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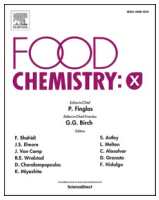

Effect of coating on flavor metabolism of fish under different storage temperatures

Xin Li [a,c], Zongcai Tu [a,b,*], Xiaomei Sha [a,*], Zhongying Li [a], Jinlin Li [a], Mingzheng Huang [d]

[a] National R&D Center for Freshwater Fish Processing, College of Life Science, Jiangxi Normal University, Nanchang, Jiangxi 330022, China
[b] State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, Jiangxi 330047, China
[c] School of Public Health, The Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education, Guizhou Medical University, Guiyang 550025, China
[d] College of Food and Pharmaceutical Engineering, Guizhou Institute of Technology, Guiyang 550003, China

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ABSTRACT

Two edible coatings (gelatin coating and ginger essential oil-gelatin coating) were prepared to maintain the flavor quality of fish fillets at two storage temperatures (4 °C and 25 °C). The effects of coating on fish fillets were evaluated by detecting the physical properties, microstructure, microbial properties, volatile flavor and taste flavor of fish. In the same coating method, fish fillets stored at 4 °C showed better effect than that at 25 °C on maintain water content, color and texture, however, fish fillets stored at 25 °C were closer to fresh fish in volatile flavor and taste flavor than that at 4 °C; whatever the storage temperature, coating could slow down the growth of fish microorganisms, maintain water content, color, texture, volatile flavor and taste flavor of fish fillets; GGC exhibited better effect on maintain flavor quality than GC.

1. Introduction

Edible film has attracted more and more attention in the field of food packaging due to its resource availability and environmental degradability. The barrier effect of edible coating significantly extended the shelf life of food, and maintained the water content, mechanical properties and sensory perception of food (Shahidi & Hossain, 2020); in particular, the addition of essential oils to the preparation of edible coatings improved the antibacterial and antioxidant properties of the coatings (Shahidi & Hossain, 2018). Fish products were prone to corruption under the joint action of chemical reaction, lipid oxidation, endogenous enzymes and microorganisms (Lou et al., 2021), thus, researchers tried a series of coating methods to extend the shelf life of fish. Feng et al. studied coating effect on the fish myofibril under refrigerate condition, the results showed that coating groups (0.4% TP and 1.2% gelatin group) exhibited the most intact nano structure after 17 days of cold storage (Feng et al., 2017). Sun et al. investigated the effect of fish gelatin coating enriched with curcumin/β-cyclodextrin (CUR/βCD) on fish physicochemical properties, the result showed fish gelatin coating enriched with CUR/βCD could maintain the quality of fish fillets and extend its shelf life under 4 °C (Sun et al., 2019). The previous research most focused on the physicochemical changes and microbial changes of coated meat during storage, the research about the multiple flavors of coated meat was rare and shallowed, especially on the metabolic mechanism of coating on meat flavor.

Food flavor is an important index to measure the quality of food, and it is closely related to the intuitive feeling of consumption. Traditional studies on food flavor mainly focus on smell and taste, while Spence (Spence & Charles, 2015) believed that flavor was a comprehensive experience, it included the multi-sensory integration of sight, hearing, touch, smell and taste. Therefore, it was more scientific way to evaluate food flavor combining color, texture, volatile flavor and taste flavor. The main components of meat were water, protein and fat (Wen et al., 2019), changes in these ingredients were closely related to changes in flavor. Changes in water would cause changes in the texture and color of meat (Giorgio et al., 2019); protein metabolism would cause changes in the texture and taste of meat (Sharedeh et al., 2015); and lipid oxidation would mainly cause changes in the volatile flavor of fish (Sharedeh et al., 2015). Fish aroma was a precious quality of fish, life experience told us that there were great differences in the flavor of fish preserved in different temperature. For example, naturally dried fish had a stronger fish smell, while fish with cold storage got a more similar chewing

* Corresponding authors at: National R&D Center for Freshwater Fish Processing, College of Life Science, Jiangxi Normal University, Nanchang, Jiangxi 330022, China (Zong-Cai Tu).
E-mail addresses: tuzc_mail@aliyun.com (Z. Tu), shaxiaomei1987@sina.com (X. Sha).

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experience to fresh fish. Therefore, we suspect that the metabolic mechanism of fish flavor was quite different at different storage temperatures.

In order to prolong the shelf life of fish and understand the flavor change mechanism of fish under different storage conditions, this paper prepared gelatin coating (GC) and ginger essential oil-gelatin coating (GGC) respectively and compared the effect of coating on fish flavor under two different storage temperatures (4 °C and 25 °C). Combined with the multi-data processing method, the effect mechanism of coating preservation on fish flavor were analyzed through the detection of weight loss, color, texture, microstructure, volatile flavor (volatile flavor detection and electronic nose detection) and taste flavor (free amino acids detection and electronic nose detection). Coating may become a practical commercial method for fish preservation, this study may provide a new theory for coating preservation of fish flavor.

2. Materials and methods

2.1. Materials

Fish samples (*Ctenopharyngodon idellus*) were obtained from Yaohu lake aquaculture plant. Fish gelatin was purchased from Jiliding biotechnology company, 270 bloom (Suzhou, China). Ginger essential oil (GEO) was got from Yumei Cosmetics Company (Jiangxi, China). Tween-80 and glycerol were obtained from Sigma-Aldrich (Shanghai, China).

2.2. Preparation of coating solution

GGC solution: firstly, 8% fish gelatin (w/v, based on distilled water) was dissolved in distilled water at 60 °C for 90 min; then, GEO and Tween-80 were mixed at the ratio of 1:1, this mixture was added to melted gelatin solution; finally, the GEO was added to mixture so that its concentration was 0.5% (v/v, based on distilled water), the final solution was stied at room temperature for 30 min.

GC solution: this solution was prepared according to the method of GGC solution without adding GEO.

All coating solutions were ready for immediate use. The preparation method of coating was based on the previous experiments (Li et al., 2020).

2.3. Fish samples preparation

Fish were slaughtered immediately after they arrived at the laboratory. Fish were removed from the water individually and given a sharp blow to the head, removing the gills or severing the caudal vessels of the tail in order to obtain fish without blood odor. Fish samples were taken from back muscles and cut into small pieces (4 cm × 3 cm × 2 cm) for the experiment.

Fish fillets were immersed into two coating solutions at room temperature, then fish fillets were placed in −20 °C for 1 min, in order to form a solid coating on the surface of fish fillets as soon as possible. Fish fillets without coating were treated as the control group. All fish fillets were placed in sterilized glass petri dishes (150 mm), covering lid on the dishes. Fish fillets placed in 4 °C were measured every 2 days, fish fillets placed in 25 °C were measured every 3 h. All materials exposed to fish fillets were sterilized, the entire process was completed in 1 h (Li et al., 2020).

2.4. Physical properties

2.4.1. Weight loss

The initial weight of fish fillets was recorded as M_1, the changed weight of fish fillets during storage process was denoted as M_2. The weight loss% was calculated as follow:

$$\text{weight loss } (\%) = \frac{M_1 - M_2}{M_1} \times 100\%$$

each sample was tested 4 times (Xu et al., 2019).

2.4.2. Color

The color of fish was measured using colorimeter (CR-10, Konica Minolta Optics, Inc., Japan). The total color was calculated by the following formula:

$$\Delta E = \sqrt{(L - L^*)^2 + (b - b^*)^2 + (a - a^*)^2}$$

where the value of L*, a*, and b* were came from standard white plate (L* = 92.56, a* = −0.49, b* = −0.25), each group was tested 6 times (Ezati et al., 2020).

2.4.3. Texture analysis

Texture analysis was operated by Texture Analyzer (Stable Micro System TA.TX-plus, UK). The test conditions were set as follows: TPA model, flat cylindrical probe with 36 mm (P/36R), test speed was 1.0 mm/s, trigger force was 5 g, each type of fillets was tested 8 times (Ezati et al., 2020).

