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Article

The Overall Quality Changes of Chinese Sauced Ducks at Different Stages during Processing and Storage

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Abstract: This study revealed the physicochemical, microbial, flavor and sensory changes in sauced duck from the marinating phase to the end of storage, divided into six stages (stages A-F). The changes in color, total plate count, total volatile basic nitrogen, and thiobarbituric acid reactive substance at different stages were clarified. We utilized 16S rRNA gene sequencing, GC-IMS, and GC-MS to explore the changes in bacterial flora, fatty acid composition, and flavor characters. The dominant bacteria identified in stages A-C included *Psychrobacter*, *Flavobacterium*, and *Pseudomonas*, while *Lactobacillus* and *Staphylococcus* dominated during stages D-F. Aldehydes, esters, alcohols, and ketones emerged as the main flavor compounds. Several unsaturated fatty acids significantly ($P < 0.05$) decreased from stage A to stage F. The sensory quality of sauced duck improved. The potential reactions and correlation analysis of sauced duck samples across different stages were performed. 3-Methy-1-butanol could be a crucial indicator of sauced duck's overall quality. This research could support reference for the treatment optimization of sauced duck products.

Keywords: sauced duck; flavor change; microbial change; physic-chemical change; sensory change; flavor development

1. Introduction

China has the highest consumption of duck meat globally, accounting for over 70% of global production (Li et al., 2022; Lyu et al., 2021). In Zhejiang Province, locals typically select Shaoxing pockmark duck (*Anas platyrhynchos* var. *domestica*) as raw material for the sauced duck. Chinese consumers widely favor this traditional sauced duck product for its delicious taste, unique flavor, and desirable textural properties. Duck meat is rich in high-quality protein and poly-unsaturated fatty acids (PUFA), including C18: 2 and C18: 3 (Arshad et al., 2020). The processing methods of sauced duck vary across different regions in China. Therefore, sauced duck's flavor compounds and sensory qualities may differ due to different processing methods (Chang et al., 2023; Zhu et al., 2023). In Zhejiang Province, the cooking and drying treatments for sauced duck are performed after marinating, which brings an intense aroma and delicious taste and is highly favored by consumers

(Fan et al., 2024). Additionally, the natural air drying and sun drying during storage could strengthen the aroma and taste of sauced duck.

However, previous studies of duck meat have yet to deal with the flavor and sensory development during processing and storage. The processing properties of Shaoxing duck need to be further explored. Sauced duck is highly vulnerable to bacterial growth and spoilage due to its high moisture content (Liu et al., 2019). Also, the flavor development might be highly related to bacterial flora, especially during storage. It still need to discover the change rule of sauced duck during processing and storage, especially from the perspective of marinating to storage.

Therefore, this work aimed to investigate the physicochemical, microbial, chemical, and flavor changes in sauced ducks from the marinating treatment to the end of storage. The change in flavor compounds was clarified, and relevant potential correlations were further analyzed. This work could provide a basis for optimizing the processing and storage of sauced duck and improving its overall quality.

2. Materials and Methods

2.1. Chemicals and Reagents

The aqueous solutions were prepared using ultrapure water. All chromatographic grade reagents were purchased from Sigma-Aldrich Chemical Co., Ltd. Other analytical grade chemicals and reagents were all purchased from Aladdin Co., Ltd. All spice food additives were food-grade.

2.2. Sauced Duck Preparation

Lanhai Ecological Agriculture (Hangzhou, Zhejiang Province, China) Co., Ltd. provided fresh duck carcasses and the main production methods of sauced duck. Three-year-old laying Shaoxing pockmark ducks were slaughtered to make sauced ducks. The primary production process included marinating, cooking, drying, and preserving. Marinating ingredients contain more than ten spices (cinnamon, star anise, allspice, pepper, etc.). The carcasses were marinated in the mixture for 72 h and were flipped sides every 24 h. The packet was boiled with soy sauce and yellow wine for 10 min during the cooking process. The drying treatment was conducted within a 50 - 60 °C temperature range for 15 h. All dried samples were stored at room temperature and exposed to the air circulation and nature lights on sunny days, based on the chief engineer's experience.

2.3. Experimental Group Design

12 parallel samples were collected from 72 Shaoxing pockmark duck samples at six stages. All main body parts (including duck leg, breast, and wing) were tested for all indexes and the mean values were calculated in this study. The processing and storage treatment of all parallel samples was the same. A-F represents each of the six sampling stages in sequential order. Concretely, stage A stands for after marinating and before cooking; B stands for after cooking and before drying; C stands for samples collected after drying and before storage; D, E, and F stand for stored for 5, 10, and 15 d, respectively.

2.4. Determination of Edible Indexes

The color, total plate count (TPC), total volatile basic nitrogen (T-VBN), and thiobarbituric acid reactive substance (TBARS) values are essential indexes of evaluating whether the sauced duck is edible.

Color: We measured the surface color values of the duck sample with a Minolta colorimeter (CR-10 Plus, Minolta, Japan).

TPC: The determination of TPC was also strictly performed according to our previous study (Liu et al., 2019). Briefly, 25 g of sample was placed into 225 ml of sterile 0.85% sodium chloride (NaCl) solution and homogenized in a Bag-Mixer (Inter-science Ltd., France) for 2 min. Samples were plated

on agar plates (Land Bridge Co., Ltd., China) and incubated at 37°C for 48 h. The TPC was expressed as $\log_{10}$ CFU/g meat.

T-VBN: T-VBN was determined according to Liu et al. (2019). Briefly, 20 g of sample was homogenized in 2% trichloroacetic acid at 5000 rpm for 1 min. The mixture was filtered and added with 1% magnesium oxide (MgO) solution. The liberated T-VBN was absorbed utilizing a 2% boric acid solution, and titrated with a 0.01 N hydrochloric acid (HCl) solution. The result was expressed as mg/100 g of sample.

TBARS: We analyzed the value of TBARS with malondialdehyde (MDA) assay kit (Jiancheng Ltd., China) following the manufacturer's instruction and the result was expressed as mg of MDA per kg.

2.5. Fatty Acid Analysis

The fatty acid (FA) composition was determined according to the method of Duan et al. (2023). The minced sample was added with chloroform-methanol solution (1: 2: 0.8, v/v/v) and extracted for the fat content using saturated salt water. After saponifying fat content, we removed water using Na_2SO_4 and dissolved it with hexane. The 7890B-7000C gas chromatography-mass spectrometry (GC-MS) system (Agilent, CA, USA) equipped with a capillary column (CD-2560, 100 m $\times$ 250 μm $\times$ 0.20 μm ; CNW, Germany) was applied. The FA were identified using the NIST 14 database. In this study, FA were classified as saturated fatty acid (SFA), mono-unsaturated fatty acid (MUFA), PUFA, and cholesterol.

2.6. Sensory Evaluation

The method was referenced from the Xu et al. (2023). The sensory evaluation was conducted by ten professionally-trained panelists, aged 20 – 30, including five males and five females. We signed an agreement with all participants to use and study their information. In line with the agreement, all participants' privacy rights are committed being protected fully. All duck meat samples were water rinsed, cut into even-sized pieces, and placed into clean glass jars. In order to simulate the general consumption mode of sauced duck, these samples were steamed in boiling water until the core temperature exceeded 75 °C. All cooked samples were provided as blind samples. Panelists could not communicate with each other during the sensory evaluation. The concrete criteria are shown in Table S1. Before each tasting, all panelists should rinse their mouths thoroughly to ensure no residues interfere.

2.7. Microbial Community Analysis

The 16S rRNA gene sequencing was performed based on the previous studies (Li et al., 2022; Ma et al., 2024). Sequencing libraries were generated using Next® Ultra™ DNA Library Prep Kit for Illumina (New England Biolabs, USA). The library quality was assessed using Qubit 2.0 Fluorometer (Thermo Fisher Co., USA) and Bioanalyzer 2100 system (Agilent Co., USA). The library was sequenced on Mi-Seq platform (Illumina Co., USA). Paired-end reads from the original DNA fragments were merged using FLASH (Johns Hopkins University, USA). Sequences analysis was performed by UPARSE (Independent Investigator, California, USA), according to Edgar. (2013).

Sequences with more than 97% similarity were assigned to the same OTUs. The relative abundances were visualized using the Krona chart referred to Ondov et al. (2011). The heat map was drawn with software R 4.2.2. Effect Size (LEfSe) analysis was used for identifying biomarkers within different groups (Figure 1). To identify differences in microbial species, ANOSIM and MRPP were performed based on the Bray-Curtis dissimilarity distance matrices. Species with an LDA score of more than four were identified as biomarkers. Rarefaction curves were found based on these metrics (Figure S1). We calculated three metrics: Chao1, Simpson, and the Shannon index (Figure S2). Cluster analysis was preceded by principal component analysis (PCA) in Figure S1 using the QIIME (version

1.8.0, Knight Lab, University of California, USA). We used unweighted unifrac distance for PCoA and UPGMA clustering (Figure S2).

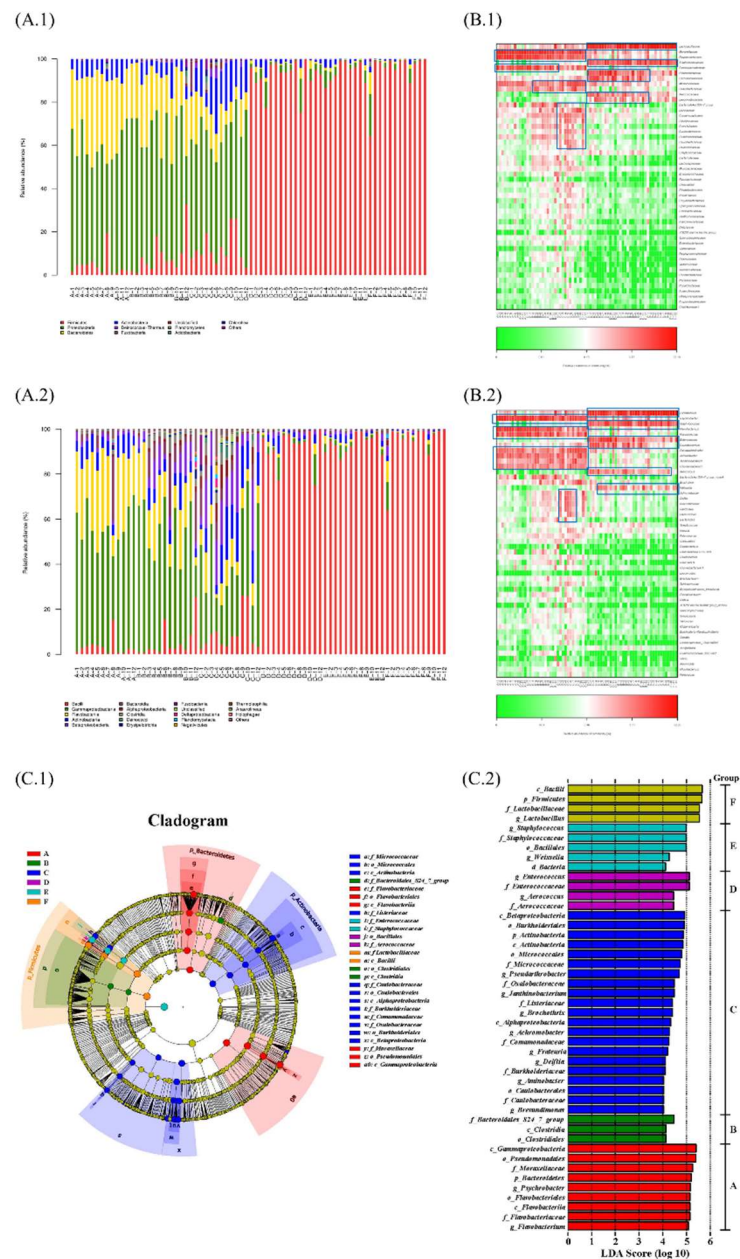


Figure 1. Bacterial flora of samples at different stages; Bar-plot at Family level (A.1) and at Genus level (A.2); Heat map at Family level (B.1) and at Genus level (B.2); Cladogram (C.1) and LDA analysis diagram (C.2) of bacterial flora at different stages. Note: Different letter (A-F) stands for different stages. Different number (1-12) after letters stands for different repetitions in each stage.

2.8. Gas Chromatography-Ion Mobility Spectrometry Analysis

The volatile flavor compounds were determined by a Flavor spec® gas chromatography-ion mobility spectrometry (GC-IMS) system (G.A.S, Germany). Samples were incubated at 60 °C for 20 min, and then 500 µL of gas was injected into the injector. After being ionized, the analyte was added to the ionization chamber. The main parameters set as 500 V/cm of linear voltage, 45 °C of drift tube temperature, and 75 ml/min of drift gas flow rate.

