Food Science of Animal Products

Research Article

Effect of modified atmosphere packaging with high concentrations of CO_2 on muscle degradation and endogenous enzyme activity of salmon fillets

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Abstract: The preservation effect of modified atmosphere packaging (MAP) on seafood has been applied widely, but packaging with high concentrations of CO_2 will have adverse effects on seafood. This study aimed to elucidate the potential mechanism of the adverse effects caused by MAP with high CO_2 concentration at 4 °C. The changes in the quality of salmon fillets under high concentrations of CO_2 packaging were demonstrated in this article through indicators such as total volatile basic nitrogen content, microbial counts, thiobarbituric acid reactive substances (TBARS) value, and drip loss. The underlying mechanisms were further explained from the perspectives of protein degradation and endogenous enzymes. The study indicated that the microbial counts in the 90% CO_2/10% O_2 group were higher than both the 75% CO_2/10% O_2/15% N_2 and 60% CO_2/10% O_2/30% N_2 groups on the 12th day, and there was also an increase in TBARS value and drip loss. On the 2nd day, the caspase-3 relative activity in the 90% CO_2/10% O_2 group (119.96%) was found to be higher than the 75% CO_2/10% O_2/15% N_2 group (111.99%). On the 12th day, the relative activities of caspase-3 in the 90% CO_2/10% O_2 group and the 75% CO_2/10% O_2/15% N_2 group were 34.13% and 25.77%, respectively, the latter exhibited the lowest cathepsin activity and the minimum myofibril fragmentation index. The results of free amino acids analysis indicated that the 90% CO_2/10% O_2 group was also accompanied by an accumulation of more bitter amino acids during storage. The MAP with 75% CO_2 had better effect on inhibiting caspases and cathepsins, which could be the main reason to a better muscle quality of salmon fillets at 4 °C.

Keywords: salmon; modified atmosphere packaging; protein degradation; cathepsin; caspase

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1 Introduction

Salmon (*Salmo salar*) is renowned as the "treasure of the water"[1]. However, the complex biochemical reactions involved in spoilage during storage and transport often lead to changes in odor and taste of salmon, which largely reduces consumer acceptance[2]. In order to better maintain salmon quality and extend its shelf life, preservation technologies that are convenient, safe and environmentally friendly are needed. Modified atmosphere packaging (MAP) is considered a highly effective preservation technique that can extend the shelf life of food[3]. The earliest MAP was achieved through vacuum packaging to preserve freshness[4]. Nowadays, the shelf life of aquatic products is primarily extended by adjusting the gas composition in MAP to inhibit microbial growth and delay protein degradation[5]. Currently, gases such as CO_2, N_2, and O_2 are commonly used, and the coordinated effect of these gases has enhanced the preservation of seafood products[6]. For example, studies revealed that MAP with high concentration of CO_2 had a particularly pronounced inhibitory effect on Gram-negative bacteria[7]. Zhang et al.[8] found that the initial dominant microbial population in grass carp was inhibited in the system of MAP (75% CO_2/25% N_2). Li et al.[9] demonstrated that the MAP system with 60% CO_2/5% O_2/35% N_2 significantly inhibited the growth of *Shewanella putrefaciens* and *Pseudomonas fluorescens*.

Although MAP plays a significant role in inhibiting the growth of microorganisms, the deterioration process of the texture of aquatic products continues. The adverse effect of extremely high CO_2 on the texture quality of salmon and other meat products have also been reported[2,10–11], but the mechanisms remain unclear. Endogenous enzymes including cathepsins and caspases, have a crucial impact on the quality of aquatic products as one of the primary factors in protein degradation[12]. After the death of fish, cathepsin B and L are released from lysosomes and contact with myofibrillar proteins, hydrolyzing actin and myosin[13]. Caspases are also demonstrated to play a significant role in improving the tenderization of meat products[14]. Studies have showed that the activities of caspase-3 and caspase-9 are negatively correlated with shear force[15], and caspase-8 is also involved in the deterioration of meat quality as an initiator caspase[16]. However, there are few studies about the mechanisms of MAP on the degradation of

protein related with these enzymes. Therefore, this study investigated the effects of air, 90% CO_2/10% O_2, 75% CO_2/10% O_2/15% N_2, and 60% CO_2/10% O_2/30% N_2 MAP at 4 °C on the quality of salmon fillets, from the perspectives of protein degradation and endogenous enzyme activity. The aim was to elucidate the reasons why the preservation effect of MAP with high concentrations of CO_2 was lacking, so as to provide new theoretical basis for the MAP preservation of salmon and other aquatic products.

