

Freshness and Shelf Life of Air Packaged and Modified Atmosphere Packaged Fresh Tilapia Fillets during Freezing-point Storage

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Abstract

The freshness and shelf life of filleted tilapia packaged in air packaging (AP) and modified-atmosphere packing (MAP) of CO₂/N₂ 30%/70% conditions, combined with freezing-point storage (-0.7°C–0°C) were investigated by sensory, textural, and physicochemical parameters, as well as microbiological and endogenous enzyme activities. The results showed that the total volatile base nitrogen, myofibril fragmentation index, sensory scores, total viable count, and pH in MAP tilapia fillets were lower than those of AP tilapia fillets, while the salt-soluble protein level and water holding capacity of MAP tilapia fillets were higher than those of AP tilapia fillets. Results showed that MAP combined with freezing-point storage exhibited a significant fresh-keeping effect on tilapia fillets, the shelf lives of AP and MAP tilapia fillets were 11th and 14th day, respectively; and total viable count and total volatile base nitrogen were 5.94 log CFU/g and 21.74 mg/100 g in MAP at 14th day, respectively. It was concluded that an application of MAP combined with freezing-point storage on tilapia fillets preservation was achieved.

Keywords: Tilapia fillets; Air Packaging (AP); Modified Atmosphere Packaging (MAP); Freezing-point storage; Freshness; Shelf life

Introduction

With increasing demand for tilapia fillets, retailers seek improved freshness and prolonged shelf life. Simultaneously, consumers demand high-quality food, with corresponding expectations of quality maintenance at a high level between the production and consumption of food. However, the shelf lives of fresh fishery products are most crucially affected by microbial activities, which are influenced mainly by the storage temperature [1]. Therefore, the food industry seeks methods to maintain the freshness and extend the shelf life of various types of fish.

The application of Modified Atmosphere Packaging (MAP) to food is an effective available method of food preservation. Use of this technology has increased recently for its effectiveness and low cost in reducing the occurrence of oxidative reactions [1]. Monteiro et al. [2] reported that the shelf life of refrigerated tilapia fillets, treated by a combination of MAP at 40% CO₂ and 60% N₂ and irradiation, was increased from 3–8 days. Erkan et al. [3] found that MAP (O₂/CO₂/N₂, 5%/70%/25%) storage could extend the shelf life of chub mackerel to 12 days, compared to the air-packaged (AP) and vacuum-packed shelf lives of 9 days each when stored at 4°C. Since fish is more highly perishable than meat, the product temperature is the most important factor for increasing its shelf life [4]. Freezing-point storage, in which the temperature is controlled between 0°C and the freezing point of the fish, has demonstrated efficacy for the refrigeration of fish of both marine and aquaculture origins [5]. According to Li et al. [6], the freezing point of tilapia is approximately -0.7°C. Zhu et al. [5] reported that controlled freezing-point storage at -0.7°C, combined with high CO₂ (60%) MAP, effectively maintained the quality of fresh catfish meat compared to traditional preservation methods. Freezing-point storage is better than refrigerated storage for extending the shelf life of fish; also, freezing-point storage may avoid formation of ice crystals, then keeping high water holding capacity. Therefore, freezing-point storage can maintain a better sensory, even though the shelf life is shorter than that of frozen storage. However, the lower amount of water frozen out leads to lower degrees of microstructural change, freeze denaturation,

and drip loss; therefore, fish freshness and integrity can be better in freezing-point storage than that in frozen storage [4]. Based on these advantages, a storage method combining (MAP) and freezing-point storage (-0.7°C–0°C) is used in the present study to investigate the sensory, textural, and physicochemical parameters of stored tilapia, in addition to the microbial and endogenous enzyme activities. The purpose of this study is to test the efficacy of the method in maintaining the freshness and extending the shelf life of tilapia fillets.

Materials and Methods

Fish samples

Tilapias were purchased from the China Resources Vanguard Shop (Guangzhou, China) in April 2016. Tilapias were put into plastic tank (volume 50 L) and transported alive to the laboratory within 30 min. Twenty fish (735 ± 37 g) were killed by knocking head, cut the dorsal meat, skinned, and filleted. Each of the forty control fillets was packaged individually in polyethylene bags and immediately kept chilled at -0.7°C–0°C. Another forty treated fillets were placed in individual MAP bags comprising PA/TIE/PE/TIE/EVOH/TIE/PE/TIE/PE and packaged under a mixed atmosphere (MA) of 30% CO₂/70% N₂. The CO₂ and N₂ were mixed using a gas mixer (Map-D400, Senrui, Suzhou China). After packaging, the fillet packages were stored at -0.7°C–0°C and analyzed after storage for 0, 3, 7, 11, and 14 days.