We analyzed measurement data in IMS using the VOCal software 0.4.03. The retention index (RI) was calculated according to the retention time and ion migration time of volatile substances in

GC. The substances were clarified by matching the GC-IMS database and NIST 2020. The description of flavor compounds was identified according to the Flavor Ingredient Library (<https://www.femaflavor.org/flavor-library>) and the LRI& odour database (<http://www.odour.org.uk>).

2.9. Statistical Analysis

The present study employed ANOVA analysis of variance and an independent t-test to assess differences in the groups, utilizing Duncan’s test from SPSS Statistics 26.0 (IBM Co., USA). The significance level for this study was established at $P < 0.05$. The Pearson correlation analysis was calculated by SAS 9.1.3 Software (SAS Institute Inc., USA). The heat maps of Pearson Correlation Analysis were generated by Graphpad Prism Software 8.3.0.

3. Results and Discussion

3.1. Chemical and Physical Changes of Samples Across Different Stages Were Analyzed

The color, TPC, T-VBN, and TBARS results are shown in Table 1. Color is a crucial sensory indicator for sauced duck and affects the human appetite. In this study, the marinating and drying treatment significantly ($P < 0.05$) influenced the L^* values. The insignificant correlations of a^* values might be due to the marinating treatment. Duck meat products are ideal mediums for microbial growth (Geeraerts et al., 2017; Liu et al., 2019; Mann et al., 2015). The unpacked samples with high water activity were vulnerable to microbial growth and contamination. Cooking and drying treatment could inhibit microbial growth by regulating temperature and humidity. The TVBN and TBARS significantly ($P < 0.05$) increased at stages D-F (Table 1). T-VBN, which is highly correlated with their off-odor, is as a critical indicator of spoilage for meat samples (Bassey et al., 2018; Senapati & Sahu, 2020). The high T-VBN and TBARS values at stage F revealed the limited shelf-life.

Table 1. Color changes of samples at different stages.

Index	Stage					
	A	B	C	D	E	F
L^*	32.1 ± 0.3^a	48.2 ± 0.8^f	46.5 ± 0.3^e	44.3 ± 0.2^d	42.5 ± 0.1^c	41.3 ± 0.1^b
a^*	8.2 ± 0.9^a	10.5 ± 0.3^b	11.2 ± 0.6^c	11.5 ± 0.1^c	12.6 ± 0.5^d	12.1 ± 0.3^d
b^*	5.8 ± 0.6^a	6.0 ± 1.2^b	7.0 ± 0.4^c	7.8 ± 0.6^d	8.1 ± 0.3^d	8.2 ± 0.3^d
T-VBN (mg/ 100 g)	5.92 ± 0.42^a	6.85 ± 0.25^b	7.12 ± 0.92^b	20.25 ± 0.89^c	24.58 ± 0.43^d	48.20 ± 1.06^e
TBARS (mg/ kg)	0.16 ± 0.00^a	0.27 ± 0.03^b	0.58 ± 0.05^c	0.96 ± 0.08^d	1.37 ± 0.02^e	2.13 ± 0.15^f
TPC (log ₁₀ CFU /g)	1.82 ± 0.02^a	2.24 ± 0.06^b	2.20 ± 0.05^b	6.85 ± 0.01^c	7.82 ± 0.12^d	8.45 ± 0.08^e

Note: The same letter means the difference is not significant, while different letter means the difference is significant ($P < 0.05$).

Xia et al. (2021) reported the relative contents of C16:0, C18:0 and SFA were 50.11 - 58.42%, 33.86 - 38.03%, and 85.59 - 92.88% in sauced duck samples, which are similar to our findings (Figure 2A). The relative content of C18: 2 (n-6) significantly ($P < 0.05$) decreased ($20.56 \pm 0.46\%$ to $13.64 \pm 2.13\%$); C18: 1 (n-9) presented insignificant change. Moreover, the relative contents of C18: 3 (n-6), C20: 4 (n-6), and C22: 6 (n-6) also significantly decreased ($P < 0.05$) from stage A to F. All referred UFA accounted for an even lower relative content at stage F. The oxidation and lipolysis of FA, especially for C18: 2 (n-6) and C18: 3 (n-6), produced alcohols, aldehydes, and ketones, which were responsible for abundant aroma as well as “rancid” off-flavors after storage (Liu et al., 2022; Zhou et al., 2022). The change of FA composition was consistent with the increase in TBARS values. Previous studies presented similar results (Shi et al., 2019; Xia et al., 2021; Zhou et al., 2022).

In our research, sensory factors included taste, tenderness, juiciness, aroma, and appearance (Figure 2B). The previous studies mainly focused on analyzing the sensory change in sauced duck during storage. The change rule of sensory quality during marinating, cooking, and drying was revealed in this study. Cooking and drying could significantly improve the score of taste, aroma, and tenderness. The high salt contents in marinating treatment and water losses could give duck meat a firm texture (Khan et al., 2014; Zhou et al., 2022). Sauced duck could have a more abundant aroma under the metabolism of microbes and enzymes after proper storage (Zhu et al., 2024). However, excessive storage for sauced duck could increase the values of T-VBN, TBARS, ammonia, and PUFA metabolites, negatively affecting aroma and taste. The total scores could reach a relatively high level after 15 d storage.

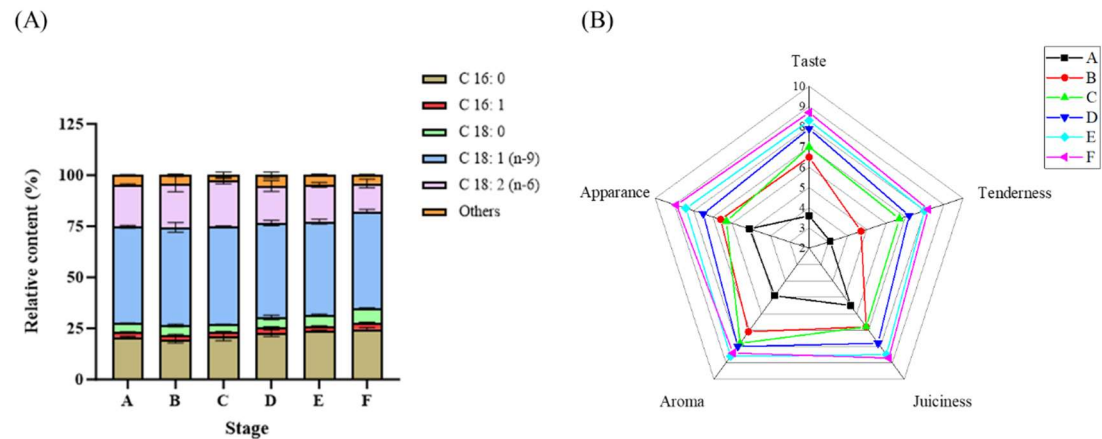


Figure 2. Free fatty acids analysis (A) and sensory evaluation (B).