2 Materials and methods

2.1 Sample preparation

The fresh salmon (each one about 6 kg) was purchased from a local supermarket (Pudong District, Shanghai, China). The salmon samples were headed, skinned and boned by skilled workers in the supermarket. The fresh salmon was cut into pieces approximately 12 cm in length, 6 cm in width and 2 cm in height. Then they were placed in a foam box filled with crushed ice and were transported to the laboratory within 0.5 h. After arriving at the laboratory, the samples were washed with cold water. After being drained of the water, the salmon fillet was put into a polyamide/polyethylene bag (Xilong Packaging Co., Ltd., Shijiazhuang, China; size: 32 cm × 22 cm; thickness: 0.32 mm; oxygen permeability: 7 $cm^3/(m^2 \cdot 24\ h \cdot 0.1\ MPa)$). Then it was filled with gas mixture with a gas-product ratio of about 3:1 (mL/g) and sealed. The samples were randomly divided into four groups: air, ambient (CK), 90% CO_2/10% O_2 (90C10O), 75% CO_2/10% O_2/15% N_2 (75C10O15N), and 60% CO_2/10% O_2/30% N_2 (60C10O30N). Finally, all samples were stored at 4 °C for 12 d and samples were taken every 2 d.

2.2 Determination of total viable count (TVC) and psychrotrophic bacterial count (PBC)

The bacterial counts were measured according to the method described by Yu et al.[17] with some modifications. The salmon fillets (25 g) were mixed with 225 mL of sterilized saline (0.85 g/100 mL) in a sterile homogenization bag and then homogenized for 1 to 2 min. After diluting the bacterial solution tenfold, 1 mL of the diluted solution was added to plate count agar containing 5 g/100 mL sodium chloride, the PBC was calculated after cultivated at 4 °C for 10 d.

2.3 Determination of physical and chemical properties

2.3.1 Determination of total volatile basic nitrogen (TVB-N) content

The TVB-N content was determined according to the method of Wang et al.[18] with some modifications. In brief, 5 g of fish meat was homogenized with 0.5 g of light magnesium oxide, and the analysis was done with an automated Kjeldahl nitrogen analyzer (Foss Analytical Co., Ltd., Höganäs, Sweden). The TVB-N content was expressed as mg/100 g of salmon. Each sample was tested in triplicate.

2.3.2 Determination of thiobarbituric acid reactive substances (TBARS) value

The TBARS value were determined according to the method of Xue et al.[19] with some modifications. In brief, the salmon pieces were mixed with 20 g/100 mL trichloroacetic acid (TCA) at a ratio of 20:1 (mL/g) and centrifuged at 8 000 × *g* for 10 min at 4 °C. The 5 mL supernatant was mixed with 0.02 mol/L 2-thiobarbituric acid and the mixture was heated in boiling water for 20 min. After cooling, the absorbance was measured at 532 nm. The TBARS value was calculated according to the following equation (1):

$$\text{TBARS value (mg/kg)} = A_{532\,\text{nm}} \times 7.8 \quad (1)$$

2.3.3 Determination of drip loss

The method for determining drip loss was based on Sun et al.[20] with some modifications. The initial sample mass was recorded as m_1, and the mass after storage was recorded as m_2. Each sample was tested in triplicate. Drip loss was expressed as a percentage of weight loss, and calculated according to the following equation (2):

$$\text{Drip loss (\%)} = \frac{m_1 - m_2}{m_1} \times 100 \quad (2)$$

2.3.4 Determination of texture parameters

The determination was conducted following the method described by Zhang et al.[21] with some modifications. The salmon samples were cut into uniform pieces of 2 cm × 2 cm × 2 cm in size, and tested using a TA.XT Plus analyzer (Stable Micro Systems Ltd., Surrey, UK). The test parameters were set as follows: 3 mm/s for pretest speed, 1 mm/s for test speed, 1 mm/s for post-test speed.

2.4 Determination of moisture distribution

The nuclear magnetic resonance imaging was conducted following the method described by Li et al.[22] with some modifications. The salmon samples were cut into pieces of 2 cm × 2 cm × 2 cm in size, and wrapped in one layer of plastic wrap and then placed directly into a 60 mm diameter nuclear magnetic tube, followed by insertion into the magnetic resonance imaging instrument (MR-60, Niumag Electric Co., Shanghai, China) for imaging analysis. The proton density weighted imaging of the gel was obtained through the MSE imaging sequence. Instrument conditions: repetition time = 500 ms, echo time = 18.2 ms. The proton density weighted map obtained was uniformly mapped and pseudo-colored using Carr-Purcell-Meiboom-Gill provided by the Newmai Co.

2.5 Determination of free amino acid (FAA) content

The determination of FAA profile was conducted following the method described by Konosu et al.[23] with some modifications. Firstly, 0.5 g of minced salmon was weighed and added to 15 mL of 5 g/100 mL TCA. After homogenization, it was allowed to stand at 4 °C for 2 h, then centrifuged at 10 000 × *g* at 4 °C for 10 min. Then, 5 mL of the supernatant was taken, and its pH was adjusted to 2.0 ± 0.2 using 1 mol/L NaOH. The final volume was adjusted to 10 mL, and the solution was filtered through a 0.22 μm aqueous phase filter membrane. Then it was subjected to instrumental analysis.