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Received October 07, 2016; **Accepted** November 04, 2016; **Published** November 11, 2016

Citation: He Y, Huang H, Li L, Yang X, Zhao Y, et al. (2016) Freshness and Shelf Life of Air Packaged and Modified Atmosphere Packaged Fresh Tilapia Fillets during Freezing-point Storage. J Nutr Food Sci 6: 564. doi: 10.4172/2155-9600.1000564

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On each sampling day, six fillet packages from the control and treatment groups were removed from storage and analyzed. The physicochemical analysis for each fillet included measurements of the pH, Total Volatile Basic Nitrogen (TVB-N), Water Holding Capacity (WHC), Myofibrillar Fragmentation Index (MFI), Salt-soluble Protein (SSP) level endogenous protease activities, and other indices such as texture, sensory evaluation, and microbiological analysis.

Physicochemical analysis

pH: The pH levels of the tilapia fillets were measured using the method described by Cyprian et al. [1] with some modifications. Three grams of minced fish were placed in 27 mL distilled water and homogenized for 1 min. The pH was measured using an Accumet glass electrode attached to an Accumet Five Easy Plus pH meter (Mettler-Toledo, Columbus, USA). The measurement was performed three times per sample; the reported results are the average of the three measurements for each sample.

TVB-N: The TVB-N values were analyzed using the method of Liu et al. [7]. Five grams of minced fish were placed in 45 mL 0.6 M perchloric acid and homogenized for 2 min. The mixture was centrifuged at 10,000×g for 10 min at 4°C. The supernatant was filtered by gauze and the filtrate was subjected to Kjeldahl steam distillation with 5 mL 30% (w/v) NaOH. Five milliliters of 30% (w/v) aqueous boric acid was used as the extraction solution. After 5 min of reaction time, 0.01 M HCl was used for neutralization titration.

WHC: The WHC was analyzed following the method described by Liu et al. [8]. Five grams of minced fish were centrifuged at 10,000×g for 15 min at 4°C. The WHC was determined as the liquid loss and expressed as the weight percentage of liquid retained in the fillets, as calculated by the formula:

$$\text{WHC (\%)} = W_2/W_1 \quad (1)$$

Where W_1 and W_2 represent the weight of the minced fish before and after centrifugation, respectively.

SSP: The SSP was extracted from the tilapia as described by Subbaiah et al. [9], with some modifications. Five grams of minced fish were placed in 50 mL of extraction buffer A (50 mM phosphate buffer, pH 7.4) and homogenized. The suspension was centrifuged at 10,000×g for 10 min at 4°C and the supernatant was decanted. Fifty milliliters of extraction buffer A was added to the precipitate and the above steps were repeated. After the second centrifugation, the sediment was re-suspended in 50 mL extraction buffer B (100 mM phosphate buffer including 1.1 M NaCl, pH 7.4) and held at 4°C for 90 min. The suspension was centrifuged a final time at 10,000×g for 10 min at 4°C and the supernatant was retained. The SSP content was stained using Coomassie Brilliant Blue dye and analyzed at 595 nm by UV3000 spectrophotometer (Mapada, Shanghai, China).

MFI: The MFI was determined using a slight modification of the procedure described by Soltanizadeh et al. [10]. Half a gram of minced fish was homogenized in 30 mL isolating buffer of 25 mM phosphate-buffered saline (PBS), including 100 mM KCl, 1 mM ethylenediaminetetraacetic acid (EDTA), and 1 mM MgCl₂ with a pH of 7.0 at 4°C for 30 s. The mixture was centrifuged at 10,000×g for 15 min at 4°C and the supernatant was decanted. Ten milliliters of buffer was added to the sediment and the mixture was passed through four layers of gauze to remove connective tissue and debris. The filtered mixture was then centrifuged at 10,000×g for 15 min and the supernatant was decanted. This process was repeated twice. Finally, the sediment

was re-suspended in 10 mL isolation buffer. The myofibril protein concentrations were determined by the Bradford method. Absorbance was measured at 595 nm. An aliquot of the myofibril solution was diluted in the isolation medium to a protein concentration of 0.5 ± 0.05 mg/mL. The diluted myofibril suspension was measured immediately at 540 nm using a UV spectrophotometer (Mapada, Shanghai, China). The measured absorbance was multiplied by 150 to produce the MFI.

Microbiological analysis

The total viable counts (TVC) of active microbes were analyzed following the method of Özyurt et al. [11] with some modifications. Five grams of fish muscle were mixed with 9 mL of Ringer's solution and homogenized for 1 min. Further decimal dilutions were made, and 1 mL of each dilution was pipetted onto the surface of plate-count agar plates. These were incubated for 72 h at 30°C. Microbiological counts were performed in triplicate and are expressed as log CFU/g.

Texture analysis

Texture profile analysis was performed using a Brookfield CT3 (Texture Analyzer, Brookfield, US) equipped with a 25 kg load cell and Texture Pro CT software. The fillets and the filleted whole fish were measured at three points, one each at the top, middle, and bottom of the fillet. A cylindrical probe of 4 mm in diameter was used to analyze the texture profiles. Testing conditions involved two consecutive cycles of 3 mm compression, cross-head movement at a constant speed of 0.5 mm.sec⁻¹, and a trigger point of 5 g. Texture variables of hardness 1, hardness 2, springiness, cohesiveness and chewiness were calculated.

Sensory evaluation

Both AP and MAP fish samples were analyzed following the methodology recommended by Liu et al. [8] with some modifications. The samples were evaluated by five trained panelists from the laboratory staff. The sensory evaluation was based on a ten-point scale, shown in Table 1. The scores for the separate characteristics of the fillets were summed to give an overall sensory score. The maximum score was 10 and the shelf-life criteria assumed that rejection would occur when the sensory score dropped below 4.

Endogenous enzyme extract and activity measurement

Cathepsins B and L activities: Minced fish (5 g) was homogenized in 20 mL of extraction buffer consisting of 50 mM sodium acetate (NaAc), 100 mM NaCl, 1 mM EDTA, and 0.2% (v/v) Triton X-100 (pH 4.0) for 1 min and then placed in a cell disruptor (Scientz Biotechnology Co., Ltd., Ningbo, China) for 2 min. The homogenate was then centrifuged at 10,000×g for 20 min at 4°C and filtered. The filtrate was used for the following measurements: Cathepsin B (CB) activity was determined at 30°C in reaction buffer A (pH 6.0), consisting of 352 mM KH₂PO₄, 48 mM Na₂HPO₄, and 4 mM Na₂EDTA. L-cysteine (8 mM) was added to the reaction buffer before use. CB + cathepsin L (CL) activity was determined in reaction buffer B (pH 5.5), consisting of 340 mM NaAc, 60 mM HAC, and 4 mM Na₂EDTA. Dithiothreitol (DTT, 8 mM) was added to the reaction buffer before use. A termination buffer (pH 4.3) containing 100 mM ClCH₂COONa, 30 mM NaAc, and 70 mM HAC was used. Z-Arg-Arg-7-amido-4-methylcoumarin hydrochloride and Z-Phe-Arg-7-amido-4-methylcoumarin hydrochloride were used as the substrates for CB and CB+CL, respectively. The reactions were initiated by adding 0.5 mL protein extract and reaction buffers A and B, respectively. The termination reagent was added last. A control using reaction buffer instead of enzyme buffer A was run in parallel. The incubation time was 30 min and the release level of 7-amino-4-

Scores	Sensory properties
10	Fresh odor and distinct meaty flavor, bright white color without mucus, stiff texture without concavities after finger pressure, clear muscle veins and compact structure
8	Normal odor, bright white color with slightly transparent or water mucus, firm and elastic texture with slight concavities after finger pressure, clear muscle veins and compact structure
6	Slight changes in odor, white color with slight dim, less elastic and slight concavities after finger pressure, clear muscle veins and flabby structure
4	Slight fishy odor, dark white color with moderate transparent mucus, soft and clammy, less elastic and concavities that disappear slowly after finger pressure, unclear muscle veins and flabby structure
2	Intense spoiled, off-odor, brownish red with slight opaque mucus, very soft and clammy, no clear muscle veins and very flabby structure
0	More intense spoiled, off-odor, yellow-green with more opaque and milky mucus, sunken and very slimy, no distinguishable muscle veins

Table 1: Sensory scheme of tilapia fillets.

methylcoumarin (AMC) was measured with an excitation wavelength of 340 nm and an emission wavelength of 440 nm. A standard curve was constructed from known concentrations of AMC in the stop buffer. The specific activity was expressed as U (units of activity) based on the release of 1 nmol AMC in 1 min. The values reported were the average of three measurements from each sample.

Statistical analysis

The results are expressed as means \pm standard errors. Data on the physiological indicators, sensory characteristics, and texture profiles were analyzed by one-way analysis of variance (ANOVA), and the significant differences were compared by Duncan's multiple comparisons. The data were analyzed by the SPSS software package (Version 17; SPSS Inc., Chicago, USA) and considered very significant at a level of $p < 0.01$ and significant at a level of $p < 0.05$.

Results and Discussion

Physiochemical effects

The measured pH values are similar in the AP and MAP tilapia fillets, as shown in Table 2. The pH values decrease upon storage time and then increase. In the present study, the initial pH is 6.85, which is higher than those detected by Liu et al. [8], and Subbaiah et al. [9], Khalafalla et al. [12] for tilapia. According to the findings of Khalafalla et al. [12], differences in initial pH values may be due to the species, diet, season, and level of stress during the catch, as well as the type of muscle. However, further studies are required to explain the nature of the high initial pH levels. The pH in both AP and MAP fillets significantly decreases over the first 7 days, reaching minimal at pH 6.49 and 6.25, respectively ($p < 0.05$). Similar results were reported by Cyprian et al. [1], Erkan et al. [3], and Sveinsdottir et al. [13] reported that MAP fish with CO₂ showed increasing exudation of the CO₂ dissolved on the fish body surface mucus, resulting in muscle acidification and decreases in the pH. Therefore, pH in MAP was lower than that of AP during the storage. Naturally, the initial reduction of pH in this study may be attributed to the decomposition of glycogen, ATP, and creatine phosphate and the dissolution of CO₂ in the fish muscle [14]. While the later increase in the pH value may relate to the production of volatile basic components, such as ammonia, by the decomposition of proteins and amino acids Subbaiah et al. [9]. Our results had similar trends with their theories. pH observed contributed significantly to SSP, springness and cathepsin L in this study, suggesting that changes of pH in the fish muscle were related to the endogenous enzymes, which may affect the degradation of salt protein. As a result, texture of fish muscle was changed.

TVB-N includes trimethylamine, dimethylamine, ammonia, and other nitrogen-containing compounds associated with seafood spoilage. The value of TVB-N increases as spoilage progresses [15]. The

initial TVB-N value is 7.81×10 mg/100g of muscle, and is significantly increased to 22.05 mg/100 g and 21.74 mg/100g at 11th and 14th day of AP and MAP, respectively, during freezing-point storage (Table 3). These increases may be due to the degradation of nitrogen-containing compounds, such as proteins, to various amines. For many fish species, the TVB-N contents increase curvilinear or linearly with time [16,17]. The shelf lives of the AP and MAP fillets are less than 11th and 14th day, respectively, according to the upper limit TVB-N value of 20 mg/100 g muscle. This difference is probably caused by the CO₂ in the MAP, which inhibits the growth of microorganisms, thus effectively slowing both protein degradation and the increase of TVB-N [16]. Kaba and Corapci [18] reported that with the increase of CO₂ ratio, the TVB-N value of product decreased, suggesting that the CO₂ was responsible for delaying the formation of TVB-N by restricting bacterial growth. Liu et al. [8] suggested that TVB-N was correlated well with storage time ($r = 0.98$), sensory acceptability ($r = -0.93$) and bacterial counts ($r > 0.90$) during tray-packed storage at 0°C. We report similar results, the correlations between TVB-N and sensory evaluations (Table 4) are very significant ($p < 0.01$). Both the sensory evaluation and TVB-N were unacceptable in AP at 11 days, but remained acceptable in MAP until 14 days.

The changes of WHC in the AP and MAP fillets are shown in Table 2. The WHC values of the samples during freezing-point storage under both storage methods are significantly increased with storage time ($p < 0.05$). This may be attributed to the denaturation of proteins and the ability of proteins to bind to water become weak throughout storage, which may increase natural exudations with increasing storage time and thereby decrease the amount of water within the organism and its cells. Our results agree with those reported by Duun and Rustad [4], Liu et al. [8] and Cheret et al. [19]. Cheret et al. underscored the increase of natural exudation with time of storage; it might appear an opposite result. The WHC values of MAP fillets are lower than those of AP fillets, suggesting that AP would be weak in preserving water bonding capacity for the high speed of protein degradation. Other researchers previously demonstrated that early postmortem events, including pH decline, proteolysis, and even protein oxidation, are key indices in the ability of a meat sample to retain moisture [1,20]. Cyprian et al. [1] indicated that minor changes in the pH drastically affect the properties of the connective tissue, which directly affect the WHC of the fish muscle. Subbaiah et al. [9] proposed that the denaturation and aggregation of the main contractile proteins responsible for the functional properties of the organism are typically caused by ice crystal growth and increasing ionic strength. A weak water bonding capacity would significantly affect the sensory quality of a specimen.

Sensory attributes

Sensory scores show significant decreases with increasing storage time for both AP and MAP fillets during freezing-point storage

Physicochemical Index	Storage Time (days)					
		0 day	3 days	7 days	11 days	14 days
pH	AP	6.85 ± 0.05 ^a	6.64 ± 0.06 ^b	6.49 ± 0.02 ^c	6.53 ± 0.01 ^{bc}	6.58 ± 0.03 ^{bc}
	MAP	6.85 ± 0.05 ^a	6.72 ± 0.03 ^b	6.25 ± 0.03 ^d	6.30 ± 0.04 ^d	6.43 ± 0.01 ^c
WHC	AP	83.65 ± 0.47 ^a	88.05 ± 0.64 ^b	89.60 ± 0.65 ^{bc}	90.05 ± 0.59 ^c	93.75 ± 0.05 ^d
	MAP	83.65 ± 0.47 ^a	85.40 ± 0.65 ^b	85.55 ± 0.38 ^{bc}	86.80 ± 0.16 ^c	88.25 ± 0.20 ^d
SSP (mg/g)	AP	7.67 ± 0.744 ^a	6.89 ± 0.534 ^{ab}	5.43 ± 1.047 ^{bc}	4.86 ± 0.051 ^c	4.55 ± 0.487 ^c
	MAP	7.67 ± 0.74 ^a	7.47 ± 1.33 ^a	5.91 ± 0.62 ^a	5.26 ± 0.59 ^a	5.03 ± 0.49 ^a
MFI	AP	58.20 ± 2.2 ^a	99.40 ± 13.8 ^b	138.70 ± 4.5 ^c	212.80 ± 7.6 ^d	264.50 ± 13.9 ^e
	MAP	58.20 ± 2.2 ^a	75.60 ± 0.4 ^a	123.50 ± 17.5 ^b	162.10 ± 11.9 ^{bc}	190.20 ± 17.2 ^c
TVB-N (mg/100 g)	AP	7.81 ± 0.47 ^a	11.66 ± 0.60 ^b	12.81 ± 0.76 ^b	22.05 ± 0.36 ^c	30.56 ± 0.60 ^d
	MAP	7.81 ± 0.47 ^a	10.71 ± 0.81 ^b	12.60 ± 0.46 ^c	17.64 ± 0.51 ^d	21.74 ± 0.60 ^e
TVC (log CFU/g)	AP	4.24 ± 0.056 ^a	4.87 ± 0.035 ^b	5.31 ± 0.018 ^c	6.21 ± 0.023 ^d	7.24 ± 0.020 ^e
	MAP	4.24 ± 0.056 ^a	4.54 ± 0.036 ^b	5.19 ± 0.028 ^c	5.49 ± 0.028 ^d	5.94 ± 0.028 ^e

AP: Air Packaging; MAP: Modified Atmosphere-Packaged. Different letters in the same line indicate significant differences ($p < 0.05$), $n = 6$

Table 2: Physicochemical indices of AP and MAP tilapia fillets during freezing-point storage.

Texture Index	Storage Time (days)					
		0 day	3 days	7 days	11 days	14 days
Hardness 1 (N)	AP	119.50 ± 9.61 ^a	117.17 ± 13.78 ^a	87.25 ± 6.88 ^b	86.17 ± 7.55 ^b	64.56 ± 5.33 ^b
	MAP	119.50 ± 9.61 ^a	118.06 ± 5.32 ^a	98.17 ± 7.61 ^{ab}	90.88 ± 4.06 ^b	89.83 ± 8.54 ^b
Hardness 2 (N)	AP	109.76 ± 8.99 ^a	108.00.17 ± 11.73 ^a	79.00 ± 6.54 ^b	77.08 ± 7.73 ^b	59.00 ± 4.67 ^b
	MAP	109.76 ± 8.99 ^a	104.13 ± 4.23 ^{ab}	84.67 ± 6.90 ^{bc}	80.75 ± 3.45 ^c	79.56 ± 7.76 ^c
Springiness (mm)	AP	2.55 ± 0.06 ^a	2.16 ± 0.08 ^b	2.16 ± 0.06 ^b	2.11 ± 0.04 ^b	2.06 ± 0.05 ^b
	MAP	2.55 ± 0.06 ^a	2.21 ± 0.05 ^b	2.11 ± 0.04 ^{bc}	2.05 ± 0.04 ^c	2.09 ± 0.04 ^{bc}
Cohesiveness (ratio)	AP	0.72 ± 0.02 ^a	0.65 ± 0.04 ^{ab}	0.63 ± 0.03 ^{ab}	0.57 ± 0.03 ^b	0.60 ± 0.03 ^b
	MAP	0.72 ± 0.02 ^a	0.61 ± 0.02 ^b	0.57 ± 0.01 ^b	0.57 ± 0.01 ^b	0.56 ± 0.01 ^b
Chewiness (N.mm ⁻¹)	AP	2.13 ± 0.44 ^a	2.05 ± 0.73 ^a	1.16 ± 0.31 ^b	1.01 ± 0.15 ^b	0.79 ± 0.25 ^b
	MAP	2.13 ± 0.44 ^a	1.65 ± 0.15 ^b	1.32 ± 0.19 ^{bc}	1.06 ± 0.06 ^c	1.05 ± 0.12 ^c

AP: Air Packaging; MAP: Modified Atmosphere-Packaged. Different letters in the same line indicate significant differences ($p < 0.05$), $n = 6$

Table 3: Texture indices of AP and MAP tilapia fillets during freezing-point storage.

