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ARTICLE

Effect of Stewing Time on the Small Molecular Metabolites, Free Fatty Acids, and Volatile Flavor Compounds in Chicken Broth

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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, gas chromatography-mass spectrometry, headspace solid-phase microextraction, and gas chromatography-mass spectrometry. 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 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), 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 (Cao et al., 2021; Wang et al., 2014). 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., 2020). 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 (Jia et al., 2023; Yu et al., 2021), 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., 2018; 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 1,607±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 (2,100 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 Supplementary Table S1, and the total scores were calculated according to the corresponding weights of the four 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, Agilent, Santa Clara, CA, USA) 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 μL of chicken broth was pipetted into a 1.5 mL EP tube with a pipette, 800 μL of methanol and 10 μL 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℃ and 24,192×g for 15 min, and 200 μL of supernatant was collected for testing. The analytical column (C18, 100 mm×2.1 mm, 1.8 μm, Agilent) was used with a set injection volume of 4 μL, autosampler temperature of 4℃, column temperature of 40℃, 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 GC-MS (Agilent 7890A-5975C, Agilent) method was used to analyze free fatty acids in chicken broth according to our previous method (Liu et al., 2019). Firstly, 200 μL 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℃ and 2,058×g for 5 min. Subsequently, the extraction was transferred to an SPE column and placed in a constant temperature water bath (90℃–95℃) 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℃ and 2,058×g 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 flame ionization detector (FID) (250℃).

Analysis of volatile compounds

The HS-SPME-GC-MS (57330U, Supelco, Bellefonte, PA, USA; 7890A-5975C, Agilent) 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 10.5×g for 15 min at 60℃ 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 mL, the injection temperature was 260℃, 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℃ for 5 min, increase to 250℃ at a rate of 5 ℃/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 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., 2017). 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 and Supplementary Table S2, 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,

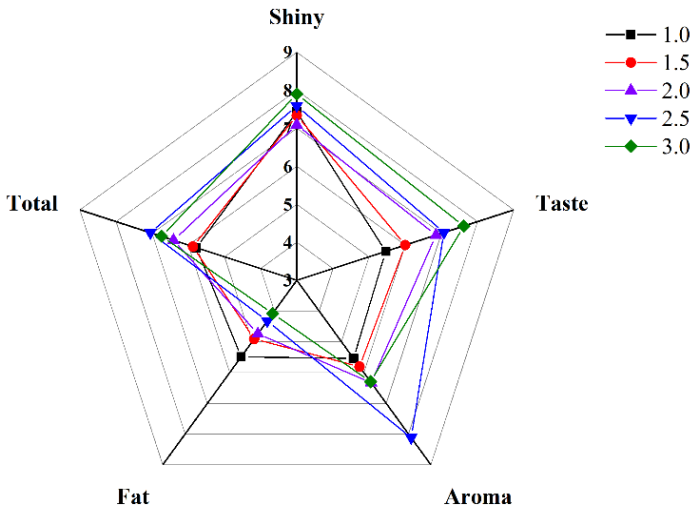

Fig. 1. Sensory evaluation score of chicken broth in different treatment groups.

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 Supplementary Table S3 and Fig. 2. Fig. 2 show the plots of 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 and Xia, 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 (AMP), xanthine nucleoside, nicotinamide, disodium 5'-inosine (IMP), hypoxanthine (Hx), inosine (I), and amino acids, among which the contents of I and IMP were significantly higher than other substances, and they occupied an important role in the contribution of the flavor.

Figs. 2C 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 diisodecyl phthalate (DIDP), xanthine nucleoside, deoxyuridine-5'-diphosphate (DUDP), adenosine, and others. Studies have shown that AMP, guanosine (GMP), I, IMP, and 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

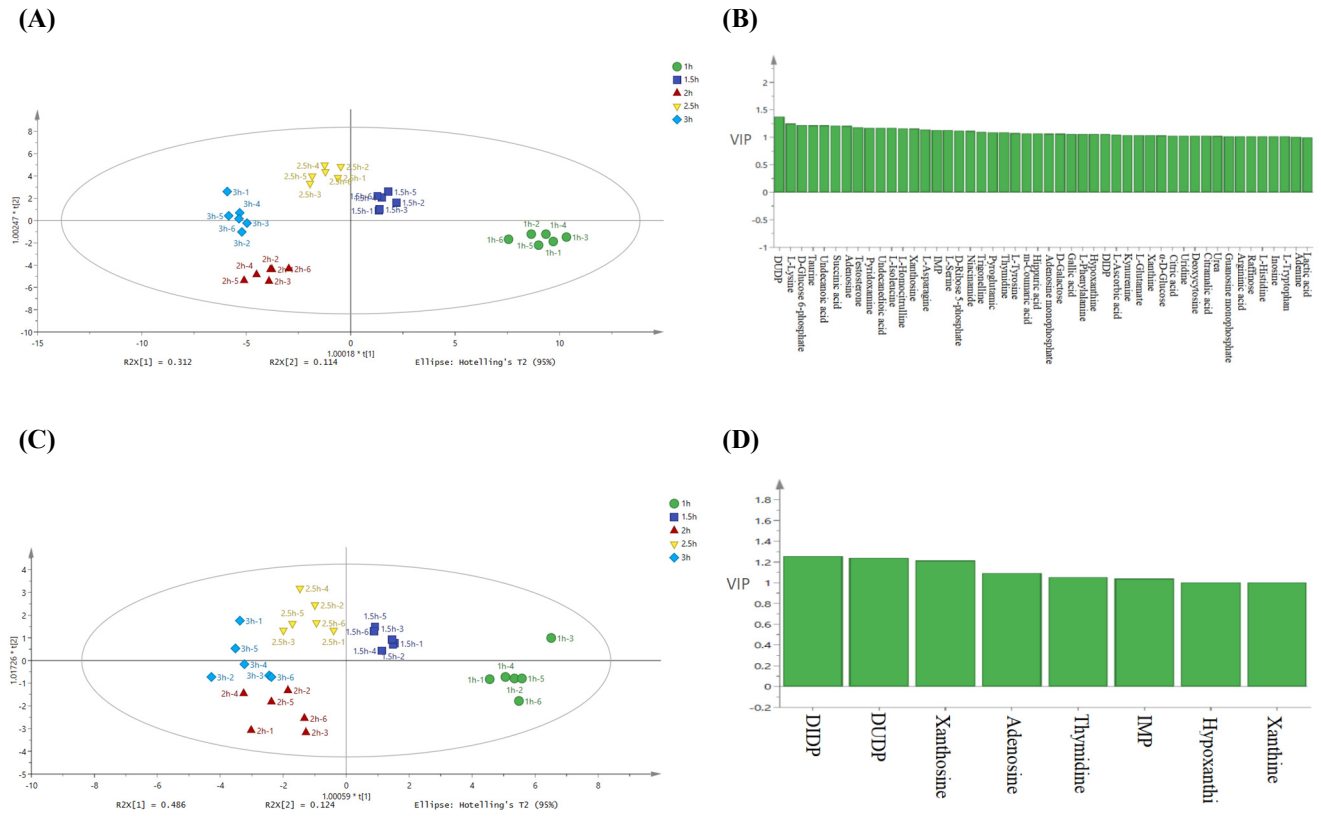

Fig. 2. Analysis of small molecular metabolites in chicken broth at different stewing times. OPLS-DA scores plot (A) and VIP scores (B) of small molecular metabolites in chicken broth at different stewing times. OPLS-DA score plot (C) and VIP plot (D) of nucleic acids in chicken broth at different stewing times. 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. VIP, variable projection importance plots; DIDP, diisodecyl phthalate; DUDP, deoxyuridine-5'-diphosphate; IMP, disodium 5'-inosine; OPLS-DA, orthogonal partial least squares discriminant analysis.

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 (Nkukwana et al., 2014; Yu et al., 2021), and there was no significant difference between C16:0 and C18:0. Xiao et al. (2018) found palmitic, stearic, oleic acid (C18:1n-9), and linoleic (C18:2n-6) acids 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

Table 1. Composition of free fatty acids in chicken soup of different treatment groups (μg/mL)

Name	Stewing times (h)				
	1.0	1.5	2.0	2.5	3.0
C10:0	1.69±0.07[a]	1.72±0.17[a]	1.92±0.08[b]	1.93±0.01[b]	2.03±0.10[b]
C12:0	3.54±0.49[a]	3.23±0.31[a]	3.52±0.06[a]	3.27±0.12[a]	3.49±0.05[a]
C14:0	9.32±1.22[a]	9.82±0.96[a]	10.04±0.74[a]	10.12±1.17[a]	9.31±0.56[a]
C14:1n5	2.35±0.38[a]	2.42±0.02[a]	2.61±0.13[a]	2.44±0.09[a]	2.32±0.29[a]
C15:0	3.12±0.20[a]	3.16±0.32[a]	3.4±0.42[a]	3.34±0.10[a]	3.31±0.20[a]
C16:0	447.73±21.59[a]	459.08±35.39[a]	499.45±75.26[a]	511.63±52.53[a]	503.92±67.97[a]
C17:0	4.14±0.19[a]	4.4±0.53[ab]	4.56±0.55[ab]	4.9±0.34[ab]	5.02±0.51[b]
C18:0	300.59±50.31[a]	303.46±117.65[a]	360.05±61.81[a]	362.86±54.41[a]	357.32±61.32[a]
C18:1n9c	17.13±1.98[ab]	17.13±1.63[ab]	17.43±1.13[ab]	19.49±0.40[b]	15.32±2.56[a]
C18:2n6c	51.46±2.11[a]	52.4±0.28[a]	55.37±2.28[a]	52.95±0.98[a]	51.46±3.04[a]
C20:0	1.61±0.30[a]	1.64±0.53[a]	1.99±0.15[a]	1.98±0.26[a]	1.86±0.22[a]
C20:1	1.23±0.05[a]	1.25±0.02[a]	1.32±0.27[a]	1.17±0.09[a]	1.15±0.07[a]
C22:1n9	32.25±1.32[a]	32.49±0.89[a]	32.56±0.99[a]	33.06±1.96[a]	32.63±1.88[a]
C22:2	7.28±0.23[a]	7.33±0.85[a]	7.36±0.34[a]	7.37±0.50[a]	6.4±0.31[a]
C24:0	1.72±0.31[a]	1.72±0.09[a]	1.74±0.25[a]	1.82±0.20[a]	1.69±0.15[a]
TF	885.16±66.02[a]	901.26±157.23[a]	1,003.32±139.75[a]	1,018.32±111.36[a]	997.25±126.92[a]
SFA	773.46±67.56[a]	788.24±155.15[a]	886.67±137.90[a]	901.85±108.37[a]	887.96±129.65[a]
MUFA	52.96±2.11[ab]	53.29±1.69[ab]	53.93±1.68[ab]	56.15±2.25[b]	51.42±1.61[a]
PUFA	58.74±1.89[ab]	59.73±0.66[ab]	62.73±2.57[ab]	60.32±1.45[b]	57.86±3.08[a]

[a,b] Different letters in the same row indicate significant differences ($p<0.05$).
TF, total fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

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 (PUFA), monounsaturated fatty acids (MUFA), 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 SFA, which were about 7 times the concentration of UFA. 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 Supplementary Table S3. These compounds collectively affect the flavor quality of chicken broth (Lorenzo and Franco, 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 (Fan et al., 2018; Feng et al., 2018; Sun 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 8,840.82 ng/100 mL, 1,012.98 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 (Supplementary Table S4; Barola et al., 2020; Qi et al., 2017). Among the aldehydes, hexanal had the largest concentration rise, reaching 5,393.02 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). Shen et al. (2016) and Yu et al. (2021) 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,

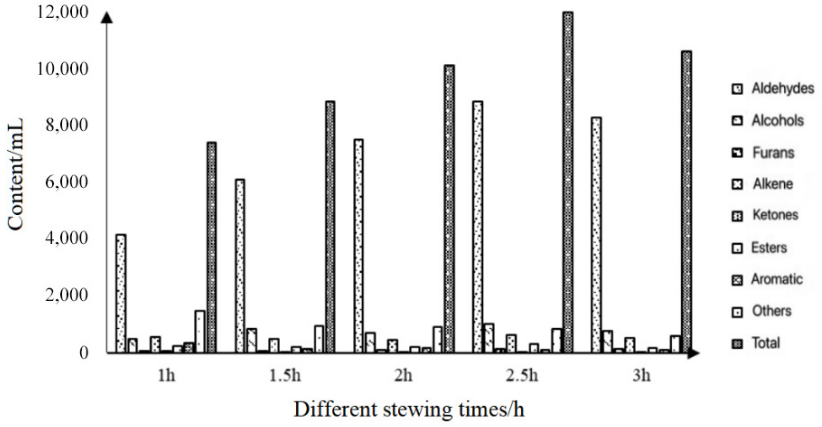

Fig. 3. The change of volatile compounds in chicken broth at different stewing time.

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 SFA, which were about 7 times higher than UFA. 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 5,393.02 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.

Supplementary Materials

Supplementary materials are only available online from: https://doi.org/10.5851/kosfa.2024.e9.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

Conceptualization: Jia R, Yang Yucai, Liao G. Data curation: Jia R, Yang Yucai, Liao G, Gu D. Formal analysis: Jia R, Yang Yucai, Yang Yuan, Wang G. Methodology: Jia R, Yang Yucai, Liao G, Gu D, Wang G. Software: Jia R, Yang Yucai, Yang Yuan, Gu D. Validation: Jia R, Yang Yucai, Liao G. Investigation: Jia R, Yang Yucai, Gu D. Writing - original draft: Jia R, Yang Yucai, Liao G. Writing - review & editing: Jia R, Yang Yucai, Liao G, Yang Yuan, Gu D, Wang G.

Ethics Approval

The experimental procedure and protocol were approved by the Animal Care Committee of the College of Animal Science and Technology, Yunnan Agricultural University.

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Supplementary Materials

Table S1. Criteria for sensory evaluation of chicken soup

Evaluation criteria	Score			
Standard (weight)	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 S2. 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^{a}	7.34±0.34^{a}	7.09±0.16^{a}	7.59±0.46^{a}	7.90±0.42^{a}
Taste (40%)	5.46±0.32^{c}	6.00±0.15^{c}	6.85±0.23^{b}	7.07±0.28^{ab}	7.62±0.59^{a}
Aroma (30%)	5.54±0.41^{c}	5.81±0.41^{bc}	6.33±0.05^{b}	8.12±0.18^{a}	6.30±0.45^{b}
Fat (15%)	5.49±0.51^{a}	4.91±0.19^{b}	4.74±0.18^{bc}	4.32±0.26^{cd}	4.08±2.24^{d}
Total	5.78±0.15^{c}	5.87±0.28^{c}	6.41±0.15^{b}	7.05±0.15^{a}	6.74±0.29^{ab}

$^{a–d}$ Different letters in the same row indicate significant differences ($p<0.05$).

Table S3. List of small molecular metabolites in chicken soup of different treatment groups

NO.	Metabolite	NO.	Metabolite	NO.	Metabolite
71	Hypoxanthine	96	L-Methionine	184	Salicyluric 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	β-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	α-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 S4. 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
Aldehyde					
Acetaldehyde	30.71±3.67^{a}	31.1±3.46^{a}	26.32±4.57^{a}	42.93±5.81^{b}	27.31±3.58^{a}
Propanal	5.73±0.73^{a}	8.87±0.66^{c}	7.7±0.26^{bc}	8.9±1.08^{c}	7.18±0.28^{b}
2-Methyl-Butanal	4.56±0.60^{d}	2.69±0.26^{c}	1.87±0.21^{b}	2.03±0.15^{b}	1.22±0.15^{a}
3-Methyl-Butanal	7.17±0.62^{d}	3.93±0.40^{c}	2.67±0.38^{b}	2.58±0.38^{ab}	1.89±0.14^{a}
Pentanal	145.44±5.62^{a}	187.12±11.38^{b}	245.58±8.72^{c}	278.87±29.89^{d}	238.2±15.01^{c}
(E)-2-Butenal	4.13±0.49^{a}	7.8±1.39^{b}	5.08±0.62^{a}	7.93±0.28^{b}	7.34±0.91^{b}
Hexanal	2,673.97±168.71^{a}	3,981.09±151.19^{b}	4,676.16±214.37^{c}	5,393.02±286.98^{d}	4,880.09±230.10^{c}
Heptanal	101.34±9.70^{a}	166.66±9.09^{b}	212.9±15.24^{c}	241.44±8.38^{d}	231.88±18.27^{cd}
(E)-2-Hexenal	3.31±1.67^{a}	8.72±1.19^{b}	8.36±0.14^{b}	10.88±0.20^{c}	10.73±0.49^{c}
Octanal	113.97±16.48^{a}	154.69±102.15^{a}	203.98±135.11^{ab}	301.79±21.07^{b}	330.17±20.08^{b}
(Z)-2-Heptenal	62.42±4.35^{a}	145.51±18.56^{b}	151.11±8.36^{b}	206.68±7.18^{c}	213.22±3.11^{c}
Nonanal	443.63±47.44^{a}	609.54±53.71^{b}	873.24±66^{b}	1,003.38±61.28^{c}	1,096.66±82.29^{c}
(E)-2-Octenal	4.27±0.40^{a}	6.79±1.05^{ab}	9.39±1.85^{bc}	14.41±3.76^{d}	11.45±0.94^{cd}
Benzaldehyde	126.39±10.50^{c}	109.36±7.89^{b}	81.15±3.45^{a}	143.31±11.73^{d}	86.43±4.71^{a}
(E)-2-Nonenal	36.22±5.73^{a}	63.13±9.77^{b}	90.56±7.16^{c}	106.17±4.47^{d}	102.83±2.34^{d}
(E)-2-Decenal	170.05±28.43^{a}	304.74±49.16^{b}	450.64±39.06^{c}	533.82±29.26^{c}	499.27±63^{c}
(E,E)-2,4-Nonadienal	29.61±3.19^{a}	37.22±6.80^{a}	59.34±6.82^{b}	78.39±6.67^{c}	73.46±10.15^{c}
3-Ethyl-Benzaldehyde	3.08±0.39^{a}	7.37±0.32^{c}	6.11±0.47^{b}	8.17±0.26^{d}	8.49±0.35^{d}
2-Undecenal	129.45±21.19^{a}	212.58±38.48^{b}	319.6±20.16^{c}	356.34±26^{c}	339.27±27.84^{c}
(E,E)-2,4-Decadienal	40.86±11.95^{a}	48.17±12.97^{a}	67.11±6.07^{b}	99.79±5.88^{c}	92.85±4.27^{c}
Total	4,136.35±311.89^{a}	6,097.06±413.94^{b}	7,498.86±205.82^{c}	8,840.82±444.27^{d}	8,259.97±349.74^{d}
Alcohols					
2-Hexanol	1.85±0.09^{a}	2.19±0.61^{a}	2.03±0.68^{a}	3.44±0.86^{b}	2.9±0.21^{ab}
Ethanol	29.61±2.46^{d}	17.33±2.09^{c}	7.75±1.24^{b}	5.35±0.51^{ab}	3.69±0.61^{a}
3,5,5-Trimethyl-1-hexanol	ND	4.09±1.46^{b}	8.96±4.20^{c}	11.79±0.67^{c}	19.08±0.94^{d}
(S)-(-)-1,2,4-Butanetriol	2.95±1.00^{c}	2.15±0.39^{bc}	1.68±0.56^{b}	ND	ND
1-Pentanol	12.46±0.49^{a}	35.06±9.10^{bc}	25.78±9.46^{b}	38.42±3.72^{c}	30.55±2.52^{bc}
2-Ethoxy-ethanol	2.5±0.18^{c}	2.37±0.27^{c}	1.49±0.31^{ab}	1.7±0.10^{c}	1.22±0.30^{a}
2,2'-Oxybis-ethanol	39.62±20.11^{b}	37.45±8.66^{b}	27.23±4.72^{ab}	12.24±0.16^{a}	11.07±4.80^{a}
1-Hexanol	19.68±4.52^{a}	78.62±100.71^{a}	103.45±150.77^{a}	141.58±123.45^{a}	20.27±2.51^{a}
3-Methyl-2-butanol	7.86±0.45^{a}	6.44±0.75^{a}	8.8±3.11^{a}	7.23±0.60^{a}	6.2±0.21^{a}
1-Octen-3-ol	85.34±7.91^{a}	353.75±8.04^{c}	253.51±18.10^{b}	406.5±45.35^{d}	381.76±8.92^{cd}