2.5. Total viable counts (TVC)

25 g fish fillet was transferred into 225 mL sterile buffered peptone water (BPW) solution. LB agar medium was used as cultivate medium and 0.1 mL diluent was spread on the surface of medium. TVC value were calculated by plant gradient dilution method and recorded as log cfu/g. The incubation time was 2 days at 30 °C, each group was operated 3 times (Ezati et al., 2020). As an important index of fish freshness, TVC value provided selection basis for series of tests (SEM, GC–MS, e-nose, FFAs and e-tongue).

2.6. SEM analysis

Fish samples were sliced into thin slices and dried by freeze-drying. The microstructure analysis was operated by a scanning electron microscope (S-3400 N, Hitachi, Japan) (Egelandsdal et al., 2019).

2.7. Analysis of volatile flavor

2.7.1. Gas chromatography-mass spectrometry (GC–MS) analysis

Sample treatment: fish fillets was cut into small pieces, then grounded into minced fish. 8 g minced fish and 2 μL cyclohexanone were added into 20 mL headspace bottle, PTEF spacer was used to seal this bottle. The volatile components of fish were balanced at 55 °C for 10 min and extracted at the same temperature for 50 min. The extraction head was inserted into GC injector and desorbed for 2 min.

GC–MS equipment: The volatile components of fish samples were analyzed by GC–MS (Trace1300/ISQ, Thermo Fisher, USA) coupled to a InertCap Wax column (60 m × 0.25 mm × 0.25 μm), and the program was set as follows: the injector temperature was 250 °C; the initial oven temperature was 40 °C, keeping 3 min, raised to 230 °C at a rate of 3 °C/min; the mass detector was operated in an electron impact mode with an ionization energy of 70 eV, using He as the carrier gas at a constant flow rate of 1 mL/min. All samples were measured triplicate. The detection method was based on Li et al and modified appropriately (Li et al., 2017).

2.7.2. Electronic nose (e-nose) analysis

Electronic nose system (PEN3, AIRSENSE Company, Germany) was used to analyze the flavor difference of fish samples. 15 g sample was placed into a glass cup and sealed with double plastic. Fish samples were equilibrated at room temperature for 30 min to generate balanced

headspace samples. The parameters of e-nose were set as follows: the flow rate was 400 mL/min, the test time was 100 s, and the clean time was 100 s. E-nose equipment possessed 10 metal oxide semiconductor (MOS) sensors, thus each sample had ten response values. Radar map and principal component analysis (PCA) were based on the response values of 10 sensors. All samples were measured triplicate (Huang et al., 2019).

2.8. Analysis of taste flavor

2.8.1. Free amino acids (FAAs) analysis

FAAs analysis was operated with automatic amino acid analyze (Hitach, RD001931, Japan). Fish samples were homogenized with ultrapure water for 3 times, then trichloroacetic acid (5%) was added to fish samples and stored at 4 °C for 12 h. 4 mol/L potassium hydroxide was used to adjust the pH to 6. The injection volume was 20 μL, the area of each peak was used to calculate the content of free amino acid (Li et al., 2017). All samples were operated triplicate.

2.8.2. Electronic tongue (e-tongue) analysis

The electronic tongue system (TS-5000Z, INSENT, Japan) was used to detect taste differences of fish samples. 50 g fish was minced with food processor for 1 min, then 200 mL distilled water (40 °C) was added to minced fish, continue stirring the mixture for 1 min. The mixture solution was centrifuged at 3000 rpm for 10 min, then 35 mL clarified liquid was used for e-tongue test, and the tested temperature was 20 °C. E-tongue equipment possessed eight sensors, thus each sample obtained eight response values. Radar map and principal component analysis (PCA) were based on the eight response values. All samples were tested triplicate (Xu, Wang, & Zhu, 2019).

2.9. Statistical analysis

Statistical tests were performed using Statistical Package for Social Science (SPSS). Variance analysis, Venn analysis, heatmap cluster analysis and principal component analysis (PCA) were used for data analyses.

3. Results and discussion

3.1. Physical properties

3.1.1. Weight loss

Weight loss of coated fish under different treatment is shown in Fig. 1 (A). With or without coating, weight loss increased over time, and the weight loss of fish fillets at 25 °C was much higher than that at 4 °C. Meat muscle contained 75% water, only 10% −15% of which was bound water, thus fish fillets were easily lose water over time (Listrat et al., 2016), and water in muscles evaporated more easily at higher temperature. Water content was an important indicator of meat tenderness, the poor moisture represented poor tenderness, which affected the mouthfeel of fish fillet.

Compared with control group, coating groups were better at retaining water in fish fillets, this is because the coating acted as water vapor barrier, effectively preventing the evaporation of water from fish. Compared the two groups of coated fish, the effect of GGC groups was better than that of GC groups. Gelatin is a hydrophilic material, the barrier performance of gelatin film to water vapor is poor; ginger essential oil in GGC coating was a hydrophobic component, the addition of ginger essential oil enhanced the effect of water vapor barrier. Ana et al obtained similar result, coating prevented the weight loss of fish during storage, and the effect of the film group adding essential oil was better than that without the film group (Vital et al., 2018).

3.1.2. Color

Fish color are illustrated from Fig. 1(B) to Fig. 1(E). Color was expressed through 4 parameters: ΔE L a and b. L value represented lightness, a value stood for red/blue, b value represented yellow/green, and ΔE value was the total color. Whether coated or not, a value, b value and ΔE value increased whereas L value decreased as storage time prolonged; in addition, fish samples stored at 4 °C exhibited higher L value than that at 25 °C. Oxidation of lipids, pigments, protein, carbohydrates and vitamins produced oxidation products, these products caused the loss of color; enzymatic autolysis of carbohydrates, fats and proteins resulted in greenish discoloration of meat (Dave & Ghaly, 2011). Therefore, the color of fish turned green and discoloration as the storage time increased. The loss water of fish fillets due to decrease of lightness, fish stored at 25 °C exhibited lower lightness because fish was easily dehydrated under this environment.

Compared with control group, the changed of coating groups at two storage temperature was smaller than control group. The coatings maintain the color of the fish fillets in two ways: it acted as a water vapor barrier to keep the lightness of fish; and it acted as an oxygen barrier to prevent the production of oxidation products in the fish, thus preventing the deterioration of the fish color. GGC was better than GC on color retention. Due to the addition of ginger essential oil, GGC had a better barrier effect on water vapor than GC, so the brightness of GGC is better than GC. The antioxidant effect of ginger essential oil more effectively inhibits the production of fish oxidation products, and GGC has a better effect to maintain the color of fish than GC. Cardoso et al (2019) obtained a similar conclusion that coated meat maintained color stability during storage.

3.1.3. Texture analysis

The texture of coated fish under different treatment are listed from Fig. 1(F) to Fig. 1(J). Hardness, springiness, cohesiveness, gumminess and chewiness were five characteristic parameters of meat. Hardness (N/cm^2), springiness (cm) and cohesiveness (A_2/A_1) were three independent variables, gumminess and chewiness were dependent variables, gumminess (N/cm^2) = hardness × cohesiveness, chewiness (N/cm) = hardness × cohesiveness × springiness. Whether fish fillets were coated or not, hardness exhibited different trend at two storage temperatures, hardness increased with time at 4 °C, and decreased with time at 25 °C; springiness and cohesiveness decreased as time goes; under 25 °C condition, gumminess and cohesiveness fluctuated up and down with time; under 4 °C condition, gumminess and cohesiveness decreased with time. Texture change was related to the change of water loss, microbial metabolism and enzyme autolysis during fish storage. Microbial growth in meat could result in slime formation and structural degradation, thereby reducing the capacity of holding water in fish fillets; the autolysis of enzymes in protein, carbohydrate and fat caused soft change in meat (Wei et al., 2021). For the above two reasons, the hardness of fish fillets would change soft during the storage time. Therefore, harness of fish fillets showed downward trend at 4 °C. However, the hardness of fish fillets rose obviously at 25 °C, higher temperature was the reason that caused the fish to lose water quickly, which increased the hardness of the fish. Meanwhile, fish myofibrils were made up of a series of sarcomeres, each sarcomere in myofibrils was separated by a Z-line. Z-line had the effect of maintain structure of myofilament. As storage time extended, Z-line became indistinct and fragmentation, myofibril released, ultimately due to myofibrillar protein disintegrated, cohesiveness decreased with the disintegration and fragmentation of myofibrils (Feng et al., 2017). Gumminess and chewiness were two dependent variables, they varied according to changes in hardness, cohesiveness and springiness. Gumminess and chewiness fluctuated up and down at 25 °C, and showed decreased trend at 4 °C. They exhibited different trends at different temperatures, this is the result of a sharp increase in the hardness of fish at 25 °C.

Compared with control group, all coated groups showed gradual trend as the storage time prolongs; GGC was better than GC in preserving fish texture, the difference between GC groups and GGC groups were not significant. The important function of the coating was to act as a water

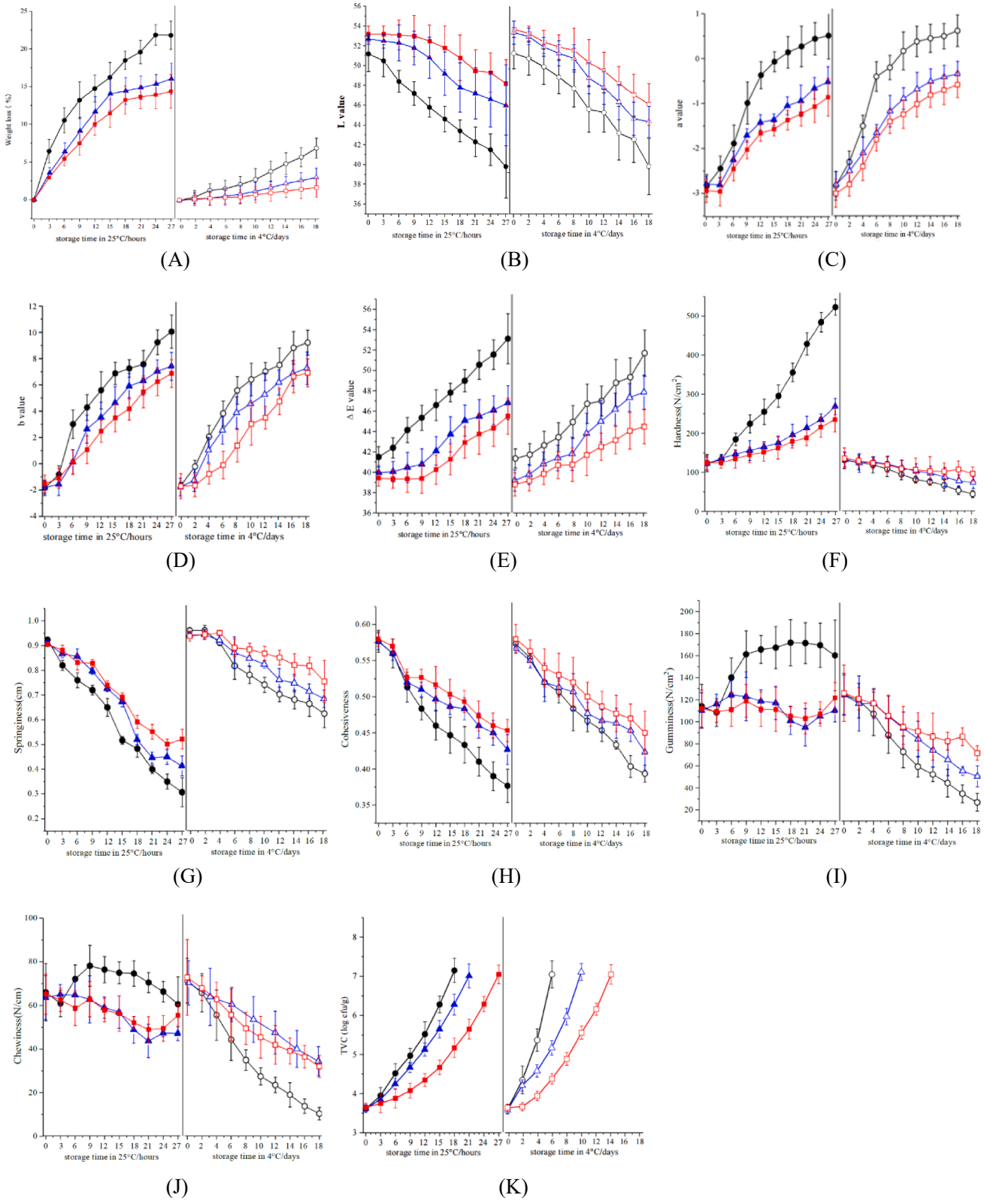


Fig. 1. (A). Weight loss of coated fish with different treatment. (B)-(E). Color of coated fish under different treatment, (B): L value; (C): a value; (D): b value; (E): ΔE value. (F)-(J). Texture of coated fish under different treatment, (F): Hardness; (G): Springiness; (H): Cohesiveness; (I): Gumminess; (J): Chewiness. (K). TVC value of coated fish with different treatment. (●:25℃ control; ▲:25℃-GC; ■:25℃-GGC; ○:4℃ control; △: 4℃-GC; □:4℃-GGC).

vapor barrier, enhance the water holding capacity of fish. Due to the addition of the essential oil, GGC is more resistant to water vapor. In addition, the antibacterial activity of essential oil inhibits the growth of fish microbes, and the antioxidant activity impedes autolysis of proteins, carbohydrates, and fats, these biological activities of the essential oil can maintain the water holding capacity of the fish. However, the difference between GC groups and GGC groups were not significant, the texture might more depend on the shape of the fish.

3.2. Total viable counts (TVC)

The TVC of fish fillets with different treatment is exhibited in Fig. 1 (K). The number of microorganisms was one of the basic indicators to evaluate the quality and safety of fish, 7 log cfu/g was considered as the upper limit for fresh meat (Moreira et al., 2019). At 4 °C storage condition, the initial TVC of fresh fish was 3.61 log cfu/g; the growth rate of bacterial in control group was the fastest, reaching 7.05 log cfu/g at day 6; it took 10 days for GC to reach 7.11 log cfu/g; and it spend 14 days for GGC to reach 7.05 log cfu/g. GC and GGC extended storage time of fish fillets for 4 days and 8 days respectively. At 25 °C storage condition, the initial TVC of fresh fish was 3.61 log cfu/g; control group rapidly reached 7.15 log cfu/g at hour 18; GC took 21 h to reach 7.01 log cfu/g; GGC spent 27 h to reach 7.05 log cfu/g. GC and GGC retarded storage time of fish fillets for 3 h and 9 h respectively. All coating groups obtained longer shelf life of fish than control groups, especially, GGC has a better effect than GC. Coating could hinder the contact between fish fillets and oxygen, therefore, the microbial growth of fish in coating groups was slow. The longer shelf life of GGC groups was due to the antibacterial properties of ginger essential oil. The antibacterial mechanism of essential oil involves the destruction of cell walls by the active compounds which penetrated into the cells, denatured the proteins, and eventually caused cell death (Oussalah et al., 2007). Although TVC was not directly related to the comprehensive flavor of fish, but TVC was an evaluation index of fish freshness and also an important basis for time point selection in the following test. Therefore, fish stored for 6 days at 4 °C and fish stored for 18 h at 25 °C were selected, (including SEM test, volatile flavor analysis and taste flavor analysis), because control samples reached the upper limit at two temperatures respectively. This operation had two purposes, one was to compare the coating effect on the fish flavor; the other was to compare the difference on fish flavor at different storage temperatures.

3.3. SEM analysis

SEM micrographs of coated fish under with different treatment are exhibited in Fig. 2. The micromorphology of control groups at 4 °C and 25 °C was very different. Tiny and uniform porosity structure was exhibited in control group at 4 °C, whereas rough and shrinkage surface was observed in control group at 25 °C. Aksoy et al. considered (Aksoy et al., 2019), small and uniform pores enhance the water retention of meat, less damage done to the porosity and open structure of meat during processing, the quality of meat was better. The control group at 25 °C was damaged by the high temperature and showed a rough and wrinkled surface, which affected the perception of the fish in the mouth. Regardless of the temperature, the coating groups presented a smooth surface. Gelatin had film-forming properties, so the coating film can play a protective role on the surface of the fish, tiny pore of fish was protected by coating and water holding capacity of fish were maintained. There is no difference between the GC groups and GGC groups showed no difference in SEM micromorphology, because gelatin had good compatibility and formed a uniform and stable solution with ginger essential oil. SEM micromorphology of fish meat can be used to explain the results of its texture. Fish fillets with tiny and uniform pores had better water holding capacity, and fish fillets had better gumminess and chewiness. The function of the coating film is to form a water vapor barrier on the surface of the fish meat, thus maintain better texture of fish. GC groups and GGC groups exhibited minor difference in SEM, explaining the minor difference in texture.

3.4. Volatile flavor analysis

3.4.1. GC–MS analysis of volatile flavor

Aldehydes and alcohols in fish are shown in Table 1, a total of 8 aldehydes and 20 alcohols were detected. The flavor of fresh fish was tested in order to compare the difference in volatile compounds between fresh fish and fish samples. The subtotal of aldehydes in fresh fish was

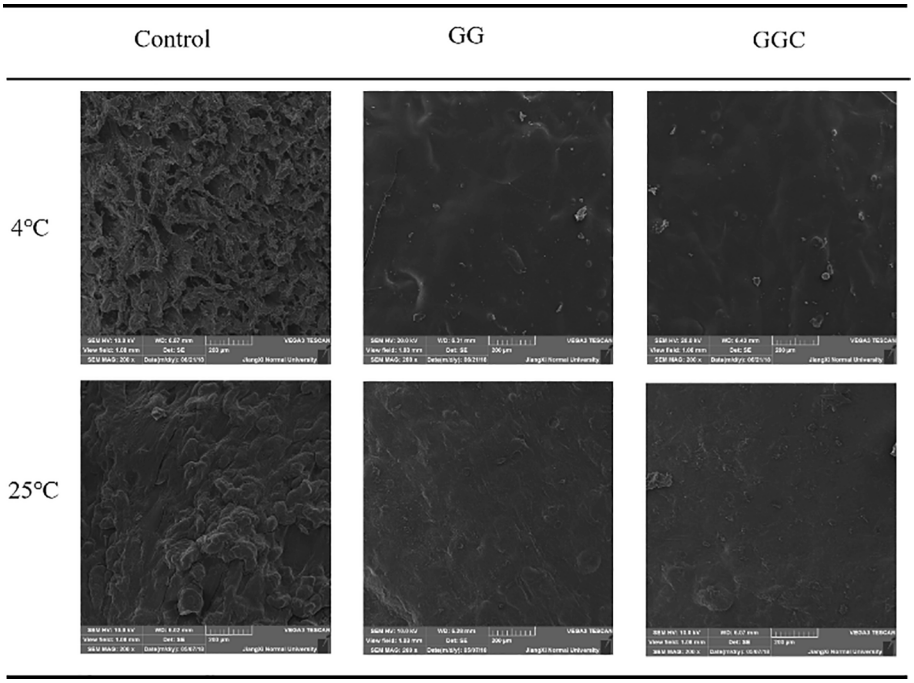


Fig. 2. SEM micrographs of coated fish under different treatment.

Table 1
Volatile compounds and their content of coated fish under different temperatures. (μg/L).

Number	Name	Fresh fish	25℃-control	25℃-GC	25℃-GGC	4℃-control	4℃-GC	4℃-GGC	Flavor*
	Aldehydes								
1	Pentanal	0	0	0	0	126.35 ± 29.51	0	0	almond, malt, pungent
2	Hexanal	425.88 ± 47.99d	829.87 ± 66.70e	64.01 ± 7.93a	74.26 ± 7.04b	1964.67 ± 306.83 g	944.29 ± 12.06f	340.48 ± 30.40c	fat, grass, tallow
3	(E)-2-Heptenal	— —	83.11 ± 11.89b	— —	— —	93.93 ± 6.50c	69.76 ± 4.86a	— —	green
4	Nonanal	167.46 ± 29.58a	777.09 ± 76.97e	212.26 ± 65.23b	326.11 ± 29.09c	901.90 ± 60.89 g	462.70 ± 40.25d	820.80 ± 77.32f	fatty, citrus, rose
5	(E)-2-Octenal	40.78 ± 4.81a	132.81 ± 19.28e	55.19 ± 7.80b	— —	100.86 ± 12.68c	123.72 ± 17.71d	180.98 ± 8.77f	fat, green,nut
6	Octanal	— —	62.88 ± 7.09b	— —	— —	91.05 ± 2.73c	17.21 ± 2.26a	— —	fat, soap, lemon, green
7	Decanal	— —	457.11 ± 45.01c	— —	310.42 ± 22.39b	214.79 ± 32.99a	— —	— —	soap, orange peel, tallow
8	(E,E)-2,4-Decadienal	— —	— —	— —	— —	44.56 ± 0.06	— —	— —	fat,fishy
	subtotal	634.12 ± 82.38b	2342.87 ± 226.94f	331.46 ± 73.99a	710.79 ± 58.52c	3538.11 ± 452.19 g	1617.68 ± 77.14e	1342.26 ± 116.49d	
	alcohols								
1	1-Penten-3-ol	45.13 ± 4.16a	85.08 ± 7.79d	— —	— —	53.07 ± 3.68b	81.73 ± 8.04c	— —	
2	1-Pentanol	560.58 ± 58.20e	640.22 ± 73.41 g	138.11 ± 9.75b	33.77 ± 1.69a	515.76 ± 9.11d	616.74 ± 1.93f	480.14 ± 76.80c	
3	1-Hexanol	5841.68 ± 886.82c	11111.23 ± 1212.35f	3158.65 ± 80.95b	683.47 ± 2.82a	6785.58 ± 864.30d	11473.29 ± 543.33 g	9618.79 ± 1098.30e	
4	1-Octen-3-ol	2024.20 ± 162.05b	4926.83 ± 353.19f	2423.97 ± 70.26c	605.3 ± 28.25a	5771.25 ± 496.33 g	3387.79 ± 250.70d	4571.89 ± 690.69e	
5	1-Heptanol	147.94 ± 33.81b	579.19 ± 14.32 g	251.63 ± 9.63c	83.27 ± 10.73a	341.59 ± 31.42d	443.58 ± 20.20e	451.51 ± 16.18f	
6	2,4-Dimethylcyclohexanol	27.19 ± 7.94c	30.29 ± 3.25d	— —	— —	20.01 ± 1.67b	10.20 ± 1.39a	— —	
7	2-Ethyl-1-hexanol	56.55 ± 1.51b	42.59 ± 2.02a	— —	— —	59.82 ± 7.40c	104.67 ± 5.75d	— —	
8	4-Ethylcyclohexanol	— —	20.36 ± 0.72b	— —	— —	94.15 ± 2.88c	13.61 ± 0.73a	— —	
9	2,4-Dimethyl-cyclohexanol	— —	— —	— —	— —	52.63 ± 1.07	— —	— —	
10	1-Octanol	117.83 ± 8.95a	440.82 ± 2.63f	344.61 ± 22.86e	154.57 ± 8.31b	315.96 ± 31.75d	312.5 ± 5.12c	519.85 ± 90.21 g	
11	2-Octen-1-ol	34.75 ± 3.23a	325.39 ± 22.74d	0	38.96 ± 2.39b	440.48 ± 45.34e	171.6 ± 12.99c	0	
12	4-Methyl-5-decanol	— —	— —	— —	— —	45.55 ± 6.50	74.77 ± 4.78	— —	
13	1-Nonanol	287.46 ± 25.69b	795.16 ± 35.73e	— —	271.76 ± 28.82a	538.09 ± 36.74c	669.18 ± 8.56d	— —	
14	1-Decanol	— —	— —	— —	— —	128.25 ± 20.00	— —	— —	
15	3-Methyl-1-butanol	102.36 ± 13.16b	963.71 ± 76.34d	316.74 ± 1.04c	2332.94 ± 135.86e	— —	22.99 ± 2.46a	— —	
16	3-Octanol	25.78 ± 3.70	— —	— —	— —	— —	— —	— —	
17	2,3-Butanediol	39.39 ± 1.57	— —	— —	133.98 ± 7.72	— —	— —	— —	
18	(E)-2-Octen-1-ol	84.16 ± 5.06	— —	— —	— —	— —	— —	100.14 ± 3.78	
19	(Z)-3-Nonen-1-ol	23.62 ± 2.03	— —	— —	— —	— —	65.60 ± 5.56	— —	
20	2-Nonanol	— —	— —	— —	— —	— —	60.05 ± 10.03	575.38 ± 29.05	
	subtotal	9418.62 ± 1271.88c	19960.87 ± 1804.4 g	6633.71 ± 194.49b	4338.02 ± 226.59a	15162.19 ± 1558.19d	17508.3 ± 881.57f	16317.7 ± 2005.01e	
	others								
1		— —	160.06 ± 14.34b	— —	76.13 ± 4.56a	488.75 ± 51.59e	271.45 ± 24.11d	164.58 ± 5.51c	
2	2-Octanone	— —	15.41 ± 0.78	— —	— —	— —	17.60 ± 3.29	— —	
3	2-Pentanone	38.15 ± 4.48a	10814 ± 15.29c	— —	— —	— —	71.63 ± 3.33b	— —	
4	D-Limonene	518.51 ± 64.95b	468.30 ± 60.70a	8123.18 ± 153.05e	1283.34 ± 82.18d	— —	595.40 ± 30.99c	9366.72 ± 859.22f	
5	2-Heptanone	81.78 ± 3.10	— —	— —	— —	220.89 ± 2.99	— —	— —	
6	2-Pentylfuran	46.89 ± 0.80a	98.72 ± 7.24c	— —	— —	98.38 ± 5.33c	91.22 ± 3.66b	112.99 ± 20.42d	
7	Ethyl Acetate	27.33 ± 0.92d	13.34 ± 1.99b	— —	9.84 ± 1.33a	— —	20.93 ± 2.38c	— —	

Note: $p < 0.05$.
*obtained from literature (Li et al., 2017).

634.12 ug/L. The subtotal of aldehydes in control groups were 3538.11 ug/L (4 °C) and 2342.87 ug/L (25 °C), the subtotal of alcohols in GC groups were 1617.68 ug/L (4 °C) and 331.46 ug/L (25 °C), the subtotal of alcohols in GGC groups were 1342.26 ug/L (4 °C) and 710.79 ug/L (25 °C). The subtotal of alcohols in fresh fish was 9418.62 ug/L. The subtotal of alcohols in control groups were 15162.19 ug/L (4 °C) and 19960.87 ug/L (25 °C), the subtotal of alcohols in GC groups were 17508.3 ug/L (4 °C) and 6633.71 ug/L (25 °C), the subtotal of alcohols in GGC groups were 16317.7 ug/L (4 °C) and 4338.02 ug/L (25 °C). The subtotal of alcohols and aldehydes in control groups were all increased, coating decreased the subtotal of alcohols and aldehydes in fish samples. By comparing the subtotal of alcohols and aldehydes under different temperatures, it was found that the subtotal of 25 °C coating groups were lower than that of 4 °C coating groups. Fish was rich in unsaturated fatty acid (PUFA), lipid oxidation products of PUFA included aldehydes, ketones, acids and alcohols (Aksoy et al., 2019). Aldehydes and alcohols were considered to contribute to the flavor of fish due to their lower relative thresholds (Yu et al., 2018). At the same time, the oxidation process of fats was accompanied by the formation of odorous compounds. Coating as an oxygen barrier reduced the rate of lipid oxidation, thus the accumulation of aldehydes and alcohols in coating groups were lower than those in control group. Gingerol was the main ingredient in GEO, which was a kind of phenolic with antioxidant property, it could terminate the chain radical reaction, thereby preventing harmful outcomes produced by lipid oxidant (Ghafoor et al., 2020). Thus, GGC groups reduced the oxidation rate of fish fat, reduced the generation rate of aldehydes and alcohols in fish, and effectively inhibited the generation of odor components. Similar results were observed from Maqsood et al. (Maqsood et al., 2014), the addition of plant extracts containing phenols compound inhibited the relative content of aldehydes and aldehydes in aquatic products. In the same coating way, fish stored at 4 °C exhibited higher total volatile content than that at 25 °C, which may be because the lipid oxidation reaction was more sufficient at 4 °C. Lipid degradation produced volatile compounds, lipid was considered as the source of specific flavor (Shahidi & Oh, 2020). The content and composition of fat was related to the flavor of meat, fish possessed lower fat content and weaker volatile flavor compared with other meat (Ding et al., 2020). The threshold of aldehyde was low, so aldehydes were important ingredients that affected the flavor of fish. 6 kinds of components were detected to have fatty odor (Hexanal, Nonanal, (E)-2-Octenal, Octanal, Decanal, (E, E)-2,4-Decadienal), and the levels of these components were higher in the control groups; besides, (E, E)-2,4-Decadienal had the flavor of fishy, it was the resource of fishy smell in the storage process.

Fig. 3(A) is a clustering heat map of the volatile flavor in fish samples with different treatment. The heat map showed the composition and content of volatile flavor in each fish sample by different colors. Cluster analysis could cluster fish samples according to the volatile flavor, 7 groups were divided into 4 sub-categories. As shown in Fig. 3A, 25 °C-GC, 25 °C-GGC and the fresh fish were located in a small class, indicating that the volatile flavor of 25 °C coating groups were closest to fresh fish; 4 °C-GGC as a separate category; 25 °C control group and the 4 °C-GC were located in a small class, and they were far away from fresh fish, indicating the significant change in volatile flavor; 4 °C-control as a separate category, it was far away from fresh fish. Fig. 3(B) and Fig. 3(C) were Venn diagrams of fish stored at 25 °C and at 4 °C respectively. Venn diagram represented the amounts of volatile components in fish with the same components under different treatments. The larger the value of the overlapping area, the greater the number of common components. 25 °C-control and fresh fish had 20 kinds of common components, 25 °C-GC and fresh fish owned 11 kinds of common components, 25 °C-GGC and fresh fish obtained 15 kinds of common components; 4 °C-control and fresh fish had 11 kinds of common components, 4 °C-GC and fresh fish owned 13 kinds of common components, 4 °C-GGC and fresh fish obtained 12 kinds of common components. Venn diagram results showed that common ingredients in the group of fresh fish and 25 °C-GC was less than that of fresh fish and 4 °C-GC. However, the result of cluster heat-map analysis showed that 25 °C-GC was closer to fresh fish than 4 °C-GC. Veen diagram and clustering heat map were two analysis method based on the same GCMS data. The above analysis results showed that Veen analysis conclusions were not absolutely consistent with clustering heat map analysis conclusions.

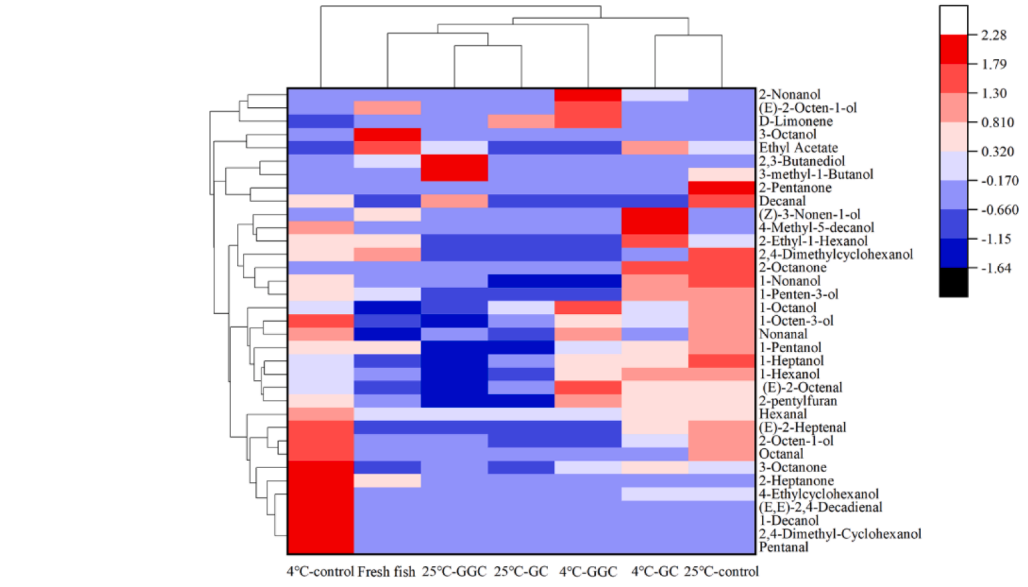
3.4.2. Radar diagram and principal component analysis (PCA) of e-nose

