DOI:10.3724/SP.J.1238.2012.00221

Microstructure, chemical and physical changes of vacuum-packed grass carp (*Ctenopharyngodon idella*) fillets stored in low temperature

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Abstract: This study was carried out to evaluate the microstructure, chemical and physical changes of vacuum-packed grass carp (*Ctenopharyngodon idella*) fillet stored in low temperature (−18, 0, 4, 10 ℃) during 15 d of storage. The chemical changes were demonstrated by significant increase of *K*–value during storage. Chilling temperature, higher temperature and longer storage time gave bigger spaces between the muscle fibers, while freezing temperature gave the biggest gapping due to ice crystallization between muscle fibers. Water holding capacity(*WHC*) was decreased significantly after storage with frozen fish having the smallest *WHC*. 0 and 4 ℃ storage had higher *WHC* than 10 ℃ storage. *L**, *a** and *b** value were higher at higher temperature storage and longer storage time, indicating that the fish were lighter, more reddish and yellowish at the end of storage. Only fish stored in frozen temperature (−18 ℃) were considered still fresh until 15 d, but had poor texture quality.

Key words: grass carp; low temperature storage; microstructure; water holding capacity; color

真空包装草鱼片低温贮藏后微观结构及理化性质的变化

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摘 要：为研究真空包装草鱼片在低温条件下的变化，将真空包装草鱼片分别于−18、0、4、10 ℃贮藏 15 d，以贮藏期间 *K* 值(ATP 分解指数)的变化来评价其化学变化。结果表明：在 0、4、10 ℃下贮藏 15 d 后，草鱼肌纤维间的空隙变大，由于鱼肉内水的结晶，在−18 ℃下肌纤维间的空隙达到最大(约 100 μm)；随着贮藏时间的延长，鱼片的持水能力显著下降，−18 ℃下贮藏鱼片的持水能力最低，0、4 ℃贮藏鱼片的持水能力高于 10 ℃贮藏鱼片；于较高温度(0、4、10 ℃)贮藏后，鱼肉的亮度值(*L**)、红绿值(*a**)和黄蓝值(*b**)均较高，在−18 ℃低温贮藏后鱼肉的亮度值增加；随贮藏时间的延长，鱼片颜色(色差仪分析结果)趋向于红色值和黄色值增大；在−18 ℃贮藏 15 d 后，鱼片仍然新鲜，但质构变差。

关 键 词：草鱼；低温贮藏；微观结构；持水能力；色泽

中图分类号：S965.112 文献标志码：A 文章编号：1007−1032(2012)02−0221−04

Immediately after death, several chemical and biological changes take place in dead fish which lead to rejection for human consumption. Enzymes present in the living fish remain active after a fish dies and cause breakdown of the flesh by self digestion. Bacteria also break down the complex chemical substances of the flesh, and the spoilage process continues until the flesh becomes putrid and inedible. These two major causes of fish spoilage affect freshness, texture, color and water holding capacity of fish meat. The purpose of low temperature storage is to slow down the spoilage rate with indistinguishable

收稿日期：2012−02−15

基金项目：长沙市科技局项目(K1104018−21)

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changes after thawed[1].

The grass carp fish (*Ctenopharyngodon idella*) is one of four important domestic fishes in China and known as "Asian carps". It has been introduced into more than 50 countries for control of aquatic vegetation and for culture as a food fish because it grows very fast and considered a delicacy by many seafood lovers. Despite that, there are few studies that evaluate post-mortem changes in its muscle during low-temperature storage. Fish in ice box usually only can be stored for less than a week[1]. Therefore, studies on chemical and physical changes of grass carp fish fillet were done first during 15 d in different low temperature storage (−18, 0, 4, 10 ℃). Vacuum packaging was done to reduce contamination during storage.

The main objective of this research was to investigate the effect of different low temperature storages on the chemical and physical attributes during 15 d of storage. Reducing fish losses and preserving its quality over extended storage period will improve food security and income without more catches.

1 Materials and methods

1.1 Preparation and treatment of fish samples

Fresh grass carp fish were obtained from the local market at Hunan Agricultural University. Fish weighed (3±0.2) kg was immediately gutted, cleaned, filleted to fillet of (120±20) g and vacuum packed. The vacuum packed fish fillets were stored in different temperatures (−18, 0, 4, 10 ℃) and observed after 5, 10, 15 d of storage. During storage samples were stored in closed fridge and minimally exposed to sunlight. Temperatures were checked three times a day with thermometer.