2.6 Determination of myofibril fragmentation index (MFI)

The MFI was determined with reference to Zou[24] with some modifications. Two grams of salmon fillet was homogenized with 40 mL buffer solution (20 mmol/L K_3PO_4, 100 mmol/L KCl, pH 7.1). After that, the mixture was filtered and then centrifuged at 6 000 × *g* at 4 °C for 15 min. The above steps were then repeated. Then, the resulting precipitate was dissolved in the same buffer to a protein concentration of (0.50 ± 0.05) mg/mL. The absorbance was immediately measured at 540 nm using a ultraviolet-visible

spectrophotometer (721N, INESA Electronics, Shanghai, China). The MFI was calculated by multiplying the absorbance at 540 nm by 200.

2.7 Determination of endogenous enzyme activity

2.7.1 Determination of caspase-3, caspase-8, and caspase-9 activities

The activities of caspase-3, caspase-8, and caspase-9 were measured using the caspase-3, caspase-8, caspase-9 activity assay kits (Biyuntian Biotechnology Co., Ltd., Shanghai, China). Approximately 0.1 g of salmon sample was taken and added to 1 mL of extraction buffer, followed by homogenization in an ice bath. The sample was centrifuged at 4 °C and 12 000 × *g* for 10 min. The supernatant was collected and placed on ice for further testing. The remaining steps were carried out according to the instructions provided. The microplate reader was preheated for more than 30 min, and the wavelength was adjusted to 405 nm to measure the absorbance. The relative activity was expressed as the ratio compared with the initial one.

2.7.2 Determination of cathepsin B and cathepsin L activities

The activities of cathepsin B and cathepsin L were measured using the cathepsin B and cathepsin L activity assay kits (Shanghai Jijin Chemical Technology Co., Ltd., Shanghai, China). First, 0.1 g of salmon sample was weighed and added to 1 mL of phosphate buffer saline (pH 7.4). After homogenization, the sample was centrifuged at 2 500 × *g* for 20 min at 4 °C. The remaining steps were carried out according to the instructions provided.

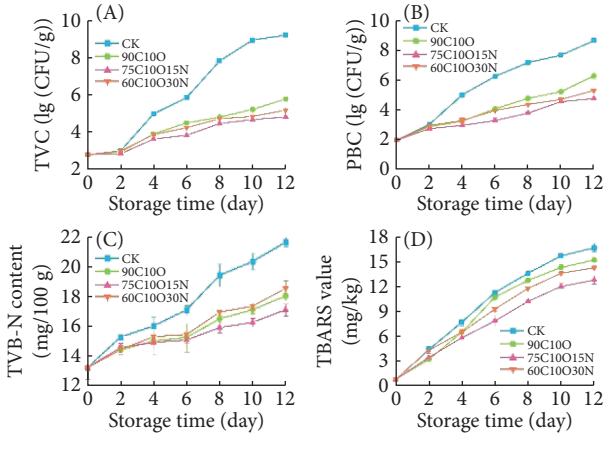
2.8 Statistical analysis

All data were processed using Excel 2019 software (Microsoft Co.). Statistical analysis was performed using SPSS 20.0 software (USA) to conduct one-way analysis of variance and Duncan's multiple-range test, with a significance level set at 0.05. Images were created using Origin 2021 software (OriginLab Co., USA).

3 Results and discussion

3.1 Microbial growth analysis

Microbial contamination is an important factor leading to the spoilage of food. Therefore, the TVC and PBC are often used as the main indicators to evaluate the freshness of aquatic products[25–26]. The results of TVC and PBC during the storage of salmon are shown in Figures 1A and B.



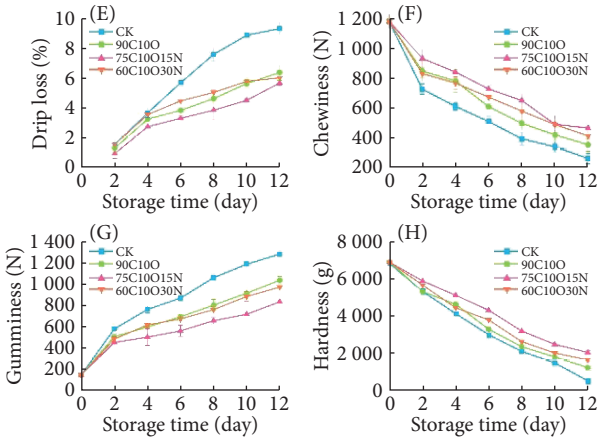


Figure 1 Changes of (A) TVC, (B) PBC, (C) TVB-N content, (D) TBARS value, (E) drip loss, (F) chewiness, (G) gumminess, and (H) hardness in salmon fillets under MAP containing different $CO_2/O_2/N_2$ ratios during storage at 4 °C. CK, ambient air; 90C10O, 90%CO_2/10% O_2; 75C10O15N, 75% CO_2/10% O_2/15% N_2; 60C10O30N, 60% CO_2/10% O_2/30% N_2.