The e-nose radar diagram of fish with different treatment is shown in Fig. 3(D). Radar diagram was used to represent the response values of ten sensors of e-nose to the fish samples. It could be observed from the diagram that there was no significant difference between 25 °C fish groups and fresh fish in 5 sensors (aromatic, polar and NOx, aromatic compounds ketones and aldehydes, H_2, low polarity aromatic and alkane), however, the difference between 25 °C fish groups and fresh fish in methane sensor was obvious. There was no significant difference between 4 °C fish groups and fresh fish in 5 sensors (aromatic, polar and NOx, aromatic compounds ketones and aldehydes, H_2, low polarity aromatic and alkane), but they were different in 3 sensors (broad-methane, sulfur organic and terpene, sulfur and aromatic). Regardless of storage temperature and coating method, 5 sensors (aromatic, polar and NOx, aromatic compounds ketones and aldehydes, H_2, low polarity aromatic and alkane) were not sensitive to fish flavor; 25 °C fish groups and 4 °C fish groups exhibited different trends in flavor trend, 25 °C fish groups were closer to fresh fish; coating could slow down the change of fish flavor during storage, and the effect of GGC was better than GC. PCA of e-nose data in fish samples is shown in Fig. 3(E), PCA analysis reflected the difference of fish samples in overall volatile flavor. The contribution rate of PC_1 was 55.56%, the contribution rate of PC_2 was 29.71%, and the cumulative contribution rate of the first two principal components was 85.37%, which surpassed 85%. 25 °C coating groups were closer to fresh fish, followed by 4 °C coating groups, then 25 °C control group, and finally 4 °C control group. This result suggested that fish samples with same coating method were fresher at 25 °C than that at 4 °C; at same storage temperature, coating samples were closer to fresh sample, and the effect of GGC was better than GC. Oxidation, microbial growth and enzymatic autolysis were three basic mechanisms in charge of meat spoilage during processing and storage, lipid oxidation and microbial growth led to change in volatile flavor (Dave & Ghaly, 2011). When the TVC values of the two control groups reached the upper limit of the fresh fish, the volatile compounds of the fish at different storage temperature were quite different, which might be due to the difference in the lipid oxidation rate of the fish at different storage temperatures. Lipid oxidation had a great influence on meat flavor, even if a small proportion of lipid was oxidized, the change in flavor could be significant (Khan et al., 2015). The addition of GEO enhanced the antioxidant activity of GGC, which inhibited the lipid oxidation of GGC fish. PCA of e-nose reflected the change in volatile compounds of fish samples, the following conclusions were obtained: the volatile flavor of fish stored at 25 °C was closer to that of fresh fish; the function of coating was to maintain volatile flavor of fish, and the effect of GGC was better than GC.

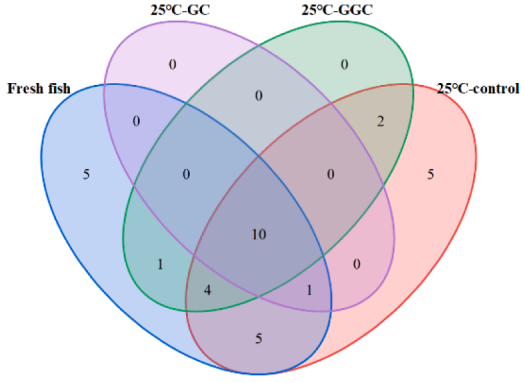
3.5. Taste analysis

3.5.1. FAAs analysis

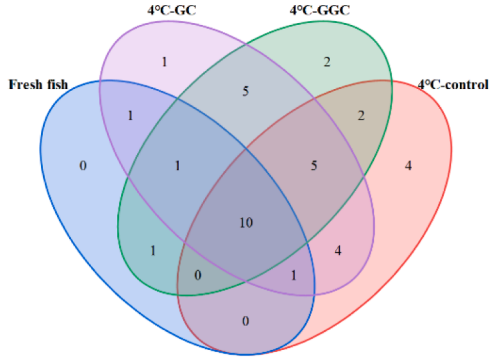
The changes of FFAs in fish with different treatment is shown in Table 2. Most of FAAs possessed a special taste, which was mainly divided into umami, sweet and bitter (Poojary et al., 2017). The composition and content of free amino acids in fish would affect the taste of fish. A total of 16 FFAs were detected in all fish samples. Under the condition of 25 °C, the content of 14 kinds of FAAs rose, and the content of 2 kinds of FFAs decreased. The total amount of FAAs representing umami, sweetness and bitterness all increased, but the increase of bitter FAAs was higher than that of sweet FAAs and umami FAAs, and the increase of umami FFAs was the least. The increase of FFAs content might be the result of autolysis of fish muscle protein (Poojary et al.,



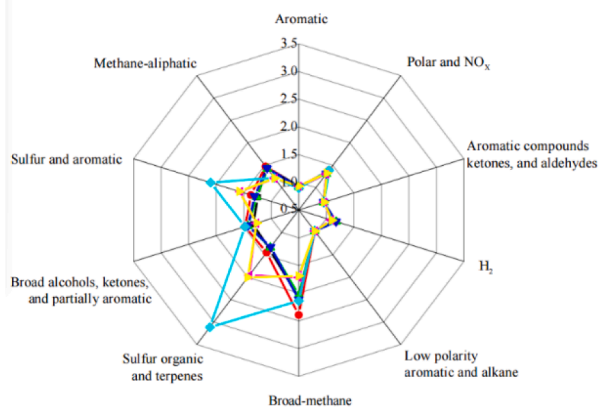
(A)



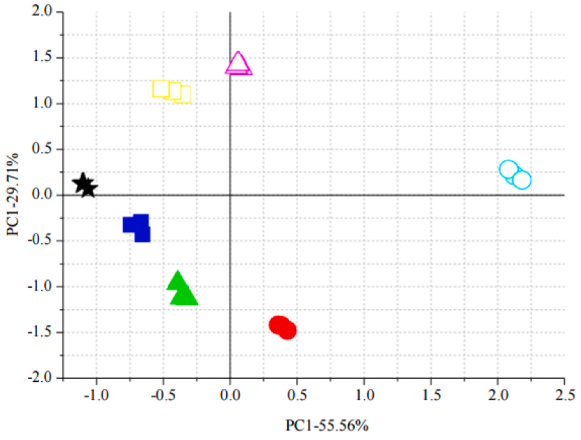
(B)



(C)



(D)



(E)

Fig. 3. (A) Heat map and cluster analysis of volatile components in fish samples; (B) Venn diagram analysis of fresh fish and 25℃ coating groups; (C) Venn diagram analysis of fresh fish and 4℃ coating groups; (D) E-nose analysis of coated fish under different treatment; (E) Principal component analysis (PCA) of e-nose. (●: 25℃ control; ▲: 25℃-GC; ■: 25℃-GGC; ○: 4℃ control; △: 4℃-GC; □: 4℃-GGC; ★: fresh fish).

Table 2
Free amino acids and their content of coated fish under different temperatures. (mmol/kg).