		pH	WHC	TVB-N	SSP	MFI	TVC	Sensory	Hardness	Springiness	CB	CL
pH	AP	-	-	-	-	-	-	-	-	-	-	-
	MAP	-	-	-	-	-	-	-	-	-	-	-
WHC	AP	-0.779	-	-	-	-	-	-	-	-	-	-
	MAP	-0.639	-	-	-	-	-	-	-	-	-	-
TVB-N	AP	-0.513	0.900*	-	-	-	-	-	-	-	-	-
	MAP	-0.649	0.981**	-	-	-	-	-	-	-	-	-
SSP	AP	0.836*	-0.924*	-0.870	-	-	-	-	-	-	-	-
	MAP	0.871*	-0.880*	-0.923*	-	-	-	-	-	-	-	-
MFI	AP	-0.651	0.931*	0.982**	-0.947**	-	-	-	-	-	-	-
	MAP	-0.776	0.946**	0.979**	-0.982**	-	-	-	-	-	-	-
TVC	AP	-0.628	0.944**	0.991**	-0.924*	0.995**	-	-	-	-	-	-
	MAP	-0.798	0.950**	0.970**	-0.979**	0.995**	-	-	-	-	-	-
Sensory	AP	0.543	-0.893*	-0.996**	0.888*	-0.988**	-0.989**	-	-	-	-	-
	MAP	0.590	-0.981**	-0.992**	0.887*	-0.958**	-0.955**	-	-	-	-	-
hardness	AP	0.693	-0.914*	-0.898*	0.954**	-0.941**	-0.940**	0.894*	-	-	-	-
	MAP	0.891*	-0.853*	-0.900*	0.998**	-0.969**	-0.966**	0.858*	-	-	-	-
Springiness	AP	0.892*	-0.915*	-0.719	0.838*	-0.786	-0.781	0.732	0.719	-	-	-
	MAP	0.862*	-0.828*	-0.766	0.820*	-0.802	-0.827*	0.725	0.816*	-	-	-
CB	AP	-0.769	0.349	0.033	-0.423	0.129	0.104	0.021	-0.348	-0.465	-	-
	MAP	-0.940**	0.853*	0.846	-0.946**	-0.911	0.924*	-0.798	-0.948**	-0.954**	-	-
CL	AP	-0.951*	0.730	0.528	-0.878*	0.677	0.632	-0.561	-0.773	0.762	0.773	-
	MAP	-0.997**	0.638	0.634	-0.850*	0.757	0.959**	-0.578	-0.869*	-0.882*	0.939**	-

AP: Air Packaging; MAP: Modified Atmosphere-Packaged. **Correlation is significant at $p < 0.01$ levels (two-tail). *Correlation is significant at $p < 0.05$ levels (two-tail)

Table 4: Correlation between freshness indicators of AP and MAP tilapia fillets.

($p < 0.05$), as shown in Figure 1. Initially, the fish freshness is excellent, but the freshness characteristics are diminished gradually with time. After 11th day, the AP fillets are found still acceptable but have a low score near the limit of score=4, and completely unacceptable at 14 (score <4), whereas MAP fillets remain acceptable at 14th day, suggesting that under the same storage time, fillets in MAP are more acceptable to consumers. The slower rate of decrease in the sensory score for MAP fillets may be attributed to CO₂ inhibiting microbial growth, slowing the rate of protein degradation, and maintaining good water retention ability. Sensory score was correlated well with WHC, TVB-N, SSP, MFI, and hardness, which may be related to juiciness, odor, veins, structure, and the softening of the sample, respectively.

Protein degradation

Variations in SSP levels of AP and MAP fillets are shown in Table 2. The initial SSP value is 7.67 mg/g fish meat, and is decreased with increasing storage time by both storage methods. This may be explained by the progressive denaturation of myofibrillar proteins during freezing-point storage; for the myofibril protein was the major component of SSP. Duun and Rustad [4] suggested that the denaturation of food muscle proteins reduces the amount of soluble proteins. Kaale and Eikevik [21] demonstrated that the variation of SSP levels during super-chilled storage could relate to different durations and speeds of blending for all experiments. A lower SSP was in MAP than that in AP during freezing-point storage, suggesting that the speed of degradation of myofibrillar proteins in MAP was lower than that in AP, indicating that the method of MAP combined with freezing-point storage on tilapia fillets may effectively prevent degradation of myofibrillar proteins. SSP was correlated well with sensory attribute ($r > 0.88$), TVC ($r > -0.92$), hardness ($r > 0.95$), springiness ($r > 0.82$), and cathepsin B and cathepsin L, indicating SSP may be degraded by bacterial growth and endogenous enzymes, and affected sensory perception the fillets, as well as contributed to texture softening.