The TVC in all groups continuously increased during storage at 4 °C. The initial TVC of CK group was 2.8 (lg (CFU/g)), which indicated that the sample was fresh[27]. The TVC in all MAP groups was significantly lower than CK group after 6 days of storage. This phenomenon might be due to the effect of CO_2 during storage[28]. The microbial acceptable threshold for seafood products is 7 (lg (CFU/g)). The TVC in CK group exceeded 7 (lg (CFU/g)) on the 8th day of storage, indicating that the sample had spoiled. The TVC in the MAP groups on the 12th day did not reach 7 (lg (CFU/g)) and was less than 62% of that in CK group, which indicated that 60% to 90% CO_2 had a significant antibacterial effect. A excessive concentration of CO_2 had a certain negative effect on microbial inhibition, because the TVC in the 90% CO_2/10% O_2 group was higher than those in the 75% CO_2/10% O_2/15% N_2 and 60% CO_2/10% O_2/30% N_2 groups. This phenomenon might have been caused by the fact that higher concentrations of CO_2 could directly interact with certain functional groups in fish muscle proteins, changing the spatial structure of the proteins, disrupting the interactions between protein molecules, increasing the solubility of the proteins, and providing nutrients for microorganisms, thus weakening the inhibitory effect of MAP on the growth of microorganisms[29].

The growth trend of PBC was similar to the TVC (Figure 1B). The initial PBC was 1.97 (lg (CFU/g)), indicating that the salmon fillets contained psychrotrophic bacteria. The PBC in the 90% CO_2/10% O_2, 75% CO_2/10% O_2/15% N_2, and 60% CO_2/10% O_2/30% N_2 groups reached 6.27, 4.76, and 5.29 (lg (CFU/g)) on the 12th day, respectively, which indicated that the 90% CO_2/10% O_2 group was less effective in inhibiting psychrotrophic bacteria compared to the other MAP groups.

3.2 Physical and chemical property analysis

3.2.1 Changes of TVB-N content

TVB-N content is one of the most widely used quality indicators for seafood products and is an important measure of the freshness of aquatic products[30]. It is also closely related to enzyme activity[31]. Figure 1C shows the changes in TVB-N contents in salmon fillets under different MAP groups. The TVB-N content in all groups gradually increased, with CK group reaching 21.67 mg/100 g after 12 days of storage. The increasing trends of TVB-N contents were

similar to the TVC. Li et al.[32] found that the TVB-N content of aquatic products could be related to the spoilage action of *S. putrefaciens*. Similar findings were also observed in the study of Gonçalves et al.[33]. This indicated that MAP could suppress the accumulation of TVB-N by inhibiting microbial proliferation.

3.2.2 Changes of TBARS value

The TBARS value reflects the extent of lipid secondary oxidation products[34]. Figure 1D shows the trends in TBARS values. The TBARS values for all groups were 0.7 mg/kg at the beginning of storage, and then continuously increased with the extension of storage time. The sudden increase in TBARS values on the second day was consistent with changes in caspase activity, possibly due to the accumulation of reactive oxygen species (ROS) during the process of cell apoptosis, leading to more severe lipid oxidation[35]. On the 12th day of storage, the TBARS value of CK group was the highest, reaching 16.6 mg/kg, while the TBARS values for the 60% CO_2/10% O_2/30% N_2, 75% CO_2/10% O_2/15% N_2, and 90% CO_2/10% O_2 groups were 14.2, 12.7, and 15.1 mg/kg, respectively, which indicated that the MAP could significantly inhibit the increase in TBARS values throughout the storage process. On the 12th day, the TBARS value of the 90% CO_2/10% O_2 group was significantly higher than the 75% CO_2/10% O_2/15% N_2 and 60% CO_2/10% O_2/30% N_2 groups, indicating that the 90% CO_2/10% O_2 group had a promoting effect on lipid oxidation.

3.2.3 Changes of drip loss

The changes of drip loss are related to the degradation and oxidation of proteins, which can be used to assess the quality of aquatic products[36]. The changes in drip loss of salmon fillets during storage are shown in Figure 1E. The drip loss of all groups increased continually during storage. The drip loss of CK group reached 9.33% after 12 days of storage, which was higher than the MAP groups. The drip loss of the 90% CO_2/10%O_2, 75% CO_2/10% O_2/15% N_2, and 60% CO_2/10% O_2/30% N_2 groups were 6.40%, 5.69%, and 6.04%, respectively. The lowest drip loss was found in 75% CO_2/10% O_2/15% N_2 group, indicating that the 75% CO_2/10% O_2/15% N_2 could maintain the muscle structure of salmon fillets more effectively. The MAP with CO_2 as high as 90% had an adverse effect on drip loss, in accordance with the previous study[2], which should be related with the degradation of muscle proteins caused by proteases[37]. The results of this experiment found that high concentrations of CO_2 did not necessarily minimize the formation of drip loss.

3.2.4 Changes of texture properties

Texture is an important quality parameter that affects the sensory and functional properties of salmon fillets. The changes in chewiness, gumminess, and hardness of salmon fillets under 4 °C storage conditions are shown in Figures 1F, G, and H. The initial chewiness of fresh salmon fillets was about 1 185.61 N. The CK group decreased to 509.41 N on the 6th day and reached 258.71 N on the 12th day. The samples from the 75% CO_2/10% O_2/15% N_2 group had the highest chewiness on the 12th day, and a similar trend was observed in hardness. The gumminess of CK group exceeded 1 200 N after 12 days of storage, while the MAP groups was less than 1 000 N. This indicated that MAP could effectively inhibit the increase of gumminess, in agreement with the previous research[2]. On the 12th day, the hardness of the MAP groups with 90% CO_2/10% O_2, 75% CO_2/10% O_2/15% N_2, and 60% CO_2/10% O_2/30% N_2 were 1 253.66, 2 058.56, and 1 661.22 g, respectively. The 75% CO_2/10% O_2/15% N_2 group had the highest hardness and chewiness, and the lowest gumminess. The changes in hardness, chewiness, and gumminess of salmon fillets are attributed to the degradation in myofibrils caused by proteases and bacterial proliferation[38]. It was hypothesized that extreme high CO_2 (90%) dissolved in the muscle, leading to a lower pH micro-environment and the changes in the microstructure of the muscle, consequently promoting the degradation of muscle protein[11], while 75% CO_2/10% O_2/15% N_2 had lower dissolving effect on muscle protein.

3.3 Moisture distribution analysis

Nuclear magnetic resonance imaging can intuitively display the moisture distribution in salmon fillets. The higher the density of water molecules, the redder the color becomes[39]. The nuclear magnetic resonance imaging of the salmon fillets under MAP is depicted in Figure 2. The color of salmon fillets in all groups gradually changed from red-yellow to blue during storage at 4 °C. This phenomenon indicated a continuous decrease in the moisture content of the flesh salmon fillets with the extension of storage time, which was consistent with the research results on drip loss. Most of the CK group had turned blue on the 6th day, indicating that the moisture had essentially been lost. Compared to the 90% CO_2/10% O_2 group, the 75% CO_2/10% O_2/15% N_2 and 60% CO_2/10% O_2/30% N_2 groups had significantly more red color, indicating that the 90% CO_2/10% O_2 group affected the salmon fillets adversely. The same result could also be observed on the 12th day. The 75% CO_2/10% O_2/15% N_2 group had the highest water retention ability. The results were consistent with the texture research. The study indicated that the 75% CO_2/10% O_2/15% N_2 group was more effective than the 90% CO_2/10% O_2 group in maintaining the tissue structure of salmon fillets, thereby inhibiting water loss.

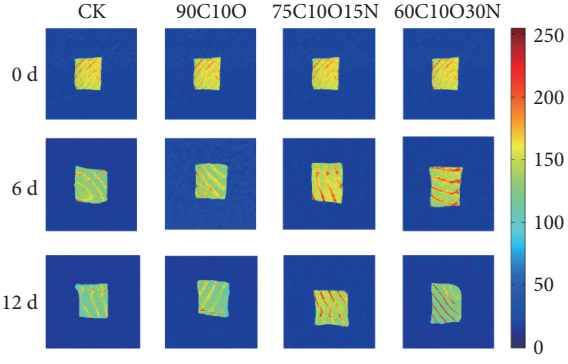


Figure 2 Nuclear magnetic resonance imaging of salmon fillets under MAP with different CO_2/O_2/N_2 ratios during storage at 4 °C. CK, ambient air; 90C10O, 90%CO_2/10% O_2; 75C10O15N, 75% CO_2/10% O_2/15% N_2; 60C10O30N, 60% CO_2/10% O_2/30% N_2.

3.4 FAA content analysis

Amino acids are the basic units that make up proteins and are an important nutritional indicator of the quality of aquatic meats[40]. Studies have revealed that FAAs are not only highly correlated with the final flavor characteristics of aquatic products, but also reflect the degradation of protein[41]. The contents of FAAs are shown in Table 1. A total of 17 amino acids were detected in fresh salmon fillets, and different FAAs imparted various flavors to the samples. The initial content of total FAAs in the fresh sample fillets was

Table 1 Changes of FAA content of salmon fillets under MAP with different $CO_2/O_2/N_2$ ratios at 4 °C (mg/100 g).

