37±0.27 <sup>c</sup>	1.49±0.31 <sup>ab</sup>	1.7±0.10 <sup>c</sup>	1.22±0.30 <sup>a</sup>
2,2'-oxybis- Ethanol	39.62±20.11 <sup>b</sup>	37.45±8.66 <sup>b</sup>	27.23±4.72 <sup>ab</sup>	12.24±0.16 <sup>a</sup>	11.07±4.80 <sup>a</sup>
1-Hexanol	19.68±4.52 <sup>a</sup>	78.62±100.71 <sup>a</sup>	103.45±150.77 <sup>a</sup>	141.58±123.45 <sup>a</sup>	20.27±2.51 <sup>a</sup>

Name	Stewing times /h				
	1.0	1.5	2.0	2.5	3.0
3-methyl-2-Butanol	7.86±0.45 <sup>a</sup>	6.44±0.75 <sup>a</sup>	8.8±3.11 <sup>a</sup>	7.23±0.60 <sup>a</sup>	6.2±0.21 <sup>a</sup>
1-Octen-3-ol	85.34±7.91 <sup>a</sup>	353.75±8.04 <sup>c</sup>	253.51±18.10 <sup>b</sup>	406.5±45.35 <sup>d</sup>	381.76±8.92 <sup>cd</sup>
1-Heptanol	23.81±3.36 <sup>a</sup>	66.9±1.33 <sup>b</sup>	66.08±2.82 <sup>b</sup>	104.2±11.71 <sup>d</sup>	92.45±3.88 <sup>c</sup>
2-ethyl-1-Hexanol	141.34±12.95 <sup>c</sup>	71.25±3.83 <sup>b</sup>	67.13±5.19 <sup>b</sup>	71.65±14.97 <sup>b</sup>	41.34±2.98 <sup>a</sup>
Linalool	68.71±2.96 <sup>b</sup>	69.13±5.41 <sup>b</sup>	32.07±5.18 <sup>a</sup>	63.01±11.13 <sup>b</sup>	38.04±1.81 <sup>a</sup>
1-Octanol	24.57±2.69 <sup>a</sup>	63.39±4.66 <sup>b</sup>	64.75±4.57 <sup>b</sup>	100.4±10.31 <sup>c</sup>	90.88±7.17 <sup>c</sup>
2-Decanol	1.53±2.64 <sup>a</sup>	ND	2.87±0.99 <sup>a</sup>	2.19±1.95 <sup>a</sup>	2.95±0.71 <sup>a</sup>
1-Nonanol	1.18±0.09 <sup>a</sup>	2.46±0.82 <sup>a</sup>	3.55±1.27 <sup>a</sup>	9.14±5.68 <sup>b</sup>	2.02±0.32 <sup>a</sup>
2-n-Propyl-1-heptanol	18.21±4.04 <sup>b</sup>	4.51±1.65 <sup>a</sup>	6.58±1.91 <sup>a</sup>	9.78±3.71 <sup>a</sup>	8.63±1.12 <sup>a</sup>
Benzyl alcohol	19.65±0.63 <sup>c</sup>	23.92±1.02 <sup>d</sup>	15.68±1.51 <sup>b</sup>	24.37±2.09 <sup>d</sup>	12.34±0.42 <sup>a</sup>
Total oxole	500.85±45.42 <sup>a</sup>	841±101.58 <sup>b</sup>	699.41±128.59 <sup>b</sup>	1012.98±118.03 <sup>c</sup>	765.38±13.71 <sup>b</sup>
Furan	12.73±2.28 <sup>a</sup>	9.95±3.89 <sup>a</sup>	8.18±0.79 <sup>a</sup>	10.3±5.59 <sup>a</sup>	7.27±1.38 <sup>a</sup>
2-pentyl-Furan	39.52±4.97 <sup>a</sup>	54.93±8.23 <sup>b</sup>	78.62±5.91 <sup>c</sup>	137.85±9.73 <sup>d</sup>	135.71±6.51 <sup>d</sup>
Total Hydrocarbons	52.25±2.70 <sup>a</sup>	64.88±5.15 <sup>a</sup>	86.79±5.42 <sup>b</sup>	148.15±12.57 <sup>c</sup>	142.98±6.32 <sup>c</sup>
