文章编号:1001-6880(2015)12-2006-08

气调包装与壳聚糖涂膜处理对鲳鱼冷藏期间品质变化的影响

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摘 要:本文研究了气调包装(MAP:80% CO_2/20% N_2)与壳聚糖(CS:10.0 g/L)涂膜处理对鲳鱼冷藏期间品质变化的影响。通过理化特性(pH 值、K 值、TVB-N 值与 TMA-N 值)、微生物特性(菌落总数)和感官评价等几个方面进行品质的综合评价。结果表明:气调包装与壳聚糖处理能延缓鲳鱼的腐败速度,抑制 K 值的升高,保持其感官品质。气调包装与壳聚糖处理能使冷藏鲳鱼的货架期分别延长 10 ~ 12 d。

关键词:鲳鱼;气调包装;壳聚糖;冷藏

中图分类号:S983 **文献标识码**:A DOI:10.16333/j.1001-6880.2015.12.002

Effects of Modified Atmosphere and Chitosan Edible Coating on Quality of Pomfret (*Pampus argenteus*) Fillets During Chilled Storage

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Abstract: The effects of modified atmosphere packaging (MAP: 80% CO_2/20% N_2) and chitosan (CS: 10.0g/L) edible coating on the quality of pomfret (*Pampus argenteus*) fillets during chilled storage were studied. Quality assessment was based on the properties of physicochemical [pH, K-value, total volatile base nitrogen (TVB-N), tri-methylamine (TMA-N)], microbiological (Total Viable Count, TVC) and sensory evaluation. The results showed that MAP and CS can delay the deteriorating speeds of *P. argenteus*, inhibit the increase of K-value and keep its sensory quality significantly. When compared with the control samples, the shelf-life of pomfret samples in MAP and CS stored at 4 ± 1 °C were 10, 12 d respectively.

Key words: *Pampus argenteus*; modified atmosphere packaging; chitosan; chilled storage

Introduction

Pomfret (*Pampus argenteus*) is extensively distributed along China coastline, as well as in Indian Ocean, Arabian Gulf and North Sea[1,2]. It is used to be considered as primary marine fishery resources with the high economic interest in China, together with *Pseudosciaena crocea*, *Trichiurus haumela* and *Sepia*. Like other seafood, fresh pomfret is highly perishable due to microbial activity and spoilage-specific chemical reactions, and thus has limited shelf-life. It is necessary to take the appropriate methods to extend the preservation period of pomfret and delay the decline of its quality.

Modified atmosphere packaging (MAP) technology is developed to slow down the growth of aerobic organisms and oxidation rate, and hence improve the quality of stored fresh foods to some extent [3-6]. MAP combined with chilled storage could prolong the shelf-life of seafood products by modifying the concentrations of O_2, N_2 and CO_2. There are several researches about the MAP of aquatic products, *e. g.* turbot (*Psetta maxima*) [7], striped red mullet (*Mullus surmuletus*) [8], Atlantic salmon (*Salmo salar* L.) [9-11], barramundi (*Lates calcarifer*) [12], bluefin tuna [13], striped catfish [14], lingcod (*Ophiodon elongates*) [15], red claw crayfish (*Cherax quadricarinatus*) [16], eel (*Anguilla anguilla*) [17] and chub mackerel (*Scomber japonicus*) [18].

In recent years, chemical preservatives are not well ac-

Received: November 14, 2014 Accepted: November 11, 2015

Foundation item: National agricultural achievement transformation funds (2014GB2C000081); Shanghai promote agriculture by applying scientific & technological advances projects (2015-4-12); special funding for the development of science and technology of Shanghai Ocean University (A2-0209-15-200061)

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cepted by consumers for the toxic effects. There has been an increasing interest in the application of various natural agents as a bio-preservative. Chitosan was extensively found in crustaceans, such as shrimp, crab and others, which was obtained by deacetylate reaction of chitin as a national food additive for having a broad range of applications in the food industry without harm and toxicity [19]. It exhibits antimicrobial activity against a range of food-borne microorganisms and has attracted attention as a potential natural food preservative consequently, which used in the field of food preservation and film packaging widely [16,20]. However, there were few studies concerning with the research on MAP and chitosan to the quality of pomfret. The aim of this study was to experimentally assess several quality indices (pH, K-value, TVB-N, TMA-N, TVC, Sensory evaluation) of fresh pomfret fillets, the impact of MAP and CS on its quality were also evaluated during chilled storage.