Number	FAAs	Fresh fish	25℃-control	25℃-GC	25℃-GGC	4℃-control	4℃-GC	4℃-GGC	Flavor*
1	Asp	0.155 ± 0.0065d	0.230 ± 0.0200 g	0.198 ± 0.0130f	0.180 ± 0.0056e	0.028 ± 0.0031a	0.057 ± 0.0045b	0.120 ± 0.0032c	umami
2	Thr	0.077 ± 0.0055a	0.066 ± 0.0481c	0.092 ± 0.0061bc	0.084 ± 0.0046ab	–	–	–	sweet
3	Ser	0.078 ± 0.0030d	0.107 ± 0.0208f	0.094 ± 0.0047ef	0.085 ± 0.0040de	0.007 ± 0.0025a	0.030 ± 0.0027b	0.059 ± 0.0021c	sweet
4	Glu	0.217 ± 0.0123f	0.152 ± 0.0040d	0.182 ± 0.0038e	0.210 ± 0.0100f	0.052 ± 0.0035a	0.081 ± 0.0067b	0.116 ± 0.0107c	umami
5	Gly	0.116 ± 0.0062d	0.152 ± 0.015f	0.163 ± 0.0040 g	0.126 ± 0.0027e	0.034 ± 0.0025a	0.061 ± 0.0030b	0.094 ± 0.0101c	sweet
6	Ala	0.128 ± 0.0031d	0.169 ± 0.0062f	0.172 ± 0.0038f	0.150 ± 0.0025e	0.021 ± 0.0025a	0.041 ± 0.0025b	0.089 ± 0.0053c	sweet
7	Cys	0.004 ± 0.0006a	0.007 ± 0.0010a	0.004 ± 0.0006a	0.004 ± 0a	0.231 ± 0.0181d	0.168 ± 0.0165c	0.092 ± 0.0035b	
8	Val	0.081 ± 0.0051bc	0.107 ± 0.0057c	0.108 ± 0.0035c	0.096 ± 0.0035a	–	0.047 ± 0.0051ab	0.079 ± 0.0047c	bitter
9	Met	0.036 ± 0.0046a	0.042 ± 0.0030ab	0.044 ± 0.0025b	0.040 ± 0.0020ab	0.100 ± 0.0053c	0.079 ± 0.0025c	0.061 ± 0.0046c	bitter
10	Ile	0.064 ± 0.0036bc	0.082 ± 0.0025bc	0.077 ± 0.0058d	0.072 ± 0.0017 cd	0.025 ± 0.0035a	0.047 ± 0.0042b	0.055 ± 0.0032bc	bitter
11	Ieu	0.131 ± 0.0056c	0.167 ± 0.0104e	0.171 ± 0.0040e	0.155 ± 0.0053d	0.049 ± 0.0060a	0.079 ± 0.0045b	0.122 ± 0.0031	bitter
12	Tyr	0.032 ± 0.0030 cd	0.044 ± 0.0067e	0.038 ± 0.0029de	0.037 ± 0.0021de	0.007 ± 0.0021a	0.018 ± 0.0031b	0.029 ± 0.0021c	
13	Phe	0.045 ± 0.0030d	0.061 ± 0.0020f	0.059 ± 0.0025f	0.055 ± 0.0011e	0.003 ± 0.0026a	0.030 ± 0.0036b	0.040 ± 0.0010c	bitter
14	Lys	0.122 ± 0.0031d	0.160 ± 0.0031f	0.155 ± 0.0065f	0.141 ± 0.0025e	0.021 ± 0.0025a	0.066 ± 0.0017b	0.089 ± 0.096c	
15	His	0.038 ± 0.0025a	0.056 ± 0.0045d	0.051 ± 0.0021 cd	0.045 ± 0.0025b	0.077 ± 0.0042f	0.062 ± 0.0025e	0.048 ± 0.0031bc	bitter
16	Arg	0.062 ± 0.0020c	0.081 ± 0.0031f	0.081 ± 0.0059f	0.072 ± 0.0025e	–	0.025 ± 0.0030a	0.049 ± 0.0025b	bitter
	Total of umami	**0.372 ± 0.0185d**	**0.382 ± 0.0183d**	**0.380 ± 0.0166d**	**0.390 ± 0.0078d**	**0.081 ± 0.0040a**	**0.137 ± 0.0111b**	**0.236 ± 0.0080c**	
	Total of sweet	**0.399 ± 0.0177d**	**0.494 ± 0.0653f**	**0.522 ± 0.0066f**	**0.445 ± 0.0127e**	**0.063 ± 0.0049a**	**0.132 ± 0.0015b**	**0.243 ± 0.0076c**	
	Total of bitter	**0.411 ± 0.0731bc**	**0.597 ± 0.0201e**	**0.592 ± 0.0137e**	**0.473 ± 0.0633d**	**0.256 ± 0.0015a**	**0.370 ± 0.0085b**	**0.455 ± 0.0155c**	
	Total	**1.339 ± 0.0819d**	**1.684 ± 0.0912e**	**1.691 ± 0.0463f**	**1.490 ± 0.0692f**	**0.660 ± 0.0117a**	**0.892 ± 0.0296b**	**1.143 ± 0.0299c**	

Note: $p < 0.05$.
*obtained from literature (Poojary, Orlien, Passamonti & Olsen, 2017).

2017). FAAs in coating groups exhibited lower increase rate compared with control group, and the effect of GGC was better than that of GC. Under the condition of 4 °C, the content of 3 kinds of FAAs increased, and the content of 13 kinds of FAAs decreased. The total amount of FAAs representing umami, sweetness and bitterness all decreased. FFAs in coating groups decreased slightly compared with control group, and the effect of GGC was better than that of GC. Therefore, in the 25 °C environments, the changing taste of fish was characterized by a rapid increase in off-taste over time; in the 4 °C environments, the changed taste of fish taste was characterized by a decrease in various tastes over time.

Fig. 4(A) is a clustering heat map of FAAs in fish samples with different processing way. The heat map showed the composition and content of FAAs in each fish samples by different colors. Cluster analysis could cluster each group of fish samples based on FAAs. All fish samples were divided into two categories according to the map, one category was fresh fish, 25 °C-GGC, 25 °C-GC, and 25 °C control group, indicating that the FAAs content and composition of 25 °C fish groups were closer to fresh fish; the other category included 4 °C control fish, 4 °C-GC and 4 °C-GGC, this result suggested that the content and composition of FAAs in 4 °C fish groups were quite different from fresh fish. Fig. 4(B) and Fig. 4(C) proposed the metabolism schematic of FAAs at 25 °C and 4 °C respectively. 8 amino acids (alanine threonine glycine serine phenylalanine tyrosine leucine lysine) decomposed to form acetyl-CoA; 3 amino acid (arginine histidine) formed α-ketoglutarate; 3 amino acid (isoleucine methionine valine) became succinyl-CoA; phenylalanine formed fumarate; aspartic acid transformed into oxaloacetic acid. These amino acids entered the TCA cycle through acetyl-CoA, α-ketoglutarate, succinyl-CoA, oxaloacetic acid and fumarate (Li et al., 2021). The difference between TCA cycle at 25 °C and TCA cycle at 4 °C was the changed content in amino acids. The content of 13 amino acids increased and the content of 2 amino acids decreased when the TCA cycle occurred at 25 °C; the content of 2 amino acids increased and the content of 13 amino acids decreased when the TCA cycle occurred at 4 °C. The content of amino acids varies at different storage temperatures, which could cause the fish taste to change in different directions. The function of coating was to slow down the changed rate of amino acids under different storage temperatures.

3.5.2. Radar diagram and principal component analysis (PCA) of e-tongue

The e-tongue radar diagram of fish samples under different processing methods is shown in Fig. 4(D), which can be used to represent the response value of eight sensors to fish samples. There was no significant change in aftertaste-astringency sensor among all fish samples, while significant change appeared between the fish samples and fresh fish in 7 sensors (sourness, bitterness, astringency, aftertaste-bitterness, umami, richness and saltiness); the change in 3 sensors (bitterness, astringency-bitterness and umami) was opposite between 25 °C fish groups and 4 °C fish groups. Regardless of the storage temperature, the value of sourness, richness and saltiness of fish samples increased, while the value of astringency decreased as the storage time extended. However, the changed trend of bitterness, aftertaste-bitterness and umami between 4 °C fish samples and 25 °C fish samples was not consistent,