Table S4. Changes in volatile substances in chicken broth of different treatment groups (ng/100 mL) (continued)

Name	Stewing times (h)				
	1.0	1.5	2.0	2.5	3.0
1-Heptanol	23.81±3.36^{a}	66.9±1.33^{b}	66.08±2.82^{b}	104.2±11.71^{d}	92.45±3.88^{c}
2-Ethyl-1-hexanol	141.34±12.95^{c}	71.25±3.83^{b}	67.13±5.19^{b}	71.65±14.97^{b}	41.34±2.98^{a}
Linalool	68.71±2.96^{b}	69.13±5.41^{b}	32.07±5.18^{a}	63.01±11.13^{b}	38.04±1.81^{a}
1-Octanol	24.57±2.69^{a}	63.39±4.66^{b}	64.75±4.57^{b}	100.4±10.31^{c}	90.88±7.17^{c}
2-Decanol	1.53±2.64^{a}	ND	2.87±0.99^{a}	2.19±1.95^{a}	2.95±0.71^{a}
1-Nonanol	1.18±0.09^{a}	2.46±0.82^{a}	3.55±1.27^{a}	9.14±5.68^{b}	2.02±0.32^{a}
2-n-Propyl-1-heptanol	18.21±4.04^{b}	4.51±1.65^{a}	6.58±1.91^{a}	9.78±3.71^{a}	8.63±1.12^{a}
Benzyl alcohol	19.65±0.63^{c}	23.92±1.02^{d}	15.68±1.51^{b}	24.37±2.09^{d}	12.34±0.42^{a}
Total	500.85±45.42^{a}	841±101.58^{b}	699.41±128.59^{b}	1,012.98±118.03^{c}	765.38±13.71^{b}
Oxole					
Furan	12.73±2.28^{a}	9.95±3.89^{a}	8.18±0.79^{a}	10.3±5.59^{a}	7.27±1.38^{a}
2-Pentyl-furan	39.52±4.97^{a}	54.93±8.23^{b}	78.62±5.91^{c}	137.85±9.73^{d}	135.71±6.51^{d}
Total	52.25±2.70^{a}	64.88±5.15^{a}	86.79±5.42^{b}	148.15±12.57^{c}	142.98±6.32^{c}
Hydrocarbons					
n-Hexane	282.85±13.67^{b}	174.26±54.46^{a}	81.48±13.72^{a}	183.69±101.90^{ab}	103.83±34.08^{a}
Heptane	3.24±0.16^{a}	4.1±0.34^{a}	4.08±0.29^{a}	7.84±1.02^{b}	10.54±3.04^{c}
1,2-Dimethyl-cyclopentane	2.25±0.50^{ab}	1.74±0.17^{a}	2.25±0.26^{ab}	2.63±0.77^{b}	2.43±0.07^{ab}
Octane	4.18±0.30^{a}	8.26±1.23^{ab}	16.25±1.31^{b}	41.6±4.13^{c}	78.82±12.35^{d}
Isopropyl cyclobutane	5.74±0.17^{b}	4.94±0.08^{b}	5.03±0.46^{b}	1.81±3.14^{a}	4.59±0.19^{b}
Nonane	ND	ND	ND	ND	3.96±0.24^{b}
2,6-Dimethyl-nonane	2.91±0.68^{b}	1.43±0.55^{a}	1.73±0.53^{a}	1.17±0.11^{a}	1.36±0.31^{a}
2,6,11-Trimethyl-dodecane	87.62±14.37^{a}	117.02±6.24^{a}	129.54±3.08^{a}	144.97±6.13^{a}	88.34±76.65^{a}
Tridecane	67.96±5.22^{ab}	78.39±9.56^{bc}	72.48±7.66^{abc}	84.57±7.57^{c}	61.38±2.79^{a}
1,1-Dimethyl-cyclopentane	3.35±0.89^{a}	8.5±0.66^{c}	7.15±0.16^{b}	9.98±0.63^{d}	9.96±0.52^{d}
Hexadecane	33.78±1.30^{d}	14.75±2.29^{a}	28.73±2.42^{bc}	30.45±1.06^{c}	26.03±1.66^{b}
3,7-Dimethyl-decane	22.44±4.38^{b}	14.2±1.72^{a}	18.13±1.72^{ab}	13.4±2.60^{a}	13.24±3.03^{a}
3,5-Dimethyl-1-hexene	ND	2.7±1.02^{b}	5.29±0.41^{c}	9.06±0.66^{d}	8.73±0.32^{d}
3-Ethyl-2-methyl-1,3-hexadiene	19.23±0.79^{a}	53.67±6.51^{b}	60.83±1.92^{c}	77.21±1.68^{d}	79.26±1.38^{d}
4-Methyl-1-undecene	17.35±0.37^{ab}	8.1±3.28^{a}	21.38±13.70^{b}	12.82±0.93^{ab}	12.36±0.73^{ab}
3-Methyl-, (E)-4-undecene	6.77±0.91^{a}	13.86±2.28^{b}	17.98±0.58^{c}	22.57±1.32^{d}	22.55±1.98^{d}
Total	559.67±3.8^{ab}	505.92±57.09^{ab}	472.33±24.61^{a}	643.77±99.56^{b}	527.39±110.34^{ab}
Esters					
Ethyl acetate	44.52±5.38^{b}	20.33±2.06^{a}	19.03±1.57^{a}	20.82±2.71^{a}	16.87±3.10^{a}