Materials and Methods

Materials

Fish sample

Fresh pomfret (*Pampus argenteus*) samples were obtained from the local fish market (Luchao Harbor, Shanghai, China) in April 2014 with a mean weight varying from 200 to 250 g. Samples were arrival at the laboratory in ice for 30 min. They were immediately gutted, washed, filleted and trimmed in a water-ice mixture, the samples had a weight about 100 g (15 cm in length and 10 cm in width), and then be randomly divided into three lots in ice and packaged using for different treatments.

Chemicals

Chitosan (in powder form, average molecular weight of chitosan was 1.6×10^5 Da and its deacetylation degree was more than 90%, Sino-pharm chemical Reagent Co. Ltd., China); Plate count agar (Land-Bridge Technology Co., Ltd., Beijing, China); 70% methanol, Trichloroacetic acid (TCA), Light magnesium oxide, boric acid, 95% ethanol, sodium chloride, potassium hydroxide (Sino-pharm chemical Reagent Co. Ltd., China).

Instruments

Sartorius PB-10 pH meter (Sartorius Scientific Instruments Co., Ltd., Germany); Unico-2100 (Unico Instruments Co., Ltd., Shanghai, China); LC-2010HT High pressure liquid chromatography (HPLC, Shimadzu Corporation, Japan); LHS-100CL Constant temperature and humidity box (Yiheng Scientific Instrument Co., Ltd., Shanghai); AUW320 Analytical balance (Shimadzu Corporation, Japan); Kjeltec8400 Kjeldahl apparatus (FOSS Co., Ltd., Shanghai, China); H-2050R High speed low temperature centrifuge (Hunan Xiangyi Laboratory Instrument Development Co. Ltd., China).

Preparation of Fish samples

One lot was stored in air (CK), the second lot was modified atmosphere packaged with 80% CO_2/20% N_2 (MAP), the final gas/sample ratio in all pouches was about 2:1 (v/w) for MAP condition, the third lot was coated with 10.0g/L chitosan (CS, Chitosan was dissolved in sterile water with 1.0% acetic acid and the final concentration was 10.0 g/L). All samples were stored in a refrigerator with controlled temperature (4 ±1 ℃) for 6-14 days. Quality assessments of samples from 3 groups were determinated by chemical (pH value, K-value, total volatile basis nitrogen and tri-methylamine), microbiological (Total Viable Count) and sensory evaluation for over 12 days at 2-day intervals.

Chemical analysis

Determination of pH value

5 g sample of the fish flesh was homogenized and mixed with 45 mL of boiled distilled water. The mixture was filtered 30 minutes later and the filtrate was measured using Sartorius PB-10 pH meter.

K value

ATP and its breakdown products were analyzed according to the modified method [21]. K-value was defined as the percentage of inosine (H_xR) and hypoxanthine (H_x) to the sum of ATP and degradation products, the formula used was as follows:

$$K - value(\%) = \frac{HxR + Hx}{ATP + ADP + AMP + IMP + HxR + Hx} \times 100$$

Determination of Total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) was estimated

by the micro-diffusion method with Kjeltec 2300. The micro-diffusion method [22] was determined by distillation after adding MgO to the homogenized samples. TVB-N values were expressed as mg TVB-N per 100 g fish muscle.

Determination of TMA-N

Tri-methylamine nitrogen (TMA-N) was measured using the method of spectrophotometer [23]. Potassium hydroxide was used to reduce the interference of dimethylamine. 1g minced fish muscle was extracted with a trichloroacetic acid solution (40%, w/v) at 8,000 rpm for 1min. The mixture was filtered. 10 mL of toluene, 1 mL of formaldehyde (10%, v/v) and 3 mL potassium hydroxide solution (45%, w/v) were added to 5 mL of filtrate. Toluene layer was transferred to another tube and 5 mL of picric acid solution (0.2 g/L) was added after shaking, the results of absorbance were read at 410 nm. A calibration curve with a trimethylamine hydrochloride solution (0.01 mg/mL) was prepared to quantify TMA-N according to the AOAC method. The results were expressed as TMA-N/100 g of fish.

