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Effects of different freezing treatments on physicochemical responses and microbial characteristics of Japanese sea bass (*Lateolabrax japonicas*) fillets during refrigerated storage

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ABSTRACT

In order to establish an effective freezing method for quality control, the present research evaluated the effects of the different freezing treatments on the quality of Japanese sea bass (*Lateolabrax japonicas*) over a period of 20 days storage at 0 ± 1 °C. Fish pH value, total volatile basic nitrogen (TVB-N), K-value, trimethylamine nitrogen (TMA-N), drip loss, hardness, color, biogenic amines, microbiological characteristics were measured. Sea bass fillets were stored at −18 °C (T1), −55 °C for 24 h and then −18 °C (T2), −55 °C (T3) for 3 months prior to refrigerated storage. T2 showed lower TVB-N, pH value, biogenic amines and drip loss than T3 and T1 did. Significant lower value of bacterial loads, b^* value and hardness were observed in T1, T2 and T3 than those of control group. No significant differences were observed among T1, T2 and T3 for TMA-N and a* value. The study demonstrated that Japanese sea bass fillets treated with −55 °C for 24 h and then −18 °C up to 3 months maintain better quality during refrigerated storage.

1. Introduction

Japanese sea bass (*Lateolabrax japonicas*), as an euryhaline fish species, is widely reared in China, Japan, Korea and Taiwan, and is one of the most important marine fish cultured in China, the yield of sea bass reached 122,964 tons in 2012, accounting for 12.8% in the total marine cultured fish of China (Hu et al. 2013). Sea bass has white flesh, mild taste, and low fat content, which made them popular around the world. However, raw fish are usually more perishable than other fresh products, and have a short shelf life under refrigerated storage (Boyd, Green, & LePors, 1992). The spoilage of raw fish is caused by endogenous enzymes and microbial activities, resulting in protein degradation, lipid oxidation or decomposition (Bohme, Fernandez-No, Gallardo, Canas, & Calo-Mata, 2011). So, extending post-mortem shelf life along with the less quality loss would benefit the seafood industry as well as consumers.

The short shelf-life of fresh live seafood is an obstruction to the sales and marketing of products, and its productivities also were influenced by the seasonal variation. Seasonality and perishability of the marine products explain the necessity of utilizing preservation technologies, such as freezing, which allows people to consume the sea products far from the production areas, is one of the most important storage methods for the seafood. Water loss, lipid oxidation, color and texture changes possibly occurred during frozen storage (Hong et al., 2013). There is still some cell disruption and destruction of muscle fiber due to the formation of ice crystals (Burgaard & Jorgensen, 2011). Ice crystal size and distribution were influenced by the freezing rate, freezing temperature and frozen storage temperature (Hansen, Trinderup, Hviid, Darre, & Skibsted, 2003). Many studies have reported various methods to decrease the ice crystals and the quality loss in different meats during frozen storage. Soyer, Ozalp, Dalmis, and Bilgin (2010) evaluated the impact of freezing at three different temperatures (−7, −12 and −18 °C) and then stored at −18 °C for up to 6 months on lipid and protein oxidations in chicken meats. Results show that decreasing the freezing temperature would reduce the oxidation of proteins during frozen storage. Thaw-rigor is often found in frozen meat, Imamura et al. (2012) investigated the application of a temperature shift technique in decrease the thaw-rigor in frozen tuna meat, which indicated that storing at −7 °C for 1 day or −10 °C for 7 days before thawing prevent thaw-rigor and met-Mb formation.

Oxidation reactions are the most important factor in quality losses, including lipid oxidation and protein oxidation. Though lipid oxidation is the major form of deterioration in stored muscle foods, fish meat contains high contents of proteins, which can also be influenced by oxidative reactions. In addition, biogenic amines

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(BAs) are natural antinutrition factors, including histamine (HIM), putrescine (PUT), cadaverine (CAD), tyramine (TYM), tryptamine (TRM), 2-phenylethylamine (2-PHE), spermine (SPM) and spermidine (SPD). BAs precursors are free amino acids provided by proteolytic changes of proteins or peptides. Generally, low concentrations of BAs in food (under 100 mg/kg) do not induce a significant risk for a human's health (Bunkova et al., 2009; Shalaby, 1996).

Previous reports focused on the lipid oxidation, protein oxidation and structural change of fish meat during frozen storage (Benjakul, Visessanguan, Thongkaew, & Tanaka, 2003; Imamura et al., 2012; Soyer et al., 2010), but little work has been done on the microbiological characteristics and formation of BAs of Japanese sea bass fillets at refrigerated condition after different freezing treatments. Therefore, in the present work, we investigated the effects of different freezing treatments prior to frozen storage on the microbiological characteristics, BAs and quality changes of refrigerated Japanese sea bass fillets.