n-Hexane	282.85±13.67 <sup>b</sup>	174.26±54.46 <sup>a</sup>	81.48±13.72 <sup>a</sup>	183.69±101.90 <sup>ab</sup>	103.83±34.08 <sup>a</sup>
Heptane	3.24±0.16 <sup>a</sup>	4.1±0.34 <sup>a</sup>	4.08±0.29 <sup>a</sup>	7.84±1.02 <sup>b</sup>	10.54±3.04 <sup>c</sup>
1,2-dimethyl-Cyclopentane	2.25±0.50 <sup>ab</sup>	1.74±0.17 <sup>a</sup>	2.25±0.26 <sup>ab</sup>	2.63±0.77 <sup>b</sup>	2.43±0.07 <sup>ab</sup>
Octane	4.18±0.30 <sup>a</sup>	8.26±1.23 <sup>ab</sup>	16.25±1.31 <sup>b</sup>	41.6±4.13 <sup>c</sup>	78.82±12.35 <sup>d</sup>
Isopropyl cyclobutane	5.74±0.17 <sup>b</sup>	4.94±0.08 <sup>b</sup>	5.03±0.46 <sup>b</sup>	1.81±3.14 <sup>a</sup>	4.59±0.19 <sup>b</sup>
Nonane	ND	ND	ND	ND	3.96±0.24 <sup>b</sup>
2,6-dimethyl- Nonane	2.91±0.68 <sup>b</sup>	1.43±0.55 <sup>a</sup>	1.73±0.53 <sup>a</sup>	1.17±0.11 <sup>a</sup>	1.36±0.31 <sup>a</sup>
2,6,11-trimethyl-Dodecane	87.62±14.37 <sup>a</sup>	117.02±6.24 <sup>a</sup>	129.54±3.08 <sup>a</sup>	144.97±6.13 <sup>a</sup>	88.34±76.65 <sup>a</sup>
Tridecane	67.96±5.22 <sup>ab</sup>	78.39±9.56 <sup>bc</sup>	72.48±7.66 <sup>abc</sup>	84.57±7.57 <sup>c</sup>	61.38±2.79 <sup>a</sup>
1,1-dimethyl-Cyclopentane	3.35±0.89 <sup>a</sup>	8.5±0.66 <sup>c</sup>	7.15±0.16 <sup>b</sup>	9.98±0.63 <sup>d</sup>	9.96±0.52 <sup>d</sup>
Hexadecane	33.78±1.30 <sup>d</sup>	14.75±2.29 <sup>a</sup>	28.73±2.42 <sup>bc</sup>	30.45±1.06 <sup>c</sup>	26.03±1.66 <sup>b</sup>
3,7-dimethyl- Decane	22.44±4.38 <sup>b</sup>	14.2±1.72 <sup>a</sup>	18.13±1.72 <sup>ab</sup>	13.4±2.60 <sup>a</sup>	13.24±3.03 <sup>a</sup>
3,5-dimethyl-1-Hexene	ND	2.7±1.02 <sup>b</sup>	5.29±0.41 <sup>c</sup>	9.06±0.66 <sup>d</sup>	8.73±0.32 <sup>d</sup>

Name	Stewing times /h				
	1.0	1.5	2.0	2.5	3.0
3-ethyl-2-methyl-1,3-Hexadiene	19.23±0.79 <sup>a</sup>	53.67±6.51 <sup>b</sup>	60.83±1.92 <sup>c</sup>	77.21±1.68 <sup>d</sup>	79.26±1.38 <sup>d</sup>
4-methyl-1-Undecene	17.35±0.37 <sup>ab</sup>	8.1±3.28 <sup>a</sup>	21.38±13.70 <sup>b</sup>	12.82±0.93 <sup>ab</sup>	12.36±0.73 <sup>ab</sup>
3-methyl-, (E)- 4-Undecene	6.77±0.91 <sup>a</sup>	13.86±2.28 <sup>b</sup>	17.98±0.58 <sup>c</sup>	22.57±1.32 <sup>d</sup>	22.55±1.98 <sup>d</sup>
Total esters	559.67±3.8 <sup>ab</sup>	505.92±57.09 <sup>ab</sup>	472.33±24.61 <sup>a</sup>	643.77±99.56 <sup>b</sup>	527.39±110.34 <sup>ab</sup>
Ethyl Acetate	44.52±5.38 <sup>b</sup>	20.33±2.06 <sup>a</sup>	19.03±1.57 <sup>a</sup>	20.82±2.71 <sup>a</sup>	16.87±3.10 <sup>a</sup>