Bacteriological analysis

Total viable count (TVC) is an indicator of degradation, which is mostly due to the growth of specific spoilage organisms (SSO) in seafood[24]. According to GB 47892-2010, 25 g Fish samples were transferred to a stomacher bag. 225 mL of 0.1% peptone water with salt (NaCl, 0.85%, w/v) were added and homogenized for 60 s with a stomacher. Other decimal dilutions were obtained from this dilution and 1 mL of three dilutions was transferred in triplicate to Petri dishes containing 15 mL commercial PCA. Total viable counts (TVC) were determined by counting the number of colony-forming units after incubation at 30 ℃ for 72 h.

Sensory evaluation

The acceptability of marine food was determinated by the changes of samples in their sensory attributes. Sensory evaluation was carried out according to SC/T 3103-2010[25], and fish samples were calculated using the quality index method (QIM) based on the freshness quality grading system[21] by five trained panelists. The sensory assessment approach evaluates the freshness by giving demerit points according to certain aspects of flesh color, odor, texture and hardness respectively (Table 1) [26]. The sensory scores were ranged from 1 to a maximum of 5, where 5 represented the freshest quality and the scores increased according to spoilage up to 3 for each parameter. Panelists were asked to describe whether the fish samples were acceptable or not, this was used to determine the shelf-life of pomfret fillets.

Table 1 Sensory evaluation of pomfret fillet

Sensory description	Best (5′)	Better (4′)	Normal (3′)	worse (2′)	worst (1′)
Color	Extremely bright	Very bright	Moderately bright	Slightly dull	Very dull
Odor	Fresh flavor	Fresh seaweed flavor	Moderate seaweed flavor	No flavor	Spoiled flavor
Texture	Extremely firm muscle	Very firm muscle	Moderately firm	Slightly soft	Very soft
Appearance	Rich in elasticity	Very elasticity	Moderate elasticity	Slightly elasticity	Inelastic

Statistical analysis

Data were expressed as mean values ($n = 3$) accompanied by standard deviation. Analyses were performed with the SPSS software (version 13.0) (SPSS, Chicago, IL, USA) to detect significant differences between lots and periods of cold storage. One-way analysis of variance was used, the Turkey multiple comparison test was used to find significant differences between lots. Significance level was set at 0.05. The figures were drawn as origin software (Version Pro V8.5).

Results and Discussion

pH value

Changes in pH values during storage were shown in Table 2. The initial pH of the fish samples was 7.23 ± 0.01. The pH values of CK and CS decreased slightly at early stages and then increased gradually. The initial decrease of pH might be related to the accumulation of

lactic acid, a product of glycolysis, while the increase during later storage may be caused by the growth of spoilage bacteria leading to the accumulation of alkaline components, such as ammonia and tri-methylamine.

Table 2 pH value of pomfret fillets from different treatments stored at 4 ± 1 ℃

Storage Time (days)	CK	MAP	CS
0	7.23 ± 0.01a	7.23 ± 0.01a	7.23 ± 0.01a
2	7.21 ± 0.01a	7.26 ± 0.01b	7.11 ± 0.02b
4	7.46 ± 0.01a	7.32 ± 0.01c	7.18 ± 0.02c
6	7.60 ± 0.02a	7.29 ± 0.03d	7.20 ± 0.01a
8		7.34 ± 0.02e	7.38 ± 0.02a
10		7.41 ± 0.03e	7.46 ± 0.02d
12		7.45 ± 0.03c	7.50 ± 0.01d

a-d Values in the same line followed by a different letter were significantly different ($P < 0.05$).
A Value represent the mean of six determinations ($n = 2 \times 3$) ± SD.
B Values on day 0 correspond to non-treated product.

The variation of pH in CS samples was slower than control samples, which revealed that chitosan could inhibit the growth of spoilage bacteria and prolong the shelf-life of pomfret samples. However, significant correlation ($P < 0.05$) was not found in MAP lot. The results suggested that examination of pH could not be a useful indicator of quality changes in pomfret fillets from MA packaging. Similar observations were reported [27-31], most fish contain only very little carbohydrate (> 0.5%) in the muscle tissue and only small quantities of lactic acid were produced post-mortem [32]. The results indicated that pH values of different methods increased after the values reached the minimum on day 2, especially the control group increased much faster because glycolysis reaction caused by the stop in circulation of blood and produced large amounts of lactic acid, succinic acid, phosphoric acid, thus leading to enhancement of organism acid and decrease of pH value. With the extension of storage time, protein decomposition produced amine and other alkali substances because of the increase in microorganisms and enzyme activity, which led to the gradual increase of pH value. CO_2 could inhibit the increase of microorganisms, enzyme activity and slow down the increase of pH value.

K-value

Variations in K-value during chilled storage were shown in Fig. 1. The amount of microorganisms was few in early storage and the effect of different preservatives on enzyme degraded from ATP in the pomfret fillet was various. Therefore, the resolution of ATP was accelerated with the mass propagation of microorganisms in later storage. K-value was the index of degradation of ATP and used as the most effective indicator for the freshness in fish. When the K-value was close to the 60% limit, the sample will reach the rejection level [33].

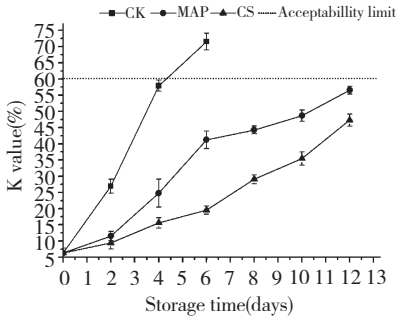

Fig. 1 K-value of pomfret fillets from different treatments stored at 4 ± 1 ℃

Initial K-value in fish samples was 6.3%, which reflected the fresh of pomfret samples. The K-value of control samples were 57.91% before day 4 and increased much faster than other groups ($P < 0.05$) during storage. It can be concluded that the lower K-value of MAP samples may result from the slower ATP degradation by the effect of CO_2/N_2 treatments on the fish samples. The result of the data also indicated that MAP was e-

qually effective in inhibiting the degradation of ATP and extending the chilled storage life of fish samples. Moreover, the K-value of CS group increased slowly and could not exceed the acceptance limit on day 12. The result showed that 10 g/L chitosan was effective in inhibiting the degradation of ATP and extending the cold storage life of fish samples.

Total volatile basic nitrogen (TVB-N)

Changes in TVB-N of pomfrets during storage were shown in Fig. 2, TVB-N includes the measurements of TMA (tri-methylamine), DMA (di-methylamine), ammonia and other volatile basic nitrogen compounds and has been reported as spoilage compounds and proposed as fish and shellfish spoilage indicator in many studies[34]. The initial TVB-N value of control samples was 5.663 ± 0.28 mgN/100 g. No significant difference between control and treated samples was detected before storage ($P < 0.05$). TVB-N values of the control samples increased rapidly and by day 6 exceeded the acceptance limits. TVB-N of MAP and CS treated samples increased slower in the first days, and a lag phase of about 2 days was observed. The shelf-life of CK, MAP and CS samples were 4, 8, 12 days. The concentration of CO_2 was act on the characteristic of microorganism effectively and inhibited the increase of microorganism.