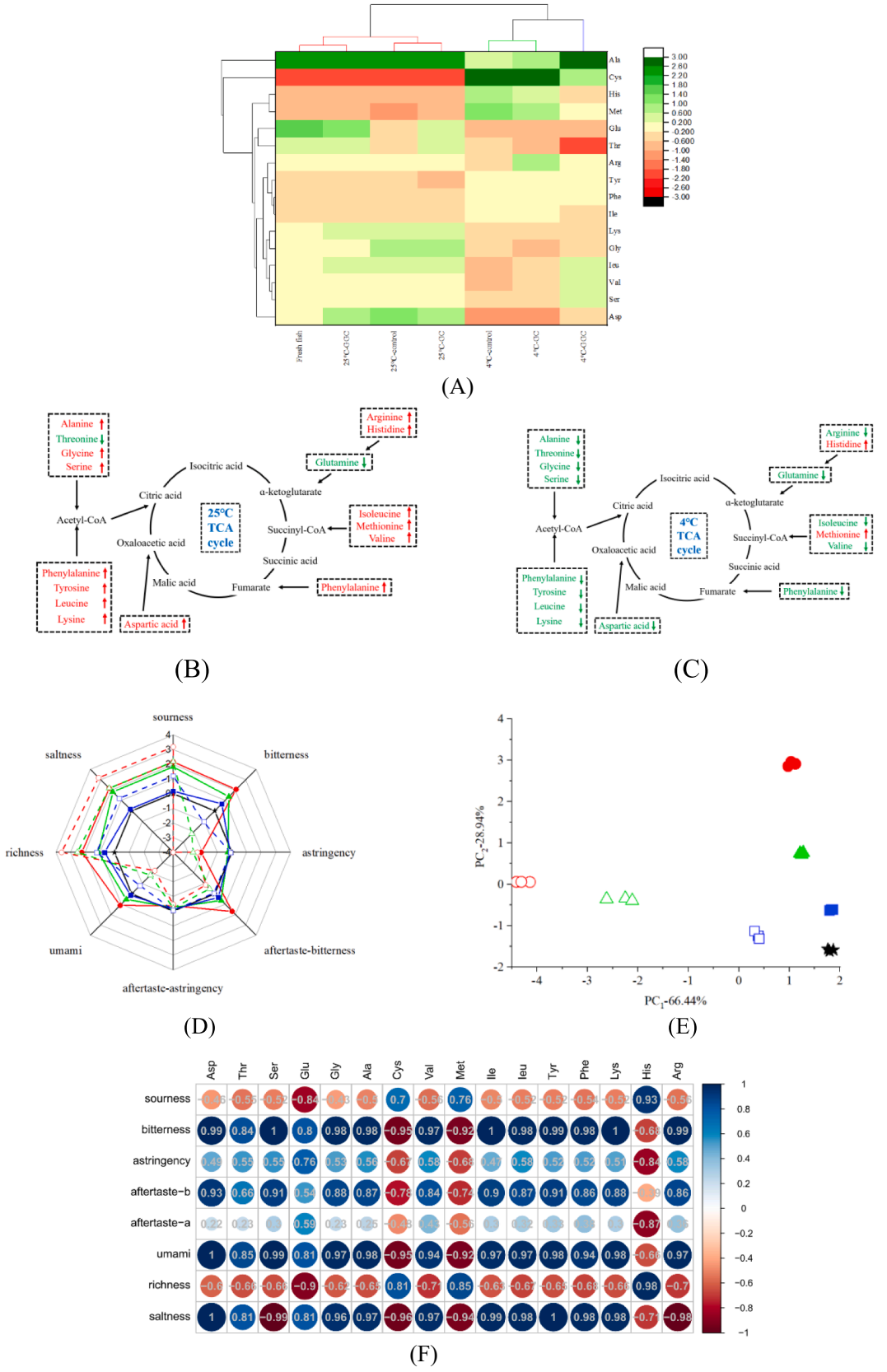


Fig. 4. (A) Heat map and cluster analysis of free amino acids in fish samples; (B) Metabolic changes of free amino acids at 25℃ storage; (C) Metabolic changes of free amino acids at 4℃ storage. Note: in the metabolism map, black color represents undetected components, green color represents decreased free amino acids, and red color represents increased free amino acids; (D) E-tongue of coated fish under different temperatures; (E) Principal component analysis (PCA) of e-tongue. (●: 25℃ control; ▲: 25℃-GC; ■: 25℃-GGC; ○: 4℃ control; △: 4℃-GC; □: 4℃-GGC; ★: fresh fish); (G) Correlation map of e-tongue properties and free amino acids. Note: Each circle indicates Pearson's correlation value, positive ($0 < r < 1$) and negative ($-1 < r < 0$) correlations are shown in blue and red, respectively.

bitterness, aftertaste-bitterness and umami of fish samples decreased at 4 °C and increased at 25 °C. Fish samples stored at 25 °C were closer to the taste of fresh fish than that stored at 4 °C; coating could slow down the changed trend of fish taste, and GGC groups was better than GC groups.

Fig. 4(E) was the PCA (principal component analysis) of e-tongue data, PCA reflected the difference between fresh fish and fish samples in taste flavor. The contribution rate of PC_1 was 66.44%, the contribution rate of PC_2 was 28.94%, and the cumulative variance contribution rate of the first two principal components is 95.38%, which represented most information about e-tongue data. 25 °C-GGC was nearest to fresh fish; followed by 25 °C-GC and 4 °C-GGC; 25 °C control, 4 °C-GG and 25 °C control were far away from fresh fish, 4 °C control was the furthest from fresh fish. With the same coating way, fish samples at 25 °C was closer to fresh fish compared with fish samples at 4 °C; at same storage temperature, coating samples were closer to fresh sample, and GGC showed better effect than gelatin coating. The changed taste of fish flavor was caused by muscle autolysis and microorganisms (Roura et al., 2010; Ruiz-Capillas & Moral, 2001; Wasson, 1993). E-tongue analysis by PCA reflected the changed taste of fish samples. When control groups reached the upper limit of TVC, the taste of fish in the coated groups were very different under different storage temperatures, which may be caused by differences in muscle autolysis. As an oxygen barrier, coating reduced muscle-to-air contact and slowed down the changed rate of FAAs produced by muscle autolysis and microorganisms. GEO possessed the activity of antioxidant and antimicrobial, it could effectively prevent the growth of microorganisms and autolysis of muscles, so GGC presented a better effect on inhibiting the metabolism of FFAs.

3.5.3. Pearson's correlation coefficients of e-tongue properties and free amino acids

Correlation map is shown in Fig. 4(F). The higher absolute value of Pearson's correlation coefficient, the stronger correlation; an absolute value of the correlation coefficient of 1 is considered to be a complete linear correlation, while an absolute value of the correlation coefficient between 0.8 and 1.0 is considered to be a strong correlation. Sourness and richness were negatively correlated with most free amino acids. Bitterness, aftertaste-b, umami and saltness were positively correlated with most free amino acids. Astringency and aftertaste-a were positively correlated with most free amino acids, and the correlation coefficient was low. Bitterness was completely linearly correlated to Ser Ile and Lys, bitterness showed an extremely stronger correlation to Asp, Thr, Glu, Gly, Ala, Cys, Val, Met, Ieu, Tyr, Phe and Arg. Aftertaste-b was strongly correlated with Asp, Ser, Gly, Ala, Val, Ile, Ieu, Tyr, Phe, Lys and Arg; umami was completely linearly correlated to Asp, umami was strongly correlated with Thr, Ser, Glu, Gly, Ala, Cys, Val, Met, Ile, Ieu, Tyr, Phe, Lys and Arg; richness was strongly correlated with Glu, Cys, Met and His; saltness was completely linearly correlated to Asp and Tyr, saltness was strongly correlated with Thr, Ser, Glu, Gly, Ala, Cys, Val, Met, Ile, Ieu, Phe, Lys and Arg.

4. Conclusion

This study showed coating effect on flavor metabolism of fish fillets at two different temperatures. Under the storage temperature of 4 °C, gelatin coating and GGC could prolong the preservation time of fish for 4 days and 8 days respectively, and under the storage condition of 25 °C, gelatin coating and GGC could extend the preservation time of fish for 3 h and 9 h respectively. Coating could slow down the changes in weight loss, color and texture of fish; fish stored at 4 °C possessed better color and texture, and less weight loss. Volatile flavor analysis (GC–MS analysis and e-nose analysis) and taste analysis (FFAs analysis and e-tongue analysis) of fish provided comprehensive information on flavor metabolism of fish fillets. Coating inhibited the formation of off-volatile flavor and off-taste flavor in the process of fish metabolism, and GGC presented better effect than gelatin coating; fish stored at 25 °C were closer to fresh fish in volatile flavor and taste flavor.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Statement

All procedures were performed in compliance with relevant laws and institutional guidelines and that the appropriate institutional committee (s) has approved.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100256.

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