Table S4. Changes in volatile substances in chicken broth of different treatment groups (ng/100 mL) (continued)

Name	Stewing times (h)				
	1.0	1.5	2.0	2.5	3.0
Isobutyl acetate	11.88±0.83^{d}	5.59±1.04^{c}	5.95±0.17^{c}	4.32±0.29^{b}	2.82±0.57^{a}
Methyl acetoacetate	4.62±0.26^{a}	10.66±2.54^{b}	13.6±0.35^{c}	12.75±0.36^{bc}	12.72±1.10^{bc}
Butyrolactone	10.93±0.79^{c}	12.04±0.56^{c}	7.29±0.96^{ab}	8.32±0.26^{b}	6.64±0.17^{a}
Total	71.94±4.01^{c}	48.62±4.94^{b}	45.88±1.92^{b}	46.22±3.05^{b}	39.06±3.44^{a}
Ketones					
Acetone	64.19±13.01^{b}	32.41±5.66^{a}	28.27±2.27^{a}	32.25±8.49^{a}	18.42±4.99^{a}
2-Butanone	11.6±0.72^{d}	5.71±0.78^{c}	4.11±0.43^{b}	5.09±0.28^{bc}	2.93±0.33^{a}
Methyl isobutyl ketone	ND	20.11±5.67^{c}	12.32±1.72^{b}	44.6±5.47^{e}	32.12±3.37^{d}
2,3-Pentanedione	12.06±1.39^{a}	12.34±2.19^{a}	15.45±0.60^{b}	18.55±1.21^{c}	14.51±0.87^{ab}
1-Octen-3-one	11.46±2.50^{a}	14.67±2.38^{abc}	12.19±1.10^{ab}	19.64±4.58^{c}	17.24±1.25^{bc}
2-Heptanone	97.84±8.78^{ab}	84.42±45.05^{b}	93.96±48.28^{ab}	145.75±1.69^{b}	52.06±11.56^{a}
3-Octen-2-one	8.76±0.15^{a}	13.01±3.05^{ab}	13.18±5.28^{ab}	17.14±1.36^{b}	16.05±3.98^{b}
Acetophenone	26.66±3.22^{c}	16.75±1.10^{b}	16.25±2.55^{b}	14.27±1.27^{b}	9.11±0.22^{a}
Total	232.57±13.62^{b}	199.43±55.52^{ab}	195.73±41.2^{ab}	297.28±8.39^{c}	162.44±17.05^{a}
Aromatic hydrocarbon					
Benzene	7.07±1.17^{c}	3.67±0.62^{b}	3.73±0.66^{b}	2.93±0.76^{ab}	2.06±0.41^{a}
Toluene	232±26.86^{c}	69.18±5.96^{a}	128.41±9.84^{b}	64.82±16.86^{a}	49.25±7.38^{a}
p-Xylene	71.52±14.04^{b}	31.78±3.53^{a}	31.45±3.91^{a}	26.77±4.19^{a}	20.99±2.14^{a}
1,3-Dimethyl-benzene	35.21±4.86^{c}	21.29±0.66^{b}	18.46±2.60^{ab}	19.06±2.15^{ab}	15.01±1.21^{a}