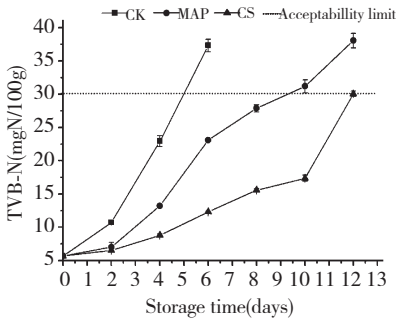

Fig. 2 TVB-N value of pomfret fillets from different treatments stored at 4 ± 1 ℃

TMA-N analyses

TMA-N values of pomfret fillets in each group were shown in Fig. 3. TMA-N contents were often used as a biochemical index to assess the keeping quality and shelf-life of fish [35]. TMA was produced during the decomposition of tri-methylamine N-oxide caused by bacterial spoilage and enzymatic activity, which was the main component responsible for the unpleasant odor of fish samples [36]. TMA-N values reached 5.0 mgN/100 g, which indicated the minimum limit of acceptability for fish samples[37].

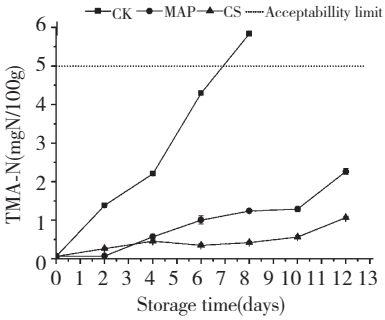

Fig. 3 TMA-N value of pomfret fillets from different treatments stored at 4 ± 1 ℃

The initial TMA-N content of pomfret fillets was low (0.061 ± 0.016 mg/N/100g of flesh) and indicated the freshness of samples. The TMA-N contents of fish sample packaged in CK were significantly faster ($P < 0.05$) than TMA-N contents of samples in MAP and CS after day 2 of storage for the amount of microorganisms was few in early stage and exceedingly increasing at later period. When ? shery products are stored in cold environment and contacted with atmospheric air directly, other enzymatic and chemical autolytic reactions were involved in the production of TMA-N compounds in the later shelf-life stage signi? cantly. Ammonia and dimethylamine may be considered the two crucial factors that influencing the rate of TMA-N [8].

Bacteriological analysis

The changes of TVC in pomfret fillets from different treatments under the cold storage were presented in Fig. 4. The initial of TVC in pomfret samples was found

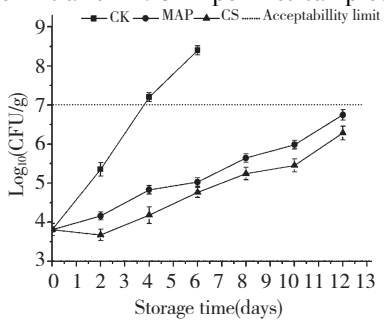

Fig. 4 TVC of pomfret fillets from different treatments stored at 4 ± 1 ℃

to be 3.812 ± 0.155 $\log_{10}$ CFU/g. The microbiota increased significantly ($P < 0.05$) after fish fillets were exposed to different treatments and stored under chilled conditions. The TVC of CK samples reached 7.0logcfu/g after 4 days of storage, which was the upper acceptability limit for freshwater and marine species by ICMSF.

The significant reduction of TVC in MAP samples can be attributed to the inhibitory effect of CO_2 on spoilage bacteria. Gas mixes with high levels of CO_2 inhibited or reduced the growth of various aerobic spoilage bacteria and provided the conditions for the growth of gram-positive bacteria [38,39]. The results of treatment indicated that samples with MAP could inhibit the growth of spoilage bacteria growth and extend the shelf life of fish samples to 8-12 days. Chitosan treatment showed no immediate bactericidal action at the very beginning, however the TVC decrease during the first 2 days storage. The TVC of samples coated with CS were always lower than the control sample ($P < 0.05$) and could not reach the acceptable limit even on day 12.

Sensory analyses

The results from the sensory analyses were presented for some selected attributes in Table 3. It indicated that sensory parameters showed significant decline in different groups with the increase of storage period. Pomfret fillets with the characteristic of fresh fish had a pleasant taste and odor at day 0. The results also indicated that the sensory properties of MAP and CS groups received a higher score than the lower acceptability limit of 3. The flesh texture and flesh odor of all packaged samples received scores above the acceptance limit of 3 at day 6. The most sensitive sensory attribute for the quality evaluation of pomfret was odor. The shelf life of pomfret fillet was 6 days for CK samples, 10 days for MAP samples, 12 days for CS samples.