2. Materials and methods

2.1. Materials and samples preparation

Live Japanese sea bass used in this study were purchased from an aquatic market in Jinzhou, China. The mean weight and length of fish were 640 ± 20 g and 28.5 ± 0.43 cm, respectively. Fish were transported to the Aquatic Products Processing Laboratory of Bohai University within 0.5 h and kept alive before being processed. The sea bass were decapitated and filleted by hand, and two fillets were obtained from each fish. A cryogenic temperature of −55 °C has been suggested as ideal storage conditions for frozen meat to prevent quality changes (Zhou, Xu, & Liu, 2010). So we packed fillets in polyethylene bags and randomly separated fillets into three groups. Three different freezing treatments were used: (T1) frozen at −18 °C and kept for up to 3 months; (T2) frozen at −55 °C by an ultra-low temperature freezer (MDF-382E, Sanyo Co., Ltd., Dalian, China) for 24 h and then moved to a refrigerator at −18 °C (as a typical frozen storage temperature) and kept for up to 3 months; (T3) frozen at −55 °C and kept for up to 3 months. After that, the sea bass fillets were stored in refrigerated chambers at 0 ± 1 °C during 20 days for subsequent quality analysis. Fresh sea bass fillets were obtained and then stored at 0 ± 1 °C as control. For each group, 18 sea bass fillets were used, and subsequently every 4 days, microbial characteristics, biogenic amines and quality changes from each group were analyzed.

2.2. Microbiological characteristics

All samples were analyzed for mesophilic bacteria, psychrophilic bacteria, *Enterobacteriaceae*, and *Shewanella putrefaciens* counts as described by Li, Li, & Hu (2013). The samples were homogenized for 2 min with a BagMixer (Model 400, Interscience, France). Mesophilic bacteria counts were determined on plate count agar (PCA, Aoboxing Bio-Tech, Beijing, China) by counting the number of colony-forming units after incubation at 35 °C for 48 h. Psychrophilic bacteria were enumerated on plate count agar (PCA, Aoboxing Bio-Tech, Beijing, China) after incubation at 7 °C for 10 days and expressed as $\log_{10}$ CFU/g. *Enterobacteriaceae* were enumerated in violet red bile glucose agar (VRBGA, Aoboxing Bio-Tech, Beijing, China) with a double layer at 30 °C for 24 h. *S. putrefaciens* were counted from the black colonies grown on iron agar (Lyngby, Aoboxing Bio-Tech, Beijing, China) at 20 °C for 72 h and a representative number of colonies were confirmed by using API 20NE (Biomerieux, France).

2.3. pH value

The pH values were measured according to Li, Li, Hu, and Li (2013) by mixing minced fish flesh (10 g) with 90 ml distilled water by a blender and the mixture was stirred for 30 min. After filtered, the pH values of the filtrate were measured using a digital pH meter (FE20, Mettler Toledo, Shanghai, China).

2.4. Total volatile basic nitrogen (TVB-N)

The total volatile basic nitrogen (TVB-N) values were determined as described by Capillas & Moral (2001). TVB-N values were measured with a Kjeltec 8400 (Foss, Sweden). TVB-N values were expressed in mg nitrogen per 100 g sample.

2.5. Trimethylamine nitrogen (TMA-N)

TMA-N was determined according to the official procedure of the AOAC (1995). Homogenized samples (10 g) were weighed and blended with 90 ml of 7.5% trichloroacetic acid solution and then filtered. Four milliliters of extract was transferred into test tubes, and then 1 ml of 20% formaldehyde, 10 ml of anhydrous toluene and 3 ml of K_2CO_3 solution were added. The tubes were shaken, and then the 5-ml toluene layer was pipetted. Five milliliters of 0.02% picric acid solution was added. The absorbance was recorded at 410 nm against a blank control and the TMA-N value was expressed as milligrams per 100 g of muscle.

2.6. K-value

The ATP and its related products were determined according to the method of Yoneda, Kasamatsu, Hatae, and Watabe (2002), with some modifications. The minced sea bass meat (4 g) was homogenized with 20 ml of 10% cold perchloric acid using a homogenizer (PRO200, Pro Scientific Inc., Oxford, USA) and centrifuged at 4000 *g* for 10 min. The precipitate was extracted by the above reagent and centrifuged again. These two supernatants were combined and neutralized with KOH solution and then stored in a −80 °C freezer (MDF-382E; Sanyo, Dalian, China) until HPLC analysis. ATP and its breakdown compounds, including ATP, ADP, AMP, IMP, HxR and Hx, were determined with a HPLC (1260 LC; Agilent, Palo Alta, CA, USA). Samples were applied to a HPLC column Agilent C18 (5 μm, 4.6 × 250 mm) and a UV detector (Agilent, 1260 LC). The isocratic mobile phase was 0.05 M phosphate buffer (pH 6.8). The sample (20 μl) was injected at a flow rate of 1 ml/min and the peaks were detected at 254 nm. The identification of nucleotides, nucleosides, and bases was made by comparing their retention times with those of commercially standards, which was obtained from Sigma Chemical Co. (St. Louis, MO). The *K*-value was calculated as the percent amounts of HxR and Hx to the sum of ATP and degradation products as follows:

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

2.7. Drip loss

Drip loss was determined according to Hansen et al. (2003) with some modifications. After the designated storage period, the sea bass fillets were taken from air-tight bags and left to drain on a stainless steel wire mesh for up to 1 h at 4 °C. The drip loss was measured by weighing the fish meat sample and comparing the weight with the initial weight before freezing. The drip loss of the fish meat sample was calculated according to: