Effects of modified atmosphere packaging combined with tea polyphenol treatment on the quality of grass carp during cold storage

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Abstract. In this study, the effect of tea polyphenols (TP) combined with modified atmosphere packaging on the quality change of Cri fish fillets in cold storage was studied, expecting to provide a new strategy for the biological preservation of fish fillets. Fresh fillets were packed in three different methods as follows: Sprayed in 0.5% tea polyphenols solution (CP group); Sprayed in 0.5% tea polyphenols combining 50% CO₂ + 50% N₂ modified atmosphere packaging (MAPC group); 50% CO₂ + 50% N₂ modified atmosphere packaging (MAP group) and fresh fillets was control group. All four groups were storaged at 0°C condition. Total bacterial count, TVB-N, TBA, K and pH were periodically assessed. The microscopic morphology of muscle tissue after 10 days cold storage was observed. After 10 d of cold storage, the TVC in the MAPC group (4.43±0.25 lg cfu/g) was obviously lower than that in the MAP group (5.04±0.65 lg cfu/g) or CP group (4.90±0.21 lg cfu/g), and even lower than that in the control group (6.14±0.17 lg cfu/g). The control group revealed the most obvious growth trend, increasing from 0.15±0.03 mg MDA/kg to 0.65±0.14 mg MDA/kg, followed by MAP group (0.15±0.08 mg MDA/kg to 0.52±0.07 mg MDA/kg), and CP group (0.13±0.05 mg MDA/kg to 0.47±0.03 mg MDA/kg), and the last was the MAPC group with the minimum amplification (0.14±0.02 mg MDA/kg to 0.47±0.13 mg MDA/kg). Tea polyphenols combining with modified atmosphere packaging is a new strategy in maintaining the quality of crisped grass carp during cold storage.

1. Introduction
Crisped grass carp is favored by the vast number of consumers due to its delicious meat, crisp taste and rich nutrition [1-3]. Studies have shown that the essential amino acids account for 39.88% of the total amino acids in Crisped grass carp, which is 60.9%, 18.7% and 36.7% higher than that of matrix protein, myofibrillar protein and collagen content in general grass carp, respectively [4]. In view of the characteristics of high moisture and endogenous enzymes content, in Crisped grass carp, spoilage easily occurs in the process of packaging and transportation. Therefore, the mode of living transportation is introduced into the sales market frequently, having a high cost which is not conducive to improving the economic benefit of Crisped grass carp [5]. With respect to the above, appropriate
preservation and cold-storage are critical for maintaining the freshness and quality of Crisped grass carp.

Tea polyphenols (TP) mainly consist of (-) epigallocatechin 3 gallate (EGCG, 50%–80%), (-) epigallocatechin (EGC, 9%–21%), (-) epicatechin 3 gallate (ECG, 7%–15%) and (-) epicatechin (EC, 3%–8%). Tea polyphenols is a new natural antioxidant, it possess some advantages, such as resist oxidation, anticorrosion, maintains freshness and safe use, etc. Fan et al. [6] alstudied the preservation effect of tea polyphenols on grass carp, silver carp and Pseudosciaena crocea, and it was found that tea polyphenols could significantly inhibit bacterial reproduction and slow down the oxidation of fish fat [7-10]

At present, the most widely used and safest methods for aquatic products preservation are fresh-keeping agents and modified atmosphere packaging at home and abroad. Dong conducted a study to evaluate the effect of vacuum packaging on the spoilage bacteria in the chilled beef, and the results showed that vacuum packaging could effectively inhibit the growth and reproduction of bacteria and other microbes [11]. However, a single preservative method can usually not achieve the ideal effect of preservation. In the study, tea polyphenols combined with modified atmosphere packaging were researched to explore the quality changes of Crisped grass carp fillets during storage, and provide a reference for the freshement of Crisped grass carp.

2. Materials and methods

2.1. Preparation of materials
Live Crisped grass carp purchased from Xiaolan Town, Zhongshan City, Guangdong. Tea polyphenol was purchased from Wuhan Kernel Bio-tech Co., Ltd (purity ≥ 98%, Wuhan, Hubei province, China).

2.2. Sample treatment
Live Crisped grass carp was slaughtered, besides, fish scales, head and tail and viscera were removed. Muscles on both sides of the back were sliced in a thickness of 5 mm and cleared for further usage. Fillets were randomly divided into 4 groups and were treated as follows: Fresh fillets without any treatment of samples (Control group), Modified atmosphere packaging with 50% CO₂+50% N₂ (MAP group), Spraying preservation with 0.5% tea polyphenols (CP group), Modified atmosphere packaging combined with 0.5% tea polyphenols, followed by gas packing with 50% CO₂+50% N₂ (MAPC group). The temperature of cold storage was preset as 4±1°C, and the cold storage duration of all groups was 10 d. The physical and chemical indexes were detected with an internal of 2 days, and all the results were repeated three times. The results were expressed with mean ± standard deviation.