Table 3 Sensory evaluation of pomfret fillets from different treatments stored at 4 ± 1 ℃

Sensory parameter	Treatment methods	Storage time (days)						
		0	2	4	6	8	10	12
Color	CK	4.726 ± 0.073a	3.970 ± 0.130a	3.678 ± 0.068a	3.210 ± 0.114a	2.435 ± 0.091a	1.682 ± 0.129a	0.827 ± 0.142a
	MAP	4.726 ± 0.073a	4.370 ± 0.104b	4.260 ± 0.074b	3.660 ± 0.108c	3.420 ± 0.084d	3.110 ± 0.164e	2.640 ± 0.096f
	CS	4.726 ± 0.073a	4.550 ± 0.071b	4.340 ± 0.108c	4.030 ± 0.045d	3.780 ± 0.104e	3.330 ± 0.104f	2.760 ± 0.195g
Odor	CK	4.690 ± 0.089a	4.250 ± 0.177a	3.600 ± 0.118a	3.120 ± 0.110a	2.179 ± 0.082a	1.434 ± 0.174a	0.663 ± 0.164a
	MAP	4.690 ± 0.089a	4.450 ± 0.112a	4.100 ± 0.094b	3.970 ± 0.076b	3.570 ± 0.180c	3.250 ± 0.160d	2.550 ± 0.158e
	CS	4.690 ± 0.089a	4.520 ± 0.045b	4.230 ± 0.120c	4.060 ± 0.065c	3.800 ± 0.061d	3.460 ± 0.129e	2.570 ± 0.110f
Texture	CK	4.720 ± 0.084a	4.240 ± 0.151a	3.780 ± 0.085a	3.140 ± 0.089a	2.348 ± 0.163a	1.459 ± 0.137a	0.915 ± 0.096a
	MAP	4.720 ± 0.084a	4.460 ± 0.065a	4.150 ± 0.224a	3.960 ± 0.082b	3.530 ± 0.065c	3.390 ± 0.107c	2.420 ± 0.076d
	CS	4.720 ± 0.084a	4.480 ± 0.045b	4.180 ± 0.084c	3.910 ± 0.089d	3.570 ± 0.076e	3.430 ± 0.152f	2.900 ± 0.124g
Appearance	CK	4.714 ± 0.050a	4.300 ± 0.109a	3.590 ± 0.138a	3.020 ± 0.084a	2.372 ± 0.128a	1.532 ± 0.141a	0.990 ± 0.103a
	MAP	4.714 ± 0.050a	4.500 ± 0.061a	4.170 ± 0.529b	3.880 ± 0.084b	3.100 ± 0.085c	2.910 ± 0.102cd	2.600 ± 0.064d
	CS	4.714 ± 0.050a	4.550 ± 0.011b	4.320 ± 0.110c	3.990 ± 0.055c	3.664 ± 0.080d	3.440 ± 0.065d	2.820 ± 0.095e

a-g Values in the same line followed by a different letter were significantly different ($P < 0.05$).

A Value represent the mean of six determinations ($n = 2 \times 3$) ± SD.

B Values on day 0 correspond to non-treated product.

From the above results it was obvious that MAP extended the shelf-life of pomfret samples as compared with the control samples. CS substantially contributed to the extension of shelf-life of pomfret samples delaying spoilage while impacting a pleasant flavor to fish products, and CS samples could be retaining the good quality characteristics in terms of sensory assessment. However, high levels of CO_2 were found to be associated with some negative sensory characteristics such as drip loss, reduced texture quality and color change [7,8].

Conclusion

The quality of pomfret fillets packaged with air (CK), MAP and CS under chilled storage for up 12 days were experimentally assessed. MA packaging had a positive effect on the shelf-life of the pomfret fillets, MAP fillets had an additional 6 days of shelf-life to the period of 4 days estimated for control samples when stored at 4 ± 1 ℃. When samples packaged under MA environment, which can inhibit the increase of K, TVB-N, TMA-N, TVC and showed a different evolution of the quality parameters. Chitosan can inhibit the growth of bacteria, keep its sensory quality and prolong the shelf-life of samples for another 8 days.

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