When muscle proteins are degraded by proteolytic enzymes, an increase in fragments of different lengths (from free amino acids to large peptides) is expected [22]. The measurement of myofibril fragmentation is reported as one of the most widely used methods to determine postmortem proteolysis by Soltanizadeh et al. [10], but is rarely used in fish, as indicated by Liu et al. [23]. The MFI shows similar trends, but is significantly higher in AP than in the MAP tilapia fillets, as shown in Table 2. The initial MFI is 51.63 and this value increases throughout freezing-point storage. Liu et al. [23] reported that the MFI of *Lateolabrax japonicus* in super-chilling combined with MAP was lower than that of non-super-chilled MAP fish, suggesting that super-chilling probably inhibited the activities of the relevant enzymes and slowed protein denaturation, thereby delaying the tenderization of flesh. MFI was correlated well with SSP ($r > -0.94$).

Microbiological analysis

The TVC from AP and MAP fillets during freezing-point storage are shown in Table 2. TVC significantly increases throughout the storage time in both AP and MAP fillets ($p < 0.05$). The initial TVC is 4.24 log CFU/g, and it reaches 6.21 log CFU/g and 5.94 log CFU/g in AP and MAP fillets at 11th and 14th day, respectively. TVC of 6 log CFU/g is considered the maximum acceptable level for chilled fish Khalafalla et al. [12]. Bacteria grow more quickly in AP than MAP fillets throughout the storage time. Our results agree with those reported by Erkan et al. [3], Parlapania et al. [16] and Yesudhasan et al. [24]. The difference in bacterial growth rate may be attributed mainly to the inhibiting behavior of CO₂ and the absence of O₂, as well as to the low

storage temperature, which can inhibit the growth of microorganisms. Duun and Rustad [4] reported that super-chilled salmon fillets stored at -2°C in combination with MAP maintained good quality based on both sensory and microbial analyses, with negligible microbial growth for more than 24 days, whereas ice-chilled reference fillets maintained good quality for only 17 days. Parlapania et al. [16] reported that MAP affected the growth rate of spoilage bacteria, and the increase of CO₂ and decrease of O₂ by MAP inhibited bacterial growth and changed microbial spoilage by suppressing mostly Gram-negative species and favoring Gram-positive ones.

Texture analysis

Texture parameters, such as hardness 1, hardness 2, springiness, cohesiveness and chewiness are reported in Table 3. The values for hardness 1, hardness 2, springiness, cohesiveness, and chewiness are found to decrease ($p < 0.05$) in both AP and MAP fillets during freezing-point storage. This indicates the gradual softening of muscle. The decreasing of springiness during storage may be related to the degeneration of protein, which decreases the binding force between muscles and the elasticity of the fish, which also indirectly reflects the decreasing quality of the fish. Hardness 1 and springiness are correlated well with SSP (Table 4), which may be explained by the denaturation of proteins and the damage of membrane structures responsible for texture. As a comprehensive evaluation parameter, chewiness is directly related to hardness and springiness, which both show decreasing trends. Excepting cohesiveness, the other texture parameters show smaller extents of decline in MAP than in AP, indicating that damage of myofibrillar proteins in AP is much more serious than that in MAP fillets. Subbaiah et al. [9] found that the softening of fish muscle during frozen storage was caused by the breakdown of the three-dimensional structures of proteins and their aggregation following the activity of proteases. Huff-Lonergan and Lonergan [20] suggested that the deterioration of fish flesh texture may relate to various intrinsic and extrinsic factors, such as the loss of water from the muscle and the destruction of muscle tissues. Protein denaturation has a profound effect on the texture qualities of meat, which is considered one of the most important sensory qualities attributes [9].

Cathepsins activities

The CB activities in both AP and MAP fillets, as well as the CL activity in AP, significantly increases at first before decreasing with increasing storage time ($p < 0.05$) (Figure 2). The CL activity of MAP fillets significantly increases over time ($p < 0.05$). These results partially agree with that by Gaarder et al. [25], who reported that CB and