2.3. Total viable counts (TVC)
TVC of weevres were performed by AOAC (2002). TVC were determined in plate count agar by the spread plate method. The results were reported as log 10 CFU (colony forming units)/g. Total volatile basic nitrogen (TVB-N), Thiobarbituric acid (TBA), pH value, K value, total volatile basic nitrogen (TVB-N, mg of TVB-N/100 g tissue), peroxide value, thiobarbituric acid reactive substances and pH, which are basic indicators of lipid oxidation and were determined in accordance with previously described methods [12-14].

2.4. Scanning electron microscopy (SEM)
Samples refrigerated for 10 days under four treatments and fresh unfrozen fillets were cut into small pieces of 1 mm×1 mm×1 mm in size in accordance with the method suggested by Zhu [15]. Samples were then soaked with freshly prepared 2.5% glutaraldehyde solution (prepared with 0.1 mol/L phosphate buffer, pH=7.4) for fixation. Samples were then washed in three times with 0.1 mol/L PBS (pH=7.4), 10 min per time. Then, dehydration was performed by using alcohol with the volume fraction of 30%, 50%, 70% and 90%, respectively, followed by dehydration with 100% ethanol for 2 times, 15 min each time. The samples were then fractured, freeze dried for 10 min and sputter sprayed
with a layer of 10 nm tungsten at 15KV. Imaging was performed after cryo-shielding and transferring the sample onto the sample stage of the field emission scanning microscope (Magellan 400, FEI, Eindhoven, the Netherlands). The magnifications used for imaging are 100.

2.5. Statistical analysis
Mean values with standard deviation (SD) were reported for each experiment. Each test was repeated in triplicate. Data were subjected to one-way ANOVA processing. Differences at $p < 0.05$ level were considered significant.

3. Results

3.1. TVC analyses
The number of spoilage microorganism reflects the degree of corruption in aquatic products [16]. The TVC of fillets at 4°C changed with the cold storage time, as shown in Fig. 1. The lowest TVC of fillets was $3.85\pm0.23$ lg cfu/g, which was increased in varying degrees of all the groups with the lengthening of the cold storage time. After 10 d of cold storage, the TVC in the MAPC group ($4.43\pm0.25$ lg cfu/g) was obviously lower than that in the MAP group ($5.04\pm0.65$ lg cfu/g) or CP group ($4.90\pm0.21$ lg cfu/g), and even lower than that in the control group ($6.14\pm0.17$ lg cfu/g). Among them, the TVC in the control group was increased faster after 4 d of cold storage, followed by the MAP group and the CP group, and the lowest was the MAPC group. The above results indicated that TP combined with modified atmosphere packaging could better inhibit the increase of TVC of fillets during storage.

![Figure 1. Effect on total number of colonies of fillets with cold storage time](image)

3.2. TBA analyses
TBA value is one of the effective indexes for evaluating the degree of lipid oxidation [17]. As illustrated in Fig. 2, TBA value was increased gradually with the prolongation of the cold storage time in all groups. The control group revealed the most obvious growth trend, increasing from $0.15\pm0.03$ mg MDA/kg to $0.65\pm0.14$ mg MDA/kg, followed by MAP group ($0.15\pm0.08$ mg MDA/kg to $0.52\pm0.07$ mg MDA/kg), and CP group ($0.13\pm0.05$ mg MDA/kg to $0.47\pm0.03$ mg MDA/kg), and the last was the MAPC group with the minimum amplification ($0.14\pm0.02$ mg MDA/kg to $0.47\pm0.13$ mg MDA/kg). During the period of 4 d~10 d, the growth rate of TBA value of the MAPC group was smaller than that of the CP group and the modified atmosphere packaging group, suggesting that the lipid oxidation of fillets could be inhibited by the combination of modified atmosphere packaging and spraying preservation.
3. TVB-N analyses

Fish products are degraded into proteins and non-protein nitrogen compounds during cold storage due to the action of some enzymes and the activity of microorganisms. The value of TVB-N is often used to assess the most important indicators of the quality of aquatic products. The change of TVB-N of Crisped grass carp fillets with the prolongation of the cold storage time was presented in Fig. 3 under different packaging treatment. As described in Fig. 3, the value of TVB-N was increasing gradually with the prolongation of cold storage time in all groups. Besides, the content of TVB-N was remarkably higher in the CP group and the modified atmosphere packaging group than that in the MAPC group, but smaller than that in the blank control group. After 10 d of cold storage, the content of TVB-N was 10.43±1.05mg MDA/kg and 11.2±0.96mg MDA/kg in the CP group and MAP group, respectively, and 9.86±0.44mg MDA/kg in the MAPC group. Possible mechanism might be correlated with the bacteriostasic activity of modified atmosphere packaging and the tea polyphenols, resulting in a slow reduction in the rate of oxidative deamination of non-protein compounds [11]. Therefore, modified atmosphere packaging coordinated with spraying preservation could achieve a better suppression of the formation of TVB-N during storage.
3.4. K value analyses