3.2. The Microbial Biomarkers of Samples Were Identified at Different Stages

Rarefaction curves in Figure S1 indicate the reliability of sequencing results. Figure 1A demonstrates the relative proportion of bacteria at different stages. At the Family level, *Moraxellaceae*, *Flavobacteriaceae*, and *Pseudomonadaceae* dominated during the stages A-C, which is consistent with the previous studies (Bassey et al., 2018; Li et al., 2022). At the Genus level, *Psychrobacter*, *Flavobacterium*, and *Pseudomonas* were the dominant genera during stages A-C. In the heat map (Figure 1B), more red spots observed during stages A-C indicate the higher bacterial abundance; *Lactobacillus*, *Staphylococcus*, and *Enterococcus* dominated stages D-F. As demonstrated in Figure 1C, 46 biomarkers were found, and most (n = 21) were detected at stage C. The α -diversity showed the most complex bacterial floras at stage B (Figure S2).

Two groups were distinguished in the cluster tree: one comprised mainly samples of stages A-C groups, and the other was almost found during stages D-F. Also, the potential pathogenic biomarkers at stages C (*f_Listeriaceae*), D (*f_Enterococcus* and *g_Enterococcus*), and E (*f_Staphylococcus* and *g_Staphylococcus*) were worthy of our vigilance. The sequencing results are consistent with previous studies (Geeraerts et al., 2017). The duck's initial microbial load, which is related to poultry health, temporal development, and rearing conditions, could be the essential source of these pathogenic bacteria (Geeraerts et al., 2019; Ma et al., 2024). Also, the variations could be caused by several treatments, such as the slaughtering, slicing, and marinating process or during storage (Liu et al., 2019). The optimization of poultry health management and the development of the hygiene level during processing and storage are essential for sauced duck production. However, disinfection and microbial change might influence the flavor development of sauced duck.

3.3. The Quantitative and Qualitative Analysis of Flavor Compounds of Samples at Different Were Performed

Aldehydes, esters, alcohols, and ketones contributed significantly to the sauced duck’s flavor (Duan et al., 2023). Rasinska et al. (2019) also reported that aldehydes, such as hexanal, octanal, heptanal, and benzaldehyde, are the most crucial compounds of sauced duck. In this study, 81 flavor compounds were clarified, including 19 aldehydes, 19 esters, 13 alcohols, 11 ketones, and four acids (Table 2 and Figure 3). Xia et al. (2021) reported 105 volatile flavor compounds, including 23 esters, 14 aldehydes, 14 ketones, and 14 alcohols, from the sauced duck samples. Ethanol was the flavor compound with the highest relative contents from stage A to F. The addition of yellow wine brought meat a mellow aroma and attractive flavor. Hexanal, ethyl acetate, acetone, acetic acid, and propanal were the main flavor compounds, constituting the characteristic flavor of sauced duck. The minimum values of acetone and hexanal as well as the maximum value of ethyl acetate were found at stage E.

Table 2. The main flavor substances in the GC-IMS results.

No.	Compound	CAS#	Formula	MW	RI	Rt (s)	Dt (RIP rel)	Comment	Description
Aldehydes (n = 19)									
1	Benzaldehyde	C100527	C ₇ H ₆ O	106.1	1553.6	1406.451	1.157		Bitter Almond, Brunt sugar, Cherry, Malt, Roasted Pepper
2	Nonanal	C124196	C ₉ H ₁₈ O	142.2	1406.4	1023.341	1.48016		Fat, Floral, Green, Lemon
3	(E)-2-Heptenal	C18829555	C ₇ H ₁₂ O	112.2	1334.5	876.019	1.26147	Monomer	Almond, Fat, Fruit
					1334.9	876.877	1.67092	Dimer	
4	(E)-2-Hexenal	C6728263	C ₆ H ₁₀ O	98.1	1234.5	711.029	1.18832	Monomer	\
					1235.1	712.002	1.51718	Dimer	
5	Heptanal	C111717	C ₇ H ₁₄ O	114.2	1199.8	662.503	1.33641	Monomer	Citrus, Fat, Green, Nut
					1199.8	662.503	1.69504	Dimer	
6	(E)-2-Pentenal	C1576870	C ₅ H ₈ O	84.1	1152.5	569.553	1.10533	Monomer	\
					1152.7	570.014	1.3599	Dimer	
7	Hexanal	C66251	C ₆ H ₁₂ O	100.2	1103.8	482.051	1.27572	Monomer	Apple, Fat, Fresh, Green, Oil
					1104.2	482.69	1.56344	Dimer	
8	Pentanal	C110623	C ₅ H ₁₀ O	86.1	1004.9	361.067	1.42311		Almond, Bitter, Malt, Oil, Pungent
	3-Methylbutanal	C590863	C ₅ H ₁₀ O	86.1	931.3	305.536	1.4039		\
10	Acetal	C105577	C ₆ H ₁₄ O ₂	118.2	910.8	292.146	1.02663		Creamy, Fruit, Pleasant, Tropical Fruit
11	Butanal	C123728	C ₄ H ₈ O	72.1	889.9	279.149	1.11717		Banana, Green, Pungent
	2-Methylpropanal	C78842	C ₄ H ₈ O	72.1	830.7	245.279	1.2818		Burnt, Caramel, Cocoa, Green, Malt

13	Propanal	C123386	C ₃ H ₆ O	58.1	821.0	240.159	1.14324	Floral, Pungent, Solvent
14	Acetaldehyde	C75070	C ₂ H ₄ O	44.1	763.5	211.803	0.97998	Floral, Green Apple
15	2-Propenal	C107028	C ₃ H ₄ O	56.1	868.1	266.153	1.05955	\
16	Methionol	C3268493	C ₄ H ₈ OS	104.2	1477.2	1192.51 2	1.0955	Cooked potato, Soy
17	3-Methyl-2-butenal	C107868	C ₅ H ₈ O	84.1	1216.3	685.268	1.09732	Almond, Roasted
18	2-Methyl-pentenal	C623369	C ₆ H ₁₀ O	98.1	1200.1	663.004	1.5201	Fruit
19	Octanal	C124130	C ₈ H ₁₆ O	128.2	1304.0	820.167	1.40635	Citrus, Fat, Green, Oil, Pungent
Esters (n = 19)								
1	Methyl octanoate	C111115	C ₉ H ₁₈ O 2	158.2	1449.1	1122.05 2	1.42791	Fruit, Orange, Wax, Wine
2	(E)-2-Octenal	C2548870	C ₈ H ₁₄ O	126.2	1441.5	1103.86 8	1.33835	Dandelion, Fat, Fruit, Grass, Green, Spice
3	Hexyl propionate	C2445763	C ₉ H ₁₈ O 2	158.2	1343.7	893.679	1.41894	Fruit
4	1-Penten-3-ol	C616251	C ₅ H ₁₀ O	86.1	1178.8	623.205	0.94299	Butter, Fish, Green, Oxidized, Wet Earth
5	Methyl 2-methylbutanoate	C868575	C ₆ H ₁₂ O 2	116.2	1016.1	372.882	1.18714	Apple, Fruit, Green Apple, Strawberry
6	Ethyl acetate	C141786	C ₄ H ₈ O ₂	88.1	898.2	284.269	1.33393	Aromatic, Brandy, Grape
7	Methyl acetate	C79209	C ₃ H ₆ O ₂	74.1	852.9	257.488	1.19263	Ester, Green
8	Ethyl propanoate	C105373	C ₅ H ₁₀ O 2	102.1	976.8	337.437	1.45329	Apple, Pineapple, Rum, Strawberry
9	Ethyl isobutyrate	C97621	C ₆ H ₁₂ O 2	116.2	984.2	342.951	1.55756	\
10	Propyl acetate	C109604	C ₅ H ₁₀ O 2	102.1	997.3	353.19	1.47936	Celery, Floral, Pear, Red Fruit
11	Ethyl 3-methylbutanoate	C108645	C ₇ H ₁₄ O 2	130.2	1084.0	453.619	1.27631	Apple, Fruit, Pineapple, Sour
12	Ethyl 2-methylbutanoate	C7452791	C ₇ H ₁₄ O 2	130.2	1071.1	437.077	1.25299	Apple, Ester, Green Apple, Kiwi, Strawberry
13	Isoamyl acetate	C123922	C ₇ H ₁₄ O 2	130.2	1139.7	545.17	1.30207	Apple, Banana, Pear

Monomer

					1139.7	545.17	1.74786	Dimer	
14	Ethyl butanoate	C105544	$C_6H_{12}O_2$	116.2	1056.6	419.151	1.55878		Apple, Butter, Cheese, Pineapple, Strawberry
15	Butyl acetate	C123864	$C_6H_{12}O_2$	116.2	1091.1	462.925	1.2589	Monomer	Apple, Banana
16	1-Methoxy-2-propyl acetate	C108656	$C_6H_{12}O_3$	132.2	1242.7	723.096	1.14241		\
17	Pentyl acetate	C628637	$C_7H_{14}O_2$	130.2	1187.5	642.203	1.31303		Apple, Banana, Pear
18	Ethyl hexanoate	C123660	$C_8H_{16}O_2$	144.2	1248.3	731.416	1.34219		Apple Peel, Brandy, Fruit Gum, Overripe Fruit, Pineapple
19	Ethyl pentanoate	C539822	$C_7H_{14}O_2$	130.2	1180.5	626.931	1.68337		Apple, Dry Fish, Herb, Nut, Yeast
Alcohols (n = 13)									
1	1-Octen-3-ol	C3391864	$C_8H_{16}O$	128.2	1488.6	1222.062	1.16669		Cucumber, Earth, Fat, Floral, Mushroom
2	1-Hexanol	C111273	$C_6H_{14}O$	102.2	1373.6	953.204	1.33238	Monomer	Banana, Flower, Grass, Herb
					1373.6	953.204	1.63689	Dimer	
3	1-Heptanol	C111706	$C_7H_{16}O$	116.2	1489.1	1223.36	1.40403		\
4	Acetoin	C513860	$C_4H_8O_2$	88.1	1302.7	817.787	1.06521	Monomer	Butter, Creamy, Green Pepper
					1303.2	818.758	1.33106	Dimer	
5	1-Pentanol	C71410	$C_5H_{12}O$	88.1	1267.5	760.526	1.25791	Monomer	Balsamic, Fruit, Green, Pungent, Yeast
					1268.1	761.497	1.51126	Dimer	
6	3-Methyl-1-butanol	C123513	$C_5H_{12}O$	88.1	1223.6	695.501	1.24185	Monomer	Burnt, Cocoa, Floral, Malt
					1224.3	696.471	1.49164	Dimer	
7	1-Butanol	C71363	$C_4H_{10}O$	74.1	1163.7	591.908	1.18249	Monomer	Fruit
					1163.7	591.908	1.37859	Dimer	
8	2-Methyl-1-propanol	C78831	$C_4H_{10}O$	74.1	1113.0	497.38	1.17445	Monomer	Apple, Bitter, Cocoa, Wine
					1113.3	498.019	1.37055	Dimer	
9	1-Propanol	C71238	C_3H_8O	60.1	1058.7	421.718	1.11306	Monomer	Alcohol, Candy, Pungent
					1058.7	421.718	1.25436	Dimer	
10	2-Butanol	C78922	$C_4H_{10}O$	74.1	1042.2	402.026	1.14873	Monomer	\

					1042.5	402.42	1.32433	Dimer	
11	Ethanol	C64175	C ₂ H ₆ O	46.1	944.7	314.594	1.04172	Monomer	\
					945.8	315.382	1.12678	Dimer	
12	2-Propanol	C67630	C ₃ H ₈ O	60.1	934.8	307.899	1.09111		Floral
	cis-2-								
13	Penten-1-ol	C1576950	C ₅ H ₁₀ O	86.1	1341.3	889.042	0.94555		\
Ketones (n = 11)									
	1-Hydroxy								
1	-2-propanone	C116096	C ₃ H ₆ O ₂	74.1	1317.8	844.962	1.05629	Monomer	Butter, Herb, Malt, Pungent
					1317.8	844.962	1.05629	Dimer	
2	2-Heptanone	C110430	C ₇ H ₁₄ O	114.2	1195.4	656.68	1.26504		Blue Cheese, Fruit, Green, Nut, Spice
	4-								
3	Methyl-3-penten-2-one	C141797	C ₆ H ₁₀ O	98.1	1147.5	559.973	1.12301		\
4	1-Penten-3-one	C1629589	C ₅ H ₈ O	84.1	1034.3	392.968	1.09385		Fish, Green, Mustard, Pungent
	4-								
5	Methyl-pentanone	C108101	C ₆ H ₁₂ O	100.2	1029.4	387.454	1.48622		\
	2-								
6	Pentanone	C107879	C ₅ H ₁₀ O	86.1	1003.4	359.492	1.35177		Fruit, Pungent
	2-								
7	Butanone	C78933	C ₄ H ₈ O	72.1	918.7	297.266	1.24339		Fragrant, Fruit, Pleasant
8	Acetone	C67641	C ₃ H ₆ O	58.1	838.7	249.612	1.11443		Pungent
	3-								
	Methyl-								
9	2-pentanone	C565617	C ₆ H ₁₂ O	100.2	1040.2	399.732	1.47772		\
	e								
10	Cyclopentanone	C120923	C ₅ H ₈ O	84.1	1202.5	666.24	1.1045		Mint, Cool
	Dihydro-								
11	2(3H)-furanone	C96480	C ₄ H ₆ O ₂	86.1	1711.3	1977.29	1.08782		Caramel, Cheese, Roasted Nut
Acids (n = 4)									
1	Propanoic acid	C79094	C ₃ H ₆ O ₂	74.1	1638.6	1689.857	1.11514		Fat, Fruit, Pungent, Silage, Soy

2	Butanoic acid	C107926	C ₄ H ₈ O ₂	88.1	1705.5	1952.665	1.16147		Butter, Cheese, Sour
3	Acetic acid	C64197	C ₂ H ₄ O ₂	60.1	1505.5	1267.521	1.05921	Monomer	Acid, Fruit, Pungent, Sour, Vinegar
					1505.9	1268.819	1.15624	Dimer	
4	2-Methylpropanoic acid	C79312	C ₄ H ₈ O ₂	88.1	1635.5	1678.655	1.15151		Burnt, Butter, Cheese, Sweat
Others (n = 18)									
1	2,6-Dimethylpyrazine	C108509	C ₆ H ₈ N ₂	108.1	1362.7	931.124	1.14579		Cocoa, Coffee, Green, Roast Beef, Roasted Nut
2	Ammonia	C7664417	H ₃ N	17.0	1273.1	769.261	0.84576	Dimer	
					1273.7	770.231	0.90642	Monomer	\
3	Methylpyrazine	C109080	C ₅ H ₆ N ₂	94.1	1279.9	779.937	1.09376		Cocoa, Green, Hazelnut, Popcorn, Roasted
4	2-Pentylfuran	C3777693	C ₉ H ₁₄ O	138.2	1247.0	729.469	1.2472		Butter, Floral, Fruit, Green Bean
5	o-Xylene	C95476	C ₈ H ₁₀	106.2	1194.0	654.739	1.08127		\
6	3-Carene	C13466789	C ₁₀ H ₁₆	136.2	1149.5	563.805	1.20821		Lemon
7	Dimethyl disulfide	C624920	C ₂ H ₆ S ₂	94.2	1072.1	438.259	1.13775		Cabbage, Garlic, Onion
8	alpha-Pinene	C80568	C ₁₀ H ₁₆	136.2	1029.4	387.454	1.23104		Cedarwood, Pine, Sharp
9	Dimethyl sulfide	C75183	C ₂ H ₆ S	62.1	795.5	227.163	0.95529		Cabbage, Organic, Sulfur, Wet Earth
10	Ethylbenzene	C100414	C ₈ H ₁₀	106.2	1135.4	537.092	1.09147		\
11	Unidentified 1	\	\	0	1119.6	508.818	1.09608		\
12	Unidentified 2	\	\	0	1148.5	561.814	1.32483		\
13	Furfural	C98011	C ₅ H ₄ O ₂	96.1	1500.0	1252.624	1.09377		Almond, Baked Potatoes, Bread, Burnt, Spice
14	Dipropyl disulfide	C629196	C ₆ H ₁₄ S ₂	150.3	1421.2	1056.408	1.26895		Cooked Meat, Garlic, Onion, Pungent, Sulfur
15	3-Ethylpyridine	C536787	C ₇ H ₉ N	107.2	1388.7	984.954	1.09723		Nuts
16	2,5-Dimethylpyrazine	C123320	C ₆ H ₈ N ₂	108.1	1354.5	914.793	1.10361		Cocoa, Roast Beef, Roasted Nut
17	Unidentified 3	\	\	0	1207.3	672.711	1.15699		

Various flavor markers at different stages of samples were exhibited (Figure 3A). There were plenty of flavor markers at stages A-C and D-F. Ethyl pentanoate, alpha-pinene, and 2-propanol were the flavor markers at stage A. Four flavor markers were screened at both stage B (furfural, 1-hexanol, isoamyl acetate, and methyl 2-methylbutyrate) and stage C (2-acetylfuran, 1-hydroxy-2-propanone, 3-carene, and ethyl hexanoate). Both lipid oxidation and Strecker degradation could produce aldehydes and alcohols, such as 1-octen-3-ol, 1-pentanol, hexanal, and (E)-2-hexenal (Zhou et al., 2022). The high hexanal content could present a rotten odor in sauced ducks (Shi et al., 2019). However, the sensory results reflected that the odor of sauced ducks at stage F was acceptable. The interaction of flavor compounds might influence the threshold of off-flavor. The esters, mainly ethyl acetate, were derived from the esterification reactions during the cooking treatment.

