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Quality changes and predictive models of radial basis function neural networks for brined common carp (*Cyprinus carpio*) fillets during frozen storage

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ABSTRACT

To investigate and predict quality of 2% brined common carp (*Cyprinus carpio*) fillets during frozen storage, free fatty acids (FFA), salt extractable protein (SEP), total sulfhydryl (SH) content, and Ca^{2+}-ATPase activity were determined at 261 K, 253 K, and 245 K, respectively. There was a dramatic increase ($P < 0.05$) in FFA and a sharp decrease ($P < 0.05$) in SH at 261 K during the first 3 weeks. SEP decreased to 67.31% after 17 weeks at 245 K, whereas it took about 7 weeks and 13 weeks to decrease to the same extent at 261 K and 253 K, respectively. Ca^{2+}-ATPase activity kept decreasing to 18.28% after 7 weeks at 261 K. Furthermore, radial basis function neural networks (RBFNNs) were developed to predict quality (FFA, SEP, SH, and Ca^{2+}-ATPase activity) of brined carp fillets during frozen storage with relative errors all within ±5%. Thus, RBFNN is a promising method to predict quality of carp fillets during storage at 245–261 K.

1. Introduction

Freshwater fish are known for their high water content, abundant nutrients, and high enzyme activity, all of which contribute to perishability. The shelf life of common carp fillets for human consumption is only 3 days stored at 288 K (Krizek, Vacha, Vejsada, & Pelikanova, 2011) and 6 days at 277 K (Hasani & Hasani, 2014). The degradation of fish was a result of complex physical, chemical, and microbial changes. The deterioration caused by microbial and enzyme activity can be limited effectively under frozen storage (Liu, Kong, Han, Chen, & He, 2014). The process of freezing is still the most effective and widely used method for the preservation of fish. However, off-odor forming, discoloration and destruction of texture caused by the denaturation of proteins, lipid oxidation and hydrolysis still occurred during frozen storage (Benjakul & Sutthipan, 2009; Osako, Hossain, Kuwahara, & Nozaki, 2005). Verma, Srikar, Sudhakara, and Sarma (1995) reported that lipid hydrolysis of *Sardinella longiceps* took place during cold storage at 253 K. Lipid oxidation and hydrolysis of *Hippoglossus hippoglossus* still occurred up to 9 months (Abreu, Losada, Maroto, & Cruz, 2011). The denaturation of proteins during frozen storage of fish is recognized widely (Saeed & Howell, 2002; Sultanbawa & Li-Chan, 1998). The integrity of myosin is important for the activity of Ca^{2+}-ATPase, especially for the globular head (Benjakul, Seymour, Morrissey, & An, 1997). The aggregation of frozen-induced fish myosin can also lead to the oxidation of sulfhydryl (Ramirez, Martin-Polo, & Bandman, 2000). Srinivasan, Xiong, and Blanchard (1997) reported that the extent of loss of quality during frozen storage depended on the initial raw materials, freezing temperature, storage duration, packaging, rate of freezing–thawing, temperature fluctuations, and freeze–thaw abuse.

Common carp (*Cyprinus carpio*) is one of the oldest commercially bred freshwater fish species in the world and it is widely distributed in rivers, lakes, ponds and ditches. Food and Agricultural Organization (FAO) ranked the yield of common carp as the third highest among freshwater fish with a production of 3,791,913 tons in 2012. Traditionally, common carp is sold alive, which produces a large amount of waste and causes environmental contamination. With the life style of consumers changing, ready-to-use, high-quality and safe food products are in high demand (Hong, Luo, Zhou, & Shen, 2012). Considerable research has therefore focused on methods to improve the quality of prepared fish products, such as modified atmosphere packaging (MAP) (Farber, 1991),

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fermentation (Kumar, Jain, Ghosh, & Ganguli, 2014), smoking (Cardinal, Cornet, Serot, & Baron, 2006), and sugaring and salting (Shi, Cui, Luo, & Zhou, 2014). Salting has a long history of being used to preserve food, and to give food a special flavor (Rodrigo, Ros, Periago, Lopez, & Ortuno, 1998). The addition of salt can reduce water activity effectively, decrease microbial metabolism, inhibit lipid hydrolysis so as to enhance the texture, and improve flavor and shelf life of fish products (Yanar, Çelik, & Akamca, 2006). However, the presence of salt can also enhance oxidation of highly unsaturated lipids, which directly lead to off-flavors and odors, protein denaturation and changes in texture. Also, the rate of oxidative rancidity accelerates with an increase in salt content (Aubourg & Ugliano, 2002). Therefore, we believe it is useful to investigate how a low concentration of salt affects the quality of fish when stored at freezing temperatures.

The RBFNN is one kind of feed-forward neural network, which can simulate the human brain to execute many complicated tasks with ease. RBFNN can illuminate the complex law between the inputs and outputs through a fitting process that allows it to approximate any nonlinear function (Han, Qiao, & Chen, 2012; Liu, Jiang, Shen, Luo, & Gao, 2015). There have been many attempts to develop and optimize RBFNN to make predictions. Link, Lemes, Marquetti, Scholz, and Bona (2014) developed a RBFNN to classify the geographic and genotypic origin of arabica coffee. Dutta, Hines, Gardner, Kashwan, and Bhuyan (2003) used a RBF neural network to achieve up to 100% accuracy in the flavor classification of five different tea samples. During the process of modeling, Wu, Zhu, Cao, and Tu (2008) used a genetic algorithm (GA) to optimize the parameters of RBFNN to guarantee that fuel utilization would operate within a safe range. The quality of aquatic products is always measured through traditional chemical methods, which require complex experiments, time-consuming operations and destructive inspections (Olafsdottir et al., 1997). However, once the RBFNN is established, the quality of aquatic products can be predicted based on the initial values of the applied indicators at operated storage temperature and time range. Thus, the complicated chemical tasks would be reduced and it might be able to provide practical industrial instructions for the quality prediction. However, there are few reports on the application of RBFNN for predicting quality of aquatic products during frozen storage.

The objectives of the present study were to quantify changes in quality of 2% brined common carp fillets during frozen storage by measuring free fatty acids (FFA), salt extractable protein (SEP), total sulfhydryl (SH) content and Ca^{2+}-ATPase activity, and to establish RBFNN predictive models based on these indicators to estimate the quality of brined common carp fillets under different frozen temperatures.

2. Materials and methods

2.1. Raw materials

Samples of artificially cultured common carps (weight 1508.36 ± 150.29 g, length 44.26 ± 1.41 cm) were purchased from an aquatic product market in Beijing, China, and were transferred alive within half an hour to the laboratory, in aerated foam boxes containing water. They were killed by a knock on the head, scaled, eviscerated, decapitated, filleted and washed in flowing tap water. Then, the fish fillets were placed on a clean steel frame for 10 min for draining. After that, the fillets were brined with 2% salt (w/w) in valve bags and stored at 277 K for 12 h to ensure the salt was dispersed fully. Then, the samples were evenly distributed and stored at 261 K, 253 K, and 245 K, respectively. During the 17 week storage, analysis was done once a week during the first three weeks, and in the following weeks, it was done once every two weeks. The initial values were determined after the samples were frozen at 261 K, 253 K, and 245 K for 5 h to ensure that they were frozen. Samples were selected randomly and thawed at 277 K for 24 h before analysis. All determinations were made in triplicate.

2.2. Method

2.2.1. Free fatty acids (FFA)

The extraction of crude fat was conducted according to Bligh and Dyer (1959) with some modifications. A 30 g sample of minced carp fillets was homogenized with 30 ml of chloroform and 60 ml of methanol for 2 min, and then 30 ml of chloroform and 30 ml of deionized water were added to the mixture and homogenized for 30 s in succession. After suction filtration, the filtrate remained in a separating funnel for 3 h until divided completely. Then, the organic phase flowed out from the bottom of the funnel and dried with nitrogen. The crude fat thus obtained was dried in a desiccator for 24 h before analysis.

The determination of FFA was modified by Sanchez-Alonso, Carmona, and Careche (2012). The crude fat sample (0.05–0.1 g) was dissolved in 5 ml of toluene, and then 1 ml of cupric acetate-pyridine reagent (5%, w/v, pH 6.0) was added for coloration. The mixture was vibrated using a vortex for 2 min and centrifuged at 3000 rpm for 5 min. Absorbance of the supernatant was observed at 715 nm, and the content of FFA was expressed as g FFA present in 100 g of lipids.

2.2.2. Salt extractable protein (SEP)

Salt extractable protein was prepared by a modified method based on Donald and Lanier (1994). Two grams of minced carp fillets was homogenized with 30 ml cold distilled water individually in an ice container, and then centrifuged at 10,000 rpm at 277 K for 10 min to remove the supernatant. As stated above, 30 ml cold 0.3% NaCl replaced distilled water to remove any water-soluble substances. After that, the sediment was homogenized with 30 ml Tris–maleate buffer (0.6 M NaCl-20 Mm Tris–maleate, pH 7.0) for 10 s and extracted at 277 K for 2 h, and the extraction was centrifuged at 10,000 rpm at 277 K for 10 min. The supernatant was collected for the determination of SEP.

The content of SEP was determined based on Gornall, Burdawill, and David (1949). One milliliter of the supernatant was vibrated with 4 ml of Biuret protein assay and allowed to react at room temperature for 20 min, and absorbance was measured at 540 nm. For the blank, 0.6 M NaCl-20 Mm Tris–maleate was used. SEP content was expressed as the ratio of protein content in the supernatant to the mass of mince (mg/g).

To prepare the myofibril protein solution, a sample of 8 ml of the SEP solution was washed twice with 32 ml of cold distilled water, and the precipitate obtained was dissolved in 3 ml 0.6 M NaCl. The concentration of the myofibril protein solution was determined through a Biuret protein assay and adjusted to 4 mg/ml.

2.2.3. Total sulfhydryl (SH) content

Total sulfhydryl content was determined according to the modified method of Ellman (1959). Briefly, 0.5 ml of 4 mg/ml myofibril protein solution was mixed with 4.5 ml buffer (8 M urea, 2% SDS, 10 mM EDTA, pH 8.0), then 4 ml of the mixture was added to 0.5 ml of 0.1% DTNB-Tris–HCl buffer and bathed at 313 K for 25 min. The blank was 0.6 M NaCl. Absorbance was measured at 412 nm using a spectrophotometer, and the concentration of SH was calculated as follows:

$$\text{SH content } (\text{mol}/10^{-5}\text{g}) = A \times D/B \times C \quad (1)$$

where A is absorbance, B is concentration of myofibrillar protein, C is molar extinction coefficient of 13,600 M^{-1} cm^{-1}, and D is dilution factor, 11.25.