FAA	0 d	12 d			
		CK	90C10O	75C10O15N	60C10O30N
Asp	0.52 ± 0.09^{e}	20.98 ± 0.68^{a}	18.50 ± 0.66^{b}	12.99 ± 0.79^{d}	17.22 ± 0.35^{c}
Thr	7.39 ± 0.55^{e}	26.17 ± 0.98^{a}	18.81 ± 0.50^{c}	16.77 ± 0.49^{d}	21.39 ± 0.57^{b}
Ser	4.77 ± 0.38^{c}	6.85 ± 0.41^{b}	12.07 ± 0.71^{a}	12.24 ± 1.38^{a}	13.06 ± 1.48^{a}
Glu	20.97 ± 1.79^{c}	16.60 ± 1.88^{d}	30.20 ± 1.87^{ab}	27.47 ± 1.22^{b}	30.96 ± 1.85^{a}
Gly	10.11 ± 0.72^{b}	11.35 ± 0.73^{ab}	12.21 ± 0.69^{a}	12.32 ± 1.38^{a}	13.01 ± 1.49^{a}
Ala	37.26 ± 2.45^{a}	43.75 ± 2.58^{a}	41.42 ± 2.06^{a}	42.41 ± 6.63^{a}	42.52 ± 3.59^{a}
Cys	1.06 ± 0.30^{a}	0.52 ± 0.12^{b}	0.15 ± 0.03^{b}	0.43 ± 0.28^{b}	0.24 ± 0.08^{b}
Val	8.00 ± 0.65^{c}	10.12 ± 0.73^{b}	11.70 ± 0.70^{ab}	12.24 ± 1.61^{a}	12.63 ± 1.43^{a}
Met	3.40 ± 0.29^{b}	4.64 ± 0.33^{b}	4.76 ± 0.31^{ab}	6.35 ± 1.63^{a}	4.58 ± 1.02^{b}
Ile	4.03 ± 0.22^{bc}	3.47 ± 0.53^{c}	4.92 ± 0.31^{ab}	5.33 ± 0.72^{a}	5.29 ± 0.76^{a}
Leu	8.53 ± 0.50^{b}	9.59 ± 0.96^{b}	12.81 ± 0.77^{a}	14.16 ± 2.32^{a}	13.74 ± 1.58^{a}
Tyr	10.25 ± 0.59^{a}	6.90 ± 1.17^{b}	7.98 ± 0.37^{ab}	9.47 ± 2.14^{a}	8.52 ± 1.14^{ab}
Phe	5.71 ± 0.34^{c}	7.82 ± 0.72^{b}	9.57 ± 0.58^{ab}	10.90 ± 2.01^{a}	9.73 ± 1.12^{ab}
Lys	200.78 ± 2.98^{e}	283.37 ± 5.71^{a}	264.21 ± 10.63^{b}	234.93 ± 6.98^{d}	251.89 ± 4.00^{c}
His	35.68 ± 3.07^{b}	60.83 ± 4.61^{a}	55.42 ± 5.88^{a}	42.80 ± 2.97^{b}	36.55 ± 2.17^{b}
Arg	1.65 ± 0.18^{d}	46.93 ± 4.72^{a}	38.37 ± 7.07^{b}	28.84 ± 2.72^{c}	44.89 ± 2.41^{ab}
Pro	3.23 ± 0.34^{b}	3.53 ± 0.14^{ab}	3.71 ± 0.36^{ab}	4.33 ± 0.91^{a}	4.36 ± 0.67^{a}
Total FAAs	363.33 ± 12.37^{d}	563.39 ± 3.11^{a}	546.83 ± 10.31^{ab}	493.98 ± 10.23^{c}	530.61 ± 11.46^{b}

Notes: Data are expressed as mean ± standard deviation. Different superscript lowercase letters in the same row represent significant differences between groups ($P < 0.05$). CK, ambient air; 90C10O, 90%CO_2/10% O_2; 75C10O15N, 75% CO_2/10% O_2/15% N_2; 60C10O30N, 60% CO_2/10% O_2/30% N_2.

363.33 mg/100 g. After 12 days of storage, the total FAA content in the CK group reached 563.39 mg/100 g, the 90% CO_2/10% O_2, 75% CO_2/10% O_2/15% N_2, and 60% CO_2/10% O_2/30% N_2 groups reached 546.83, 493.98, 530.61 mg/100 g, respectively. An increasing trend in the total FAA content was shown by all groups when compared to the initial fresh salmon fillets. The total FAA content in all MAP groups was lower than the CK group, indicating that MAP could effectively inhibit the production of total FAAs. It was known that the activity of endogenous enzymes could be inhibited by MAP, thereby reducing the formation of FAAs. On the 12th day, the total content of FAAs in the 90% CO_2/10% O_2 group was found to be 52.85 and 16.22 mg/100 g higher than the 75% CO_2/10% O_2/15% N_2 and 60% CO_2/10% O_2/30% N_2 groups, and the content of the bitter amino acid histidine was found to be higher by 12.62 and 18.87 mg/100 g, respectively, indicating that the 90% CO_2/10% O_2 group was less effective in inhibiting the generation of total FAAs and bitter amino acids compared to the 75% CO_2/10% O_2/15% N_2 and 60% CO_2/10% O_2/30% N_2 groups.