Total	345.8±46.12^{c}	125.92±10.08^{a}	182.06±11.44^{b}	113.57±20.79^{a}	87.33±11.06^{a}
Others					
Hexanoic acid	3.58±0.41^{a}	4.77±0.40^{b}	4.25±0.87^{ab}	5.13±0.60^{b}	4.1±0.54^{ab}
Mevalonic acid	12.39±0.73^{d}	2.12±0.43^{c}	1.3±0.07^{b}	1.11±0.08^{b}	ND
Rosmarinic acid	3.22±0.45^{b}	4.9±0.61^{c}	3.4±0.18^{b}	ND	ND
Phenol	4.56±0.27^{c}	1.77±0.17^{b}	ND	ND	ND
Hexamethyl-disiloxane	42.07±7.26^{b}	23.8±17.56^{a}	32.27±3.51^{ab}	46.38±2.95^{b}	24.23±5.94^{a}
Hexamethyl-cyclotrisiloxane	200.18±4.78^{d}	160.28±22^{cd}	114.35±35.58^{ab}	131.46±1.84^{bc}	75.63±28.15^{a}
Trichloromethane	491.08±53.3^{c}	176.56±18.69^{ab}	227.64±24.54^{b}	165.76±102.26^{ab}	105.61±39.97^{a}
Pentyl-oxirane	5.49±0.49^{a}	11.71±0.94^{b}	14.19±0.57^{c}	15.95±0.42^{c}	14.79±1.67^{c}
Decamethyl-cyclopentasiloxane	321.3±39.32^{c}	247.77±18.57^{b}	230.5±12.52^{b}	224.23±10.98^{b}	150.93±13.2^{a}
Tetradecamethyl-cycloheptasiloxane	113.76±32.15^{b}	96.32±9.10^{ab}	81.2±13.13^{ab}	81.24±14.12^{ab}	63.33±6.60^{a}
1-Iodo-2-methylnonane	38.76±33.72^{a}	10.71±9.46^{a}	22.48±19.49^{a}	16.81±14.98^{a}	30.18±1.73^{a}
Dodecamethyl-cyclohexasiloxane	22.6±2.06^{c}	24.61±4.18^{c}	16.86±0.71^{b}	15.39±1.73^{b}	10.56±0.45^{a}

Table S4. Changes in volatile substances in chicken broth of different treatment groups (ng/100 mL) (continued)

Name	Stewing times (h)				
	1.0	1.5	2.0	2.5	3.0
Dodecamethyl-cyclohexasiloxane	215.84±45.53^{c}	173.74±15.54^{bc}	154.35±16.03^{b}	145.29±13.70^{ab}	105.74±17.37^{a}
Decamethyl-tetrasiloxane	10.51±0.77^{c}	8.36±2.05^{bc}	6.82±1.01^{b}	6.4±1.12^{b}	3.16±0.86^{a}
Total	1,485.33±82.78^{c}	947.41±91.25^{b}	909.62±93.98^{b}	855.16±130.95^{b}	588.26±57.51^{b}

$^{a–c}$ Different letters in the same row indicate significant differences ($p<0.05$).
ND, not detected.