1.2 *K*–value analysis

K–value measures how far ATP has been degraded and whether there is a very good correlation with the storing time of fish. Thus, it is used to evaluate the freshness in fish[2]. Determinations of ATP and related compounds were carried out by a reverse phase HPLC procedure[3]. 5 g of fish muscle was homogenized for 1 min with 25 mL of chilled 0.6 mol perchloric acid at 0 ℃. Samples were then centrifuged for 10 min at 6 000 r/min. The decantate were neutralized with 1 mol KOH to pH 6.5–6.8 and kept at 2 ℃ for 30 min precipitation. 20 μL of samples were injected onto HPLC (Agilent–1100, Agilent, USA) column (250 mm×4.6 mm×5 μm, Thermo Scientific). The temperatures were held at 30 ℃. Mobile phases used were 0.04 mol KH_2PO_4 and 0.06 mol K_2HPO_4, 2 mL/min. Detection was done at 254 nm UV.

1.3 Microstructure observation using scanning electron microscope

Samples were cut and sent to Life Science College, Hunan Agricultural University for Scanning Electron Microscope (SEM) observation.

1.4 Color analysis

*L**, *a**, and *b** value were measured using a colorimeter (BOIF Colorimeter 5003/WSC–Y, Beijing Optics Instrument, China).

1.5 Water holding capacity analysis

5 g of fish meat was wrapped by filter paper and centrifuged at 500 g, 10 ℃ for 10 min. *WHC* is expressed as percentage of the total water retained in the total initial water content.

1.6 Statistical analysis

Analysis of variance (ANOVA) using the general linear models were carried out by SPSS 16.0 (SPSS Inc., Chicago, USA). The program calculated multiple comparisons using Tukey's Test ($P<0.05$).

2 Results and analysis

2.1 *K*–value

Fish products with *K*–value lower than 20% are considered as very fresh fish; less than 50% are moderately fresh; and higher than 70% as not fresh[4]. The fresh grass carp fillet had initial *K*–value of 16.87%, which is considered as very fresh fish.

During storage, frozen storage (−18 ℃) had constant *K*–value (15.50%–20.90%), and there was no significant difference in *K*–value of frozen grass carp fillet during 15 d storage. Only grass carp fillet that stored in −18 ℃ could be considered very fresh until 15 d, While chilling temperature and higher temperature gave significantly higher *K*–value. Fish kept in 0 ℃ and 4 ℃ for 5 d had *K*–value of 67.07% and

59.14%, respectively. Longer storage time gave *K*–value higher than 70% (74.77% – 80.85%) as that the rate of hydrolysis is temperature-dependant; higher temperature gives higher rate of hydrolysis. Beside enzymatic reaction, most of microorganisms associated with fish are facultative psychrophile, with a wide range of growth temperature, from about －7.5 to 30 ℃ while in freezing temperature bacteria are killed more rapidly than at the higher storage temperature[5]. Fish will spoil about two times faster at 2.8 ℃ than as at －0.3 ℃[6].

2.2　Microstructure of fish meat

The lost of texture in fish products significantly affect the consumer acceptance. Figure 1 shows the microstructure of fresh grass carp muscles which were bundled firmly, while after storage the muscle fibers were not bundled firmly anymore. The longer the storage time, the bigger the gaps between the muscle fibers were.

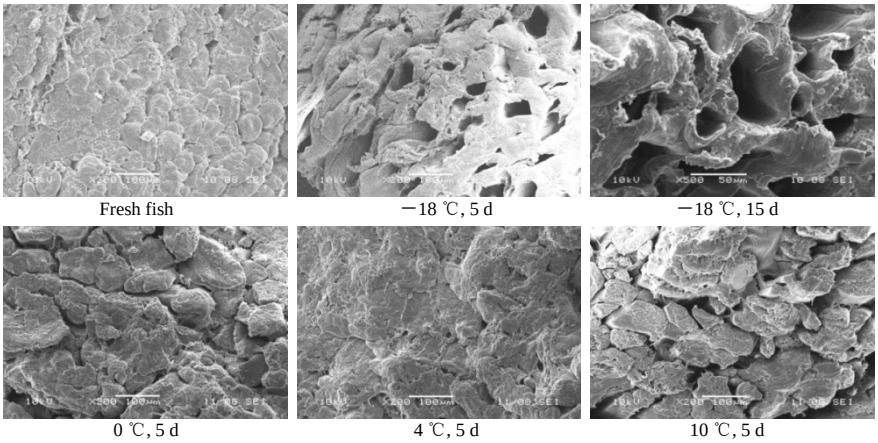

Fig.1 Microstructure of grass carp muscle samples (×200)

图 1　不同温度贮藏后草鱼片的微观结构(×200)

Autolysis hydrolyzed and broke up the muscle fibers. In chilling temperature (0, 4, 10 ℃), 0 ℃and 4 ℃ storage gave better results than 10 ℃ storage. These were happened because autolysis and bacterial spoilage were faster in higher temperature. The biggest gapping were found in fish meat stored in freezing temperature (－18 ℃). Crystallization disrupts water structures and protein conformation[7–8].