3.5 MFI analysis

MFI can reflect the degradation and extension of muscle myofibrillar proteins[42]. The changes in MFI are shown in Figure 3A. As storage time increased, the MFI were continuously rising. The rapid increase in MFI of salmon fillets during days 0–4 could be attributed to the combined effects of increased caspase activity and O_2 in MAP promoting microbial growth. After 12 days of storage, the MFI for the CK group was 170.5, while the MFI for the MAP groups did not exceed 140. MAP could retard the degradation of myofibrillar protein by inhibiting bacteria growth, in accordance with the previous study[29]. Under the storage condition of 75% CO_2/10% O_2/15% N_2, the MFI of salmon fillets was the lowest, which demonstrated that the extremely high CO_2 had an adverse effect to inhibit the myofibril degradation. It was in consistent to the results of drip loss and texture properties.

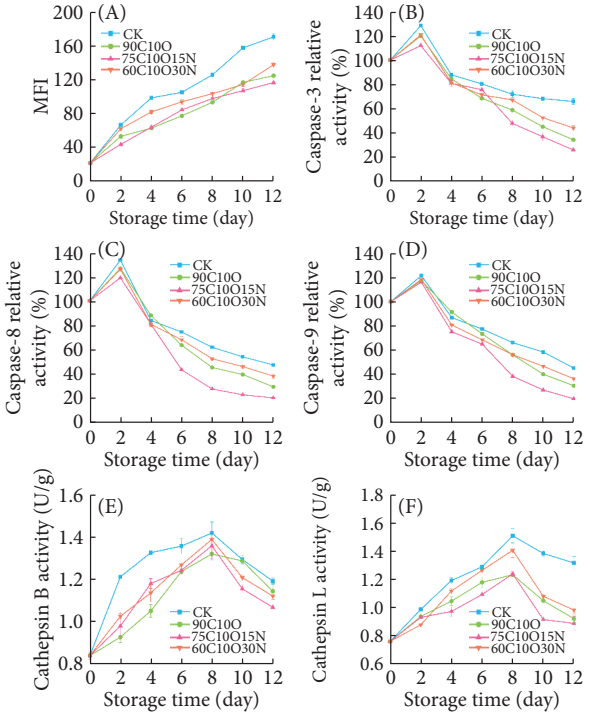


Figure 3 Changes of (A) MFI, activities of (B) caspase-3, (C) caspase-8, (D) caspase-9, (E) cathepsin B, and (F) cathepsin L in salmon fillets under MAP containing different $CO_2/O_2/N_2$ ratios during storage at 4 °C. CK, ambient air; 90C10O, 90%CO_2/10% O_2; 75C10O15N, 75% CO_2/10% O_2/15% N_2; 60C10O30N, 60% CO_2/10% O_2/30% N_2.

3.6 Enzyme activity analysis

3.6.1 *Changes in caspase-3, caspase-8, and caspase-9 activities*

Caspases are considered to be key proteases executing apoptosis, which are associated with postmortem proteolysis in skeletal

muscles[43–44]. Therefore, the relative activities of caspase-3, caspase-8, and caspase-9 of salmon fillets under MAP during storage were studied, as shown in Figures 3B, C, and D. The relative activity of caspase-3 in all samples increased in the first 2 days and then decreased gradually during storage. The relative activities of all MAP groups were lower than that in the CK group, which indicated that MAP could suppress the relative activity of caspase-3. When stored for 12 days, the caspase-3 relative activity in the 90% CO_2/10% O_2 group was 34.13%, which was higher than that in the 75% CO_2/10% O_2/15% N_2 group (25.77%). It was indicated that the activity of caspase-3 could be inhibited more effectively by the 75% CO_2/10% O_2/15% N_2 MAP than by the 90% CO_2/10% O_2 MAP. Caspase-8 and caspase-9 are a class of initiator caspases in apoptosis[45]. The change pattern of the relative activities of caspase-8 and caspase-9 were consistent with caspase-3. In general, the relative activities of caspase-8 and caspase-9 in MAPs were lower than that in the CK group, and 75% CO_2/10% O_2/15% N_2 group had the lowest relative activities of caspase-8 and caspase-9. The results showed that MAP of 75% CO_2/10% O_2/15% N_2 could retard the process of apoptosis most effectively. It was reported that caspases could be activated by ROS, which was produced from lipid oxidation[46]. Therefore, it was hypothesized that 75% CO_2/15% N_2/10% O_2 achieved the reduction of the activity of caspases by inhibiting lipid oxidation.