Isobutyl acetate	11.88±0.83 <sup>d</sup>	5.59±1.04 <sup>c</sup>	5.95±0.17 <sup>c</sup>	4.32±0.29 <sup>b</sup>	2.82±0.57 <sup>a</sup>
Methyl acetoacetate	4.62±0.26 <sup>a</sup>	10.66±2.54 <sup>b</sup>	13.6±0.35 <sup>c</sup>	12.75±0.36 <sup>bc</sup>	12.72±1.10 <sup>bc</sup>
Butyrolactone	10.93±0.79 <sup>c</sup>	12.04±0.56 <sup>c</sup>	7.29±0.96 <sup>ab</sup>	8.32±0.26 <sup>b</sup>	6.64±0.17 <sup>a</sup>
Total Ketones	71.94±4.01 <sup>c</sup>	48.62±4.94 <sup>b</sup>	45.88±1.92 <sup>b</sup>	46.22±3.05 <sup>b</sup>	39.06±3.44 <sup>a</sup>
Acetone	64.19±13.01 <sup>b</sup>	32.41±5.66 <sup>a</sup>	28.27±2.27 <sup>a</sup>	32.25±8.49 <sup>a</sup>	18.42±4.99 <sup>a</sup>
2-Butanone	11.6±0.72 <sup>d</sup>	5.71±0.78 <sup>c</sup>	4.11±0.43 <sup>b</sup>	5.09±0.28 <sup>bc</sup>	2.93±0.33 <sup>a</sup>
Methyl Isobutyl Ketone	ND	20.11±5.67 <sup>c</sup>	12.32±1.72 <sup>b</sup>	44.6±5.47 <sup>e</sup>	32.12±3.37 <sup>d</sup>
2,3-Pentanedione	12.06±1.39 <sup>a</sup>	12.34±2.19 <sup>a</sup>	15.45±0.60 <sup>b</sup>	18.55±1.21 <sup>c</sup>	14.51±0.87 <sup>ab</sup>
1-Octen-3-one	11.46±2.50 <sup>a</sup>	14.67±2.38 <sup>abc</sup>	12.19±1.10 <sup>ab</sup>	19.64±4.58 <sup>c</sup>	17.24±1.25 <sup>bc</sup>
2-Heptanone	97.84±8.78 <sup>ab</sup>	84.42±45.05 <sup>b</sup>	93.96±48.28 <sup>ab</sup>	145.75±1.69 <sup>b</sup>	52.06±11.56 <sup>a</sup>
3-Octen-2-one	8.76±0.15 <sup>a</sup>	13.01±3.05 <sup>ab</sup>	13.18±5.28 <sup>ab</sup>	17.14±1.36 <sup>b</sup>	16.05±3.98 <sup>b</sup>
Acetophenone	26.66±3.22 <sup>c</sup>	16.75±1.10 <sup>b</sup>	16.25±2.55 <sup>b</sup>	14.27±1.27 <sup>b</sup>	9.11±0.22 <sup>a</sup>
Total Aromatic hydrocarbon	232.57±13.62 <sup>b</sup>	199.43±55.52 <sup>ab</sup>	195.73±41.2 <sup>ab</sup>	297.28±8.39 <sup>c</sup>	162.44±17.05 <sup>a</sup>
Benzene	7.07±1.17 <sup>c</sup>	3.67±0.62 <sup>b</sup>	3.73±0.66 <sup>b</sup>	2.93±0.76 <sup>ab</sup>	2.06±0.41 <sup>a</sup>
Toluene	232±26.86 <sup>c</sup>	69.18±5.96 <sup>a</sup>	128.41±9.84 <sup>b</sup>	64.82±16.86 <sup>a</sup>	49.25±7.38 <sup>a</sup>
p-Xylene	71.52±14.04 <sup>b</sup>	31.78±3.53 <sup>a</sup>	31.45±3.91 <sup>a</sup>	26.77±4.19 <sup>a</sup>	20.99±2.14 <sup>a</sup>
1,3-dimethyl-Benzene	35.21±4.86 <sup>c</sup>	21.29±0.66 <sup>b</sup>	18.46±2.60 <sup>ab</sup>	19.06±2.15 <sup>ab</sup>	15.01±1.21 <sup>a</sup>