2.3　Water holding capacity

Fish after storage had lower *WHC* than fresh fish. There was no interaction effect between storage time and storage temperature, but each factor significantly affected the *WHC* as it can be seen in Table 1.

Table 1　Water holding capacity of frozen fish under different storage time and storage temperature

表 1　不同贮藏时间和贮藏温度下鱼片的持水力

storage	water holding capacity/%				
time/d	fresh fish	－18 ℃	0 ℃	4 ℃	10 ℃
5	89.903	80.000d	86.679f	87.282f	85.532e
10	89.903	79.896a	85.016c	85.778c	82.550b
15	89.903	78.375a	84.648c	84.849c	81.890b

Frozen fish had the smallest *WHC*. After storage, proteins are aggregated and denatured. When the protein is denatured, its moisture holding capacity is decreased and drip may be formed in the tissue[9]. High concentration of salt outside the cells during freezing also extracts more water from the cells and forms bigger ice crystals. These changes may affect the ability to bind water and fat, thus causing drip loss[7].

During 15 d of storage, *WHC* was decreased from (89.903±2.018)% on 0 d to (82.441±3.772)% on 15 d in average considering every storage temperature. Same result was also found in cazon fish muscle[2]. Integrated myofibrillar protein during storage caused decreased of *WHC*. Thus, longer storage time gave smaller *WHC*.

2.4　Color

The initial values of *L**, *a** and *b** were (39.82±1.30), －(0.69±2.48) and (4.01±0.75), respectively. Table 2 shows the effect of storage temperature and storage time on *L**, *a** and *b** value.

Table 2 Color of frozen fish under different storage time and storage temperature

表 2 不同贮藏时间和贮藏温度下鱼片的色泽度

storage time/d	*L**					*a**					*b**				
	Fresh fish	−18 ℃	0 ℃	4 ℃	10 ℃	Fresh fish	−18 ℃	0 ℃	4 ℃	10 ℃	Fresh fish	−18 ℃	0 ℃	4 ℃	10 ℃
5	39.82	38.88 a	40.02 ab	42.46 bc	40.82 c	–0.69	–0.22a	–0.34 a	0.24 a	0.49 a	4.01	3.28 a	3.45 a	4.11 a	2.99 a
10	39.82	40.21 d	41.74 de	43.28 ef	44.46 f	–0.69	0.89 a	1.01 a	2.50 a	2.51 a	4.01	4.82 b	5.37 b	7.25 b	7.05 b
15	39.82	39.85 d	42.37 de	43.70 ef	45.22 f	–0.69	2.51b	5.01 b	4.75 b	5.65 b	4.01	6.12 b	6.42 b	8.92 b	8.69 b

Fresh fish has a translucent appearance while spoiled fish will have an opaque surface. Translucent appearance is happened due to even scattering of incident light, while opaque appearance is happened because the incident light is unevenly scattered. Gradual disintegration of myofibrils during spoilage gives wider and more random intracellular distribution, resulting in unevenly scattered light[10].

With respect to the storage time and storage temperature, a significant increase in parameter *a** and *b** were found, indicating that the fish were more reddish and yellowish at the end of storage. The effect of storage temperature and storage time on *a** and *b** value can be seen in Table 2. Lipid oxidation and carbonyl amines reaction may results in yellowing[10], while changes in the *a** values corresponds with the changes in the metmyoglobin level, as a result of myoglobin oxidation[11]. An increase in *a** and *b** values in cazon fish muscle stored in ice for 18 d were also observed[2].

3 Conclusion

The biochemical and physical analysis observed usefully assessed the quality degradation of the grass carp fillet. Freezing temperature gave significant reductions of *K*–value and bigger gapping between muscle fibers during storage time compared to chilling temperature. *WHC* were significantly decreased after storage in all treatments. *L**, *a** and *b** values were increased during stor++ age and with higher storage temperature indicating that the fish were lighter, more reddish and yellowish at the end of storage. Only fish stored in frozen temperature (−18 ℃) were considered still fresh until 15 d, but with poor texture quality. Fish stored at chill temperature gave better texture quality but totally spoiled before 5 d. Based on this information, it is highly recommended to continue research which can prolong the shelf life of fish without frozen storage in order to preserve the texture quality of fish meat using a handy and/or emerging technology.

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