3.6.2 *Changes of cathepsin B and cathepsin L activities*

Cathepsin B exhibits strong peptidyl dipeptidase activity and is reported to involve in the degradation of myosin and sarcoplasmic proteins[47]. As shown in Figure 3E, the activity of cathepsin B in fresh salmon fillets was 0.83 U/g, and the activity of cathepsin B in salmon fillets from all groups reached its peak on day 8. The activities of cathepsin B in MAP groups were all lower than that in the CK group on the 12th day, indicating that MAP treatment could inhibit the activity of cathepsin B. The activity of cathepsin B in the 90% CO_2/10% O_2 group was lower than the other two groups within the first 8 days of storage, but was higher at the end of storage. Cathepsin L binds to hydrophobic amino acid residues and is capable of hydrolyzing various structural proteins[48–49]. As shown in Figure 3F, the initial activity of cathepsin L in fresh salmon fillets was 0.76 U/g, which also increased to the peak on day 8. The results showed that MAP significantly inhibited the activity of cathepsin L in salmon fillets. The activity of cathepsin L in the 75% CO_2/10% O_2/15% N_2 group was the lowest, indicating that higher or lower CO_2 concentrations were not conducive to inhibiting the activity of cathepsin L. In conclusion, the results of the activities of caspase-3, caspase-8, caspase-9, cathepsin B, and cathepsin L indicated that the caspases played a role in the degradation of muscle protein more probably in the early stage, while cathepsin B and cathepsin L were responsible for the degradation in the mid and later stage.

3.7 Correlation analysis

The correlations between TVC, PBC, physicochemical properties, enzyme activities were analyzed, and the heatmap are shown in Figure 4. It was observed that the MFI was strongly positively correlated to TVC, PBC, TVB-N content, TBARS value, gumminess, drip loss, and strongly negatively corelated with chewiness and hardness. It was also positively related with cathepsin B and cathepsin L. It was hypothesized that the degradation of myofibril led to the decrease of texture properties and increase of TVB-N content. The decrease of the texture was mainly attributed to the fact that the myofibrillar proteins maintaining the main part of the muscle were degraded by microorganisms and endogenous enzymes, and the muscle structure was damaged. Among the studied five enzymes, cathepsin B and cathepsin L were more likely to participate in the degradation for salmon fillets directly, while caspases might play a role in activating cathepsins the early stage[44].

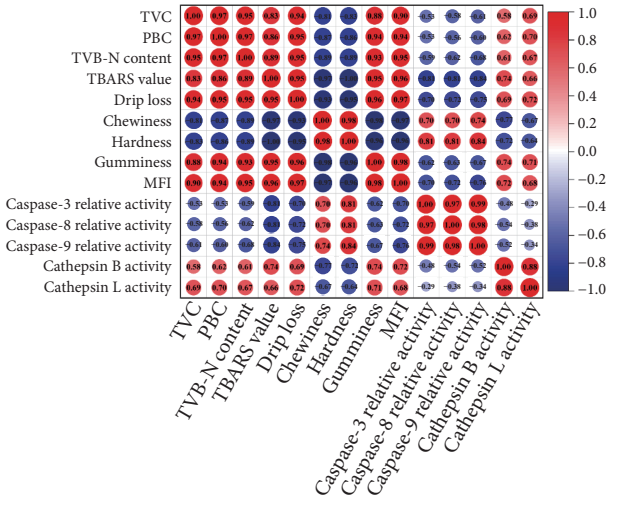


Figure 4 Pearson correlation heatmap of salmon fillets for TVC, PBC, TVB-N content, drip loss, TBARS value, texture parameters, MFI, and enzyme activity. Where the size of the circle represents the absolute value of the correlation coefficient. Positive correlations are represented in red, and negative correlations in blue.

4 Conclusion

This experiment investigated the impact of three different MAP ratios (90% CO_2/10% O_2, 75% CO_2/10% O_2/15% N_2, and 60% CO_2/10% O_2/30% N_2) on the microbial counts, physicochemical properties, and endogenous enzyme activities of salmon fillets. It was revealed through indicators such as TVC, PBC, TBARS value, and drip loss that the preservation effect of the MAP with 90% CO_2/10% O_2 was lower than the MAP with 75% CO_2/10% O_2/15% N_2. The results of endogenous enzymes also revealed that the MAP with 90% CO_2/10% O_2 did not inhibit the increase of activities of caspase-3, caspase-8, caspase-9, cathepsin B and cathepsin L as effectively as MAP with 75% CO_2/10% O_2/15% N_2, consequently leading to a worse texture properties and physiochemical qualities. Therefore, the extreme high CO_2 was not necessary for salmon fillets to inhibit bacteria and endogenous enzymes. The atmosphere of 75% CO_2/10% O_2/15% N_2 was more likely to be recommended based on this study. Besides, cathepsin B and cathepsin L might be more important in the degradation for salmon fillets under MAP conditions, which should be concerned in further study. This study proposed that MAP with 75% CO_2/10% O_2/15% N_2 was more appropriate to be applied to preserve the salmon fillet during transportation and storage process.

Conflict of 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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