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Influence of lightly salting and sugaring on the quality and water distribution of grass carp (*Ctenopharyngodon idellus*) during super-chilled storage

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ABSTRACT

Low Field Nuclear Magnetic Resonance (LF-NMR) was used to evaluate the water mobility in grass carp during super-chilled storage. Grass carps were analyzed for inosine monophosphate (IMP), total viable counts, cooking loss, drip loss, biogenic amines, and sensory. Pearson's regression analysis between LF-NMR parameters and quality indicators was done to assess the feasibility of using LF-NMR to predict fish quality. Three water populations, T_{2b} (<10 ms), T_{21} (20 ms–80 ms), and T_{22} (>100 ms), were measured. The major component T_{21}, immobilized water, shifted to longer relaxation time with added salt and sugar due to the swelling of myofibers ($P < 0.05$). T_{21} of cooked (85 °C) flesh was lowered, indicating that immobilized water mobility decreased during heating. Curing treatments predominately accelerated IMP formation, inhibited bacterial growth, retarded cooking loss, and improved the overall sensory quality. The study also showed that the LF-NMR was capable of monitoring unsalted fish deterioration.

1. Introduction

Grass carp (*Ctenopharyngodon idellus*) is the most prevalent freshwater fish in China, and is popular everywhere due to its fast growth rate, easy cultivation, high feed efficiency, high nutritional value, and relatively low price. Total global production of grass carp was about 5,500,000 tonnes in 2014 (FAO, 2014), the highest for any aquacultured species. As production increases, reduction of quality loss after harvesting becomes ever more important.

Salting has been shown to be an effective way of preserving fish by reducing water activity. However, high intakes of salt can lead to some chronic diseases, like hypertension and cardiovascular diseases (Geleijnse et al., 2007). The World Health Organization recommended that the maximum dietary intake of salt for adults should be 5 g per day (WHO, 2013). Therefore, lightly salted fish products (~2% of the weight of products) have become popular. Moreover, light salting of fish has been used to increase the flavor of food, to reduce drip loss, and to counteract the negative effects of freezing (Gudjónsdóttir et al., 2011b). On the other hand, sugar, which is widely used in Chinese cuisine, can also reduce water activity and promote good taste. Therefore, a study of the effect of

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lightly salting and sugaring on quality deterioration and water distribution of freshwater fish may prove beneficial.

Low Field Nuclear Magnetic Resonance (LF-NMR) can rapidly and non-destructively show the chemical-physical state of water in meat by providing direct information about interactions between water and proteins (Bertram et al., 2002a; Marcone et al., 2013; Carneiro et al., 2016). LF-NMR T_2 relaxation time has been successfully used to study water properties in pork, pork batters, shrimp, and fresh or salted marine fish (e.g., cod and salmon) during chilled and frozen storage (Wu et al., 2006; Shao et al., 2016; Carneiro et al., 2013; Gudjónsdóttir et al., 2011b; Aursand et al., 2008b). Super-chilling (also called partial freezing) is a method to enhance the shelf-life of fish by reducing the amount of water that can be used by bacteria, while minimizing structural damage. Thus, super-chilled products usually have a better quality than chilled or frozen products (Duun and Rustad, 2008; Gallart-Jornet et al., 2007). Nevertheless, few LF-NMR studies have been done on the water properties of super-chilled freshwater fish and how salting or sugaring might affect the outcome of super-chilled fish.

The objective of this study was to study the addition of salt and sugar on the quality and water distribution of grass carp during super-chilling, as well as to correlate the chemical, microbial, and sensory parameters with the distribution of water to determine whether LF-NMR could be used to monitor quality and/or to optimize processing.

2. Materials and methods

2.1. Experimental fish and design

A total of 33 live grass carp (weight of 1260 ± 36 g, length of 46 ± 2 cm) were purchased from Xiaoyuehe aquatic products wholesale market in Beijing, China in October 2015, and transported live to the laboratory in bags with the fish immersed in tap water. All fish were killed by a physical blow to the head using a wooden club, in accordance with the Administration of Experimental Animals issued by the China State Council in 1988 and the guidance on Treating Experimental Animals developed by China's Ministry of Science & Technology in 2006. They were then scaled, eviscerated, beheaded, washed, and cut into skin-on fillets that were then cut into two pieces (weight of 152 ± 3 g, about 8.0 × 10.5 × 2.5 cm^3).

Before starting this study, preliminary experiments with different concentrations of cure were done to determine that the most acceptable taste for consumers. After the taste tests, desirable taste was obtained with two concentrations (1.6% salt (S) and 1.6% salt + 0.8% sugar (SS)).