Total Others	345.8±46.12 <sup>c</sup>	125.92±10.08 <sup>a</sup>	182.06±11.44 <sup>b</sup>	113.57±20.79 <sup>a</sup>	87.33±11.06 <sup>a</sup>
Hexanoic acid	3.58±0.41 <sup>a</sup>	4.77±0.40 <sup>b</sup>	4.25±0.87 <sup>ab</sup>	5.13±0.60 <sup>b</sup>	4.1±0.54 <sup>ab</sup>
Mevalonic acid	12.39±0.73 <sup>d</sup>	2.12±0.43 <sup>c</sup>	1.3±0.07 <sup>b</sup>	1.11±0.08 <sup>b</sup>	ND
Rosmarinic acid	3.22±0.45 <sup>b</sup>	4.9±0.61 <sup>c</sup>	3.4±0.18 <sup>b</sup>	ND	ND

Name	Stewing times /h				
	1.0	1.5	2.0	2.5	3.0
Phenol	4.56±0.27 <sup>c</sup>	1.77±0.17 <sup>b</sup>	ND	ND	ND
hexamethyl- Disiloxane	42.07±7.26 <sup>b</sup>	23.8±17.56 <sup>a</sup>	32.27±3.51 <sup>ab</sup>	46.38±2.95 <sup>b</sup>	24.23±5.94 <sup>a</sup>
hexamethyl- Cyclotrisiloxane	200.18±4.78 <sup>d</sup>	160.28±22 <sup>cd</sup>	114.35±35.58 <sup>ab</sup>	131.46±1.84 <sup>bc</sup>	75.63±28.15 <sup>a</sup>
Trichloromethane	491.08±53.3 <sup>c</sup>	176.56±18.69 <sup>ab</sup>	227.64±24.54 <sup>b</sup>	165.76±102.26 <sup>ab</sup>	105.61±39.97 <sup>a</sup>
pentyl- Oxirane	5.49±0.49 <sup>a</sup>	11.71±0.94 <sup>b</sup>	14.19±0.57 <sup>c</sup>	15.95±0.42 <sup>c</sup>	14.79±1.67 <sup>c</sup>
decamethyl- Cyclopentasiloxane	321.3±39.32 <sup>c</sup>	247.77±18.57 <sup>b</sup>	230.5±12.52 <sup>b</sup>	224.23±10.98 <sup>b</sup>	150.93±13.2 <sup>a</sup>
tetradecamethyl- Cycloheptasiloxane	113.76±32.15 <sup>b</sup>	96.32±9.10 <sup>ab</sup>	81.2±13.13 <sup>ab</sup>	81.24±14.12 <sup>ab</sup>	63.33±6.60 <sup>a</sup>
1-Iodo-2- methylnonane	38.76±33.72 <sup>a</sup>	10.71±9.46 <sup>a</sup>	22.48±19.49 <sup>a</sup>	16.81±14.98 <sup>a</sup>	30.18±1.73 <sup>a</sup>
dodecamethyl- Cyclohexasiloxane	22.6±2.06 <sup>c</sup>	24.61±4.18 <sup>c</sup>	16.86±0.71 <sup>b</sup>	15.39±1.73 <sup>b</sup>	10.56±0.45 <sup>a</sup>
dodecamethyl- Cyclohexasiloxane	215.84±45.53 <sup>c</sup>	173.74±15.54 <sup>bc</sup>	154.35±16.03 <sup>b</sup>	145.29±13.70 <sup>ab</sup>	105.74±17.37 <sup>a</sup>
decamethyl- Tetrasiloxane	10.51±0.77 <sup>c</sup>	8.36±2.05 <sup>bc</sup>	6.82±1.01 <sup>b</sup>	6.4±1.12 <sup>b</sup>	3.16±0.86 <sup>a</sup>
Total	1485.33±82.78 <sup>c</sup>	947.41±91.25 <sup>b</sup>	909.62±93.98 <sup>b</sup>	855.16±130.95 <sup>b</sup>	588.26±57.51 <sup>b</sup>

Note: Different letters in the same row indicate significant differences ( $P < 0.05$ ).