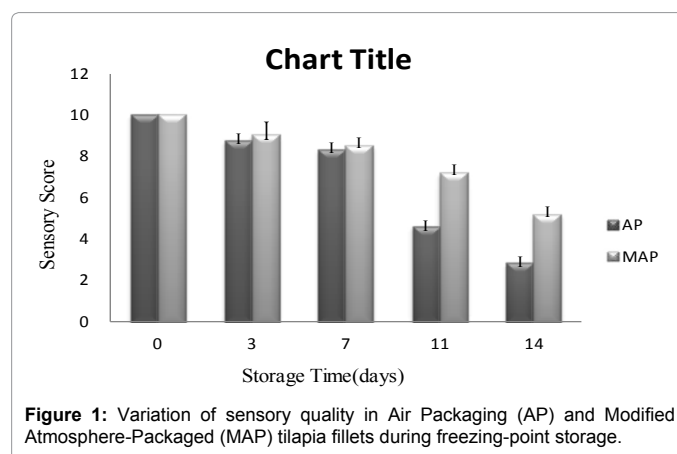
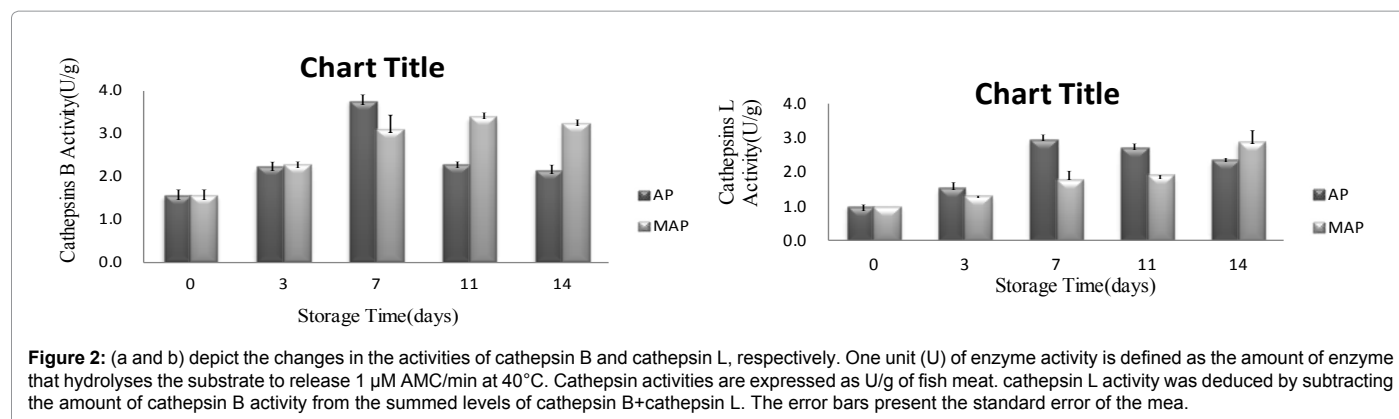


Figure 1: Variation of sensory quality in Air Packaging (AP) and Modified Atmosphere-Packaged (MAP) tilapia fillets during freezing-point storage.



cathepsin B+L activities of Atlantic salmon (*Salmo salar*) fillets increased with increasing storage time both in ice-stored and super-chilled for 114 h after slaughter. The decrease in CB and CL activities from 7 days post-mortem until 14th day post-mortem of AP might indicate that the cathepsins has been active and start to autolyse and therefore the activities decreases, similar results were found in Gaarder et al. [26], that cathepsins activities decreased at 5 days post-mortem when Atlantic salmon (*Salmo salar*) fillets stored on ice. Cathepsins are usually located in lysosomes and thus are inactive in living tissue, because they are not in contact with their substrates. However, they may be released in the cytosol by lysosome disruption after the death of the organism [27]. Gaarder et al. [25] suggested that the principal cause of post-mortem degradation of fish muscle is the hydrolytic nature of CB and CL, and that more proteolytic peptides may be released as a result. In parallel, the texture of the fillets gradually becomes more tender, and hardness 1 and springiness are correlated well with CB and CL in MAP fillets (Table 4), suggesting that the cathepsins activities were major factors determining fillet firmness. Hardness 1 and springiness are correlated well with TVC in AP fillets (Table 4), suggesting that microorganisms may be the major factor determining protein degradation that causes the increased tenderness of tilapia fillets with increased storage time. Ayala et al. [28] proposed that partially purified resolution of rigor in the post-rigor stage increases muscle tenderization by the hydrolysis of muscle proteins by endogenous calpains and cathepsins.

Conclusion

Sensory scores, SSP contents, and texture parameters were decreased with increasing storage time. TVB-N, MFI, WHC value, and TVC increased with increasing storage time. Tilapia fillets packaged under MAP showed significant delays in deterioration. AP and MAP tilapia fillets were acceptable by sensory evaluation until the 11th and 14th days of storage, respectively. The AP and MAP tilapia fillets were also acceptable by sensory, microbiological data and TVB-N until 11th and 14th day after packaging, respectively. The extension of shelf life using the suggested gas composition could be commercially exploited. However, the gas composition did negatively impact the sensory quality of the preserved tilapia fillets.

Acknowledgments

This work was supported by Natural Science Foundation of China [grant numbers 31271957, 31401563, 31601533]; National Key Technology Support Program [grant numbers 2015BAD17B03-2]; National Modern Agriculture (Tilapia) Industrial Technology System Specific [grant numbers CARS-49], and Science and Technology Program of Guangzhou, China [1561000228].

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