During stages D-F, seven, seven, and four markers were identified. At stage D, butyl acetate, propyl acetate, 1-methoxy-2-propyl acetate, cis-2-penten-1-ol, (E)-2-octenal, 2-propenal, 2-pentylfuran, were found the flavor markers. Sauced duck samples were rich in carbonyl compounds and AA compounds, which are the primary substrates of the Maillard reaction. Therefore, the contents of furans, including furfural, 2-acetylfuran, 2-pentylfuran, and dihydro-2(3H)-furanone significantly ($P < 0.05$) increased during stages C-D. At stage E, apparent increases were found in the contents of ammonia, nonanal, hexyl propionate, methyl acetate, pentyl acetate, 1-butanol, and 2-heptanone. At stage F, acetoin, propanal, 3-methyl-2-pentanone, 3-methylbutanal, butanal, and acetal increased. Figure 3C exhibited the decrease in new esters and the increase in original acids and alcohols, which might account for such PCA results. Our results differ slightly from previous findings because of different processing materials and methods (Chang et al., 2023; Zhu et al., 2023).

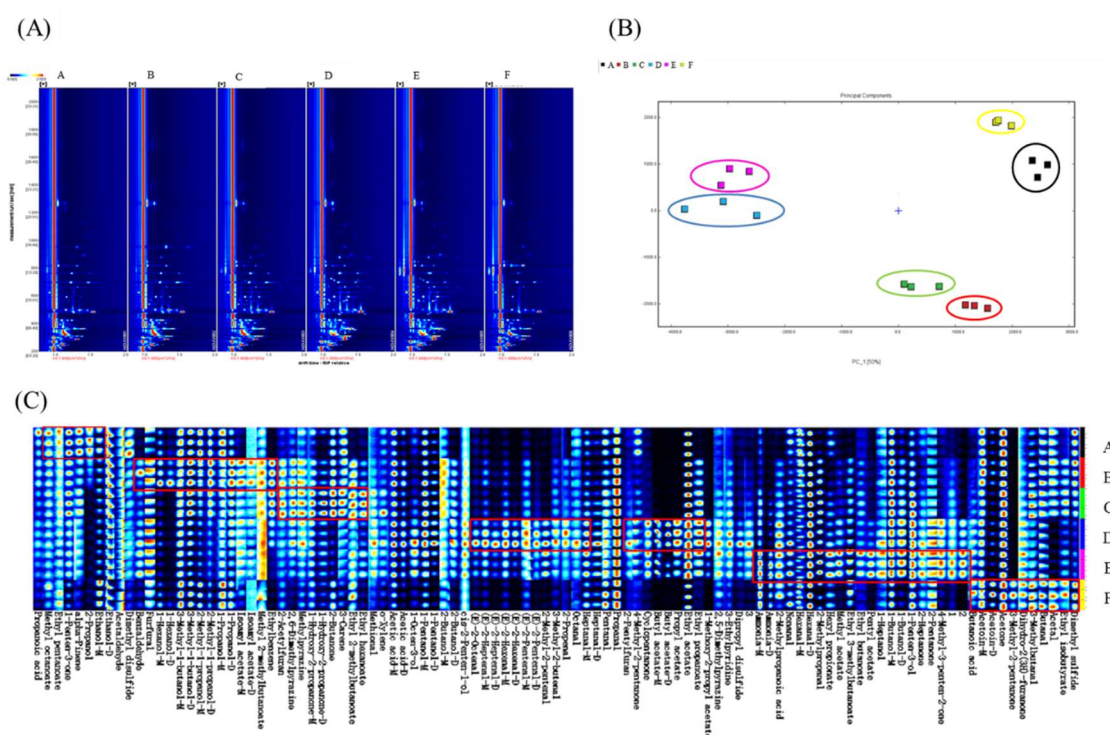


Figure 3. Two-dimensional topographic plot (A), fingerprint spectra (B) and PCA (C) in the GC-IMS results.

3.4. The Biomarker 3-Carene Were Clarified Through Correlation Analysis

The different thermal processing parameters of marinating, cooking, drying, and storage change accounted for the flavor development of sauced duck. The Pearson correlation analysis results were presented in Figure 5. All dominant bacteria showed significant correlation to the main flavor substances except for *Pseudarthrobacter* and *Enterococcus* (Figure 4A). The dominant bacteria of stages A-C exhibited a mainly positive correlation, which was different from the dominant bacteria of stages D-F. 3-carene showed significant correlations in six of the ten dominant bacteria.

In general, terpenes, such as 3-carene, were mostly derived from spices rather than the flavor metabolites during stages A-F (Álvarez et al., 2020). In this study, the change of C 18: 1 (n-9) presents a high correlation with the main flavor compounds in Figure 4B, which differed from previous studies. As discussed before, the change of C18: 1 (n-9) was insignificant during stages A-F. The different result could be attributed to the relatively stable properties of these flavor compounds as well as the addition of several spices and seasonings. Previous studies reported that C18: 2 (n-6) and C18: 3 (n-6) accounted for several flavor substances because of lipid oxidation and Strecker degradation (Shi et al., 2019; Zhou et al., 2022; Duan et al., 2023). The low relative contents of C18: 2 (n-6) and C18: 3 (n-6) could be an essential reason. The actual numerical relation and concrete molecular mechanism need further verification.

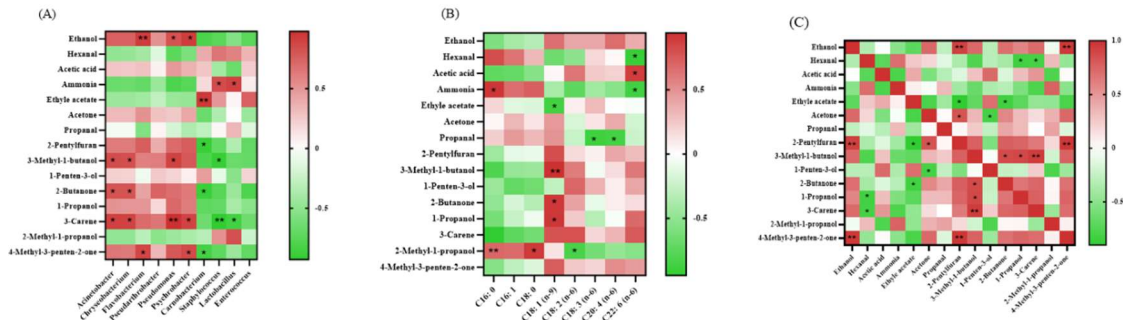


Figure 4. The correlation analysis between flavor compounds and dominant bacteria (A), free acids (B) or themselves (C). Note: * P < 0.05; ** P < 0.005. Red color stands for positive correlation; green color stands for negative correlation; white color stands for no correlation.