K value refers to the parameter of hypoxanthine and inosine decomposed by the muscle of fish under the action of endogenous cathepsin and microbes accounted for the percentage composition of ATP and related products. K value is an important index for evaluating the freshness of fish and other aquatic products [18, 19]. Figure 4 revealed changes in K value of fillets during cold storage. As prolonging the time of cold storage, the K value of each group showed a rising trend. During 0~4 d, except that in the control group, the difference in the growth trend of the K value was not significant with the gradual extension of the cold storage time in the CP group, the modified atmosphere packaging group, as well as the MAPC group. Furthermore, 4 d later, K value had been always higher in the MAP group when compared to that in the CP group and the MAPC group. However, K value of the control group was always higher than that of the other three groups throughout the whole process. K value reached 26.4±1.47 and 27.6±0.89 in the CP group and the modified atmosphere packaging group on 10 d, respectively, and it was 24.22±1.64 in the MAPC group, which were far less than that of the control group 30.28±1.77. It could be seen that modified atmosphere packaging combined with TP spraying preservation could effectively inhibit the decomposition of ATP and prolong the shelf life of the chilled fillets.

![Figure 4](image)

**Figure 4.** Effect on K value of fillets with cold storage time

3.5. Drip loss analyses

During the cold storage of fish, drip loss will occur in varying degrees, thus affecting the quality of fillets [20]. Changes in drip loss during cold storage was shown in Fig. 5. The overall rate of drop loss showed an upward trend with the lengthening of the cold storage time. Especially during the period of 0~2 d, the drip loss rate increased most rapidly, and then decreased gradually. It was speculated that muscles were stiff after death of fishes, leading to the squeezing of internal structure of muscles due to muscles contraction, accompanied by reduced water binding capacity. By comparing with MAP group and CP group, the rate of drip loss increased slowly in the MAPC group, which was 15.18±0.94 % on 10 d, followed by CP group of 18.22±1.26 % and modified atmosphere packaging group of 19.42±1.64 %, and the highest in the control group, reaching 22.08±1.76 %. The above results suggested that modified atmosphere packaging combined with spraying preservation could better inhibit drip loss of fillets compared to the other two individual processing groups during cold storage.
3.6. pH value analyses

pH value is also one of the indexes to judge the quality of fish meat. As indicated in Fig. 6, the trend of pH in all experimental groups was reduced first and then increased, which was consistent with the results reported by Gu [21]. Initially, the decrease of pH value might be due to the production of lactate from glycogen in glycolysis after the death of fishes. It might also be induced by the metabolism of microorganisms that generated carbon dioxide and formed carbonic acid into fish. Furthermore, the pH value gradually increased with the prolongation of refrigeration time. Possible reason might be associated with the decomposition of protein, resulting in the production of trimethylamine, dimethylamine, ammonia and other volatile salt compounds, eventually causing the rise of pH level of fishes due to the increase of alkaline substances [21, 22]. In addition, the change of pH value was lower in the CP group than that in the MAP group, MAPC group, and control group. As for the cause, tea polyphenols contained phenolic hydroxyl, which could dissociate H+ combining the common effect of modified atmosphere packaging on the inhibition of microbial metabolism, thus inhibiting the increase of pH value in fishes. The pH of MAPC group rise faster than CP group, may be due to promoting the growth of anaerobic microorganisms, and its mechanism still needs further researches and studies.

Figure 5. Effect on Drip loss of fillets with cold storage time

![Drip loss of fillets with cold storage time](image)

Figure 6. Effect on pH value of fillets with cold storage time

![pH value of fillets with cold storage time](image)
3.7. Microstructure
As shown in Fig. 7, the straight muscle fiber structure became looser in the fresh unchilled group than the control group, indicating the change of microstructure of muscles from straight to loose in the course of cold storage. It might be correlated with the degradation of myofibrillar protein in fillets under the action of endogenous cathepsin and microorganisms [23, 24]. The microstructure of the muscle tissue was more loose in the MAP group when compared to that in the CP group. Meanwhile, MAPC group had better muscle integrity of fillets compared with the first two groups, revealing a more satisfactory cold storage and preservation effect of modified atmosphere packaging combined with TP spraying preservation.

Figure 7. Effect on microstructure of fish fillets with different packaging methods

4. Conclusion
By comparing the quality of chilled Crisped grass carp fillets through different packaging treatments, it was found that modified atmosphere packaging combined with tea polyphenols, as well as spraying preservation and modified atmosphere packaging alone could better inhibit the lipid oxidation and the increase of TVC of fillets during the cold storage. Besides, they could better suppress the formation of TVB-N during storage and the decomposition of ATP in muscle tissues of fillets. Furthermore, in the process of cold storage, modified atmosphere packaging combined with TP spraying preservation could contribute to better inhibition of drip loss of fillets compared with modified atmosphere packaging and TP spraying preservation alone. The effect of the three packaging and cold storage methods was observed on the microstructure of musculature. 50 % CO$_2$ + 50 % N$_2$ modified atmosphere packaging combined with TP spraying preservation achieved better muscle integrity than that of modified atmosphere packaging and TP spraying preservation separately, suggesting that the former treatment was more conducive to the cold storage of fillets than that of the latter two approaches.

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