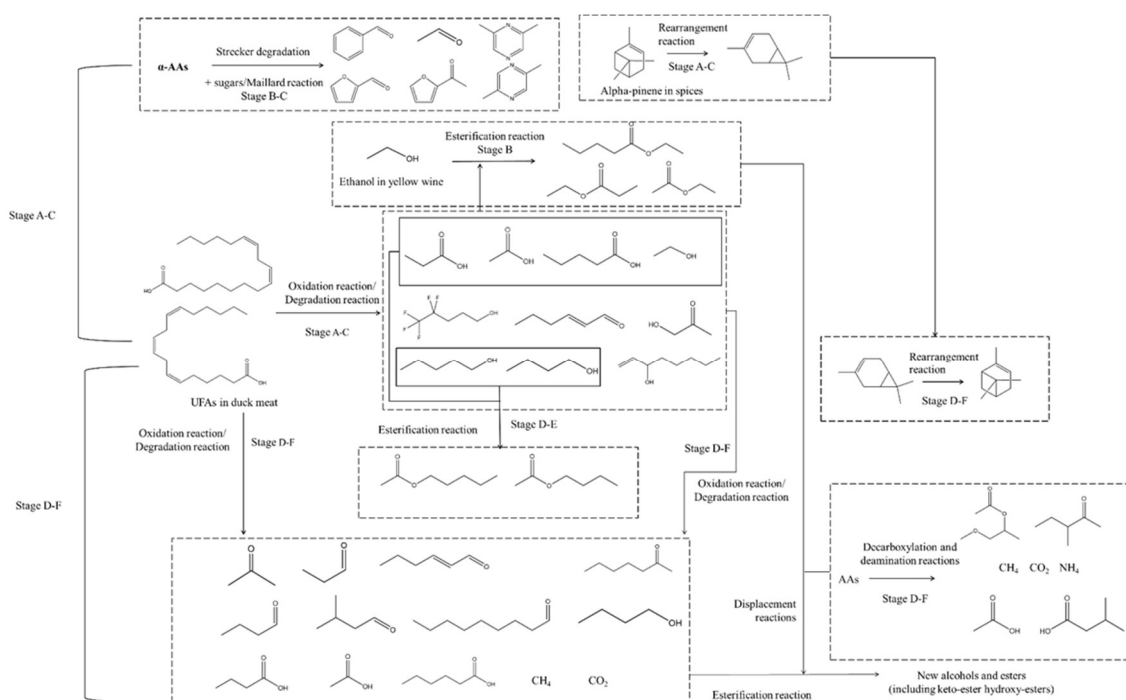


Figure 5. The potential chemical reactions of sauced duck samples during stages A-F (Shi et al., 2019; Yang et al., 2022; Zhou et al., 2022; Duan et al., 2023).

3.5. The Potential Chemical Reactions Were Predicted Based on the Previous Analysis

The correlations between main flavor compounds were presented in Figure 5C. 2-Pentylfuran and 3-methyl-1-butanol were significantly correlated ($P < 0.05$) to more than three compounds. They are regarded as the products of the Maillard reaction and Strecker degradation (Shi et al., 2019; Duan et al., 2023). As shown in Figure 4, 3-methyl-1-butanol presented a more significant correlation ($P < 0.05$ or 0.005) with FA and dominant bacteria compared to 2-pentylfuran. Based on the changes in FA and flavor composition results, the potential chemical reactions at different stages were depicted in Figure 5. The complex reactions, such as Maillard reaction, Strecker degradation, oxidation reaction, and esterification, were the essential reasons for the changes of FA and flavor. As the crucial compound in flavor development, 2-pentylfuran and 3-methyl-1-butanol have the “Butter, Floral” description. Moreover, the content of 3-methyl-1-butanol increased significantly from stage A to F, the same as the scores of sensory quality. 3-Methyl-1-butanol could be an essential indicator for the quality evaluation of sauced ducks.

4. Conclusions

The present study investigated the changes in the sauced duck across six different stages. The values of edible indices and FA composition exhibited significant change ($P < 0.05$). The dominant bacteria of stages A-C were *Psychrobacter*, *Flavobacterium*, and *Pseudomonas*. *Lactobacillus*, *Staphylococcus*, and *Enterococcus* dominated during the stages D-F. Furan compounds were the primary Maillard reaction products in sauced duck. 3-Methyl-1-butanol could serve as an indicator for the quality evaluation of sauced duck.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Methodology, Kaiyong Yao and Daxi Ren; Formal analysis, Jie Cai and Yingping Xiao; Investigation, Daodong Pan, Bindan Chen and Jinghui Fan; Resources, Jie Cai, Daodong Pan, Bindan Chen, Jinghui Fan and Yingping Xiao; Writing – original draft, Kaiyong Yao; Writing – review & editing, Daxi Ren; Supervision, Daxi Ren; Project administration, Kaiyong Yao; Funding acquisition, Yingping Xiao.

Data Availability Statement: The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Abbreviations

The following abbreviations are used in this manuscript:

AA	amino acid
ANOSIM	analysis of similarities
CTAB/SDS	cetyltrimethylammonium bromide/sodium docetyl sulfate-based
DT	drift time
FA	free fatty acid
GC-IMS	gas chromatography-ion mobility spectrometry
GC-MS	gas chromatography-mass spectrometry
HCl	hydrochloric acid
LDA	linear discriminant analysis
MDA	malondialdehyde
MgO	magnesium oxide
MRPP	multi-response permutation procedure
MUFA	mono-unsaturated fatty acid
MW	molecular weight
NaCl	sodium chloride
PCA	principal component analysis
PCoA	principal coordinate analysis
PUFA	poly-unsaturated fatty acid
RI	retention index
RIP rel	normalization treatment
RT	retention time
SFA	saturated fatty acid
Stage A	samples collected after marinating and before cooking
Stage B	samples collected after cooking and before drying
Stage C	samples collected after drying and before storage
Stage D	samples stored after 5 d
Stage E	samples stored after 10 d
Stage F	samples stored after 15 d
TBARS	thiobarbituric acid reactive substance
TPC	total plate count
T-VBN	total volatile basic nitrogen
UPGMA	unweighted pair group method with arithmetic mean
UFA	unsaturated fatty acid

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