These grass carp portions were divided into three groups. The untreated group was used as control (CK, n = 39). The treated groups were dry cured with 1.6% salt (S, n = 45), or 1.6% salt + 0.8% sugar (SS, n = 45), respectively. The dry salt or salt-sugar mixture was carefully and evenly sprinkled on both sides of the grass carp fillets. The flesh side was treated first followed by the skin side. Ordinary commercial food grade salt (CNSIC, Beijing, China) and granulated white sugar (COFCO Tunhe, Xinjiang, China) were used for dry-curing. The addition of salt and sugar was expressed as a percentage of initial weight. All portions were packed in polyvinyl chloride bags (about 250 × 200 mm, Kelinlai, Shanghai, China), and stored immediately in a refrigerator (BC/BD-62, Haier, Qingdao, China) at −3 ± 1 °C, which did lead to some crust-freezing. Three potions were taken randomly for analysis at specified time interval.

Inosine monophosphate (IMP) content was analyzed on 0, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, and 45 days. LF-NMR parameters and other quality indicators were measured every 5 days.

2.2. LF-NMR relaxation (T_2) measurements and cooking loss

LF-NMR relaxation measurements were done according to the method of Aursand et al. (2008a) with minor modifications. Briefly, two 20 mm × 20 mm × 10 mm (~3 g) cubes were removed with a sharp knife along the fiber direction from the white dorsal muscle of grass carp fillets. These cubes were individually wrapped in clear plastic wrap (Kelinlai, Shanghai, China), placed in a cylindrical test tube (25 mm in diameter), and then inserted into the probe of a Niumag Benchtop Pulsed NMR Analyzer (PQ001, Suzhou Niumag Analytical Instrument Corporation, Shanghai, China). The analyzer was operated at a resonance frequency of 18 MHz at 32 °C.

The transverse relaxation time, T_2, was measured using the Carr-Purcell-Meiboom-Gill software program (CPMG) (Carr and Purcell, 1954; Meiboom and Gill, 1958). The T_2 measurement was done with a time delay between 90° and 180° pulses (τ-value) of 83.5 μs The number of measured points was 200 for the CPMG measurements. Data from 4500 echoes were acquired as 8 scan repetitions, and the repeat time between subsequent scans was 3500 ms. The multi-exponential decay curve was obtained from NMR relaxation processing, and was done using a multi-exponential fitting analysis with the Nuimag's Multi Exp Inv Analysis software program. Three relaxation times (T_{2b}, T_{21}, and T_{22}) and the percentage of each corresponding water population (P_{2b}, P_{21}, and P_{22}) were obtained as outputs.

After the relaxation measurements, the samples were taken out from the clear plastic wrap, packed in polyethylene bags, and then immersed in a water bath at 85 °C for 15 min, followed by 30 min at room temperature (temperature = 20 ± 2 °C). The samples were weighed (m_2), and the relaxation measurements repeated. Cooking loss was determined as the difference in weight of the samples before cooking (m_1) and the weight after cooking and cooling: cooking loss (%) = $(m_1\text{-}m_2)/m_1 \times 100$.

2.3. Chemical, microbial, and sensory analysis

Preparation and detection of IMP followed the procedure of Liu et al. (2015). Raw samples were removed from the white dorsal region of fillets, and cooked samples were obtained from the cubes used to determine cooking loss. One gram of sample was homogenized (A10, IKA, Guangzhou, China) with 2 mL of cold 100 g/L perchloric acid (PCA). The homogenate was centrifuged (GL-21 M, Pingfan, Changsha, China) at 1430 g for 5 min. The sediment was washed with 2 mL of cold 50 g/L PCA, and centrifuged at 1430 g for 5 min. The wash process was repeated two times and the combined supernatant was neutralized to pH 6.3–6.5 with 10 M and 1 M KOH. The white sediment was removed by centrifugation (800 g, 3 min) and the supernatant diluted to 10 mL for further analysis. The IMP was analyzed using reverse phase high-performance liquid chromatography (HPLC) (Shimadzu, LC-10AT series, Kyoto, Japan) equipped with a SPD-10A (V) UV detector and a COSMOSIL 5C18-PAQ column (4.6ID × 250 mm) (Nacalai Tesque, Inc., Kyoto, Japan) at room temperature. All solutions were passed through a 0.22 μm membrane filter (Jinteng, Tianjin, China) prior to injecting into the column. The mobile phase was 0.05 M potassium phosphate buffer (pH 6.8); flow rate was 1 mL/min; injection volume was 50 μL; detection wave length was 254 nm.

The drip loss was estimated using the method of Yin et al. (2014) with some modifications. Portions were weighed (Da) after being drained for 1 h at 4 °C. After the designated storage period, the samples were taken from the polyvinyl chloride bags. Then they were blotted with a paper towel and reweighed (Db) to determine drip loss. Drip loss (%) = (Da-Db)/Da × 100.

Extraction and derivatization of biogenic amines (BA) of grass carp fillets used the method of Eerola et al. (1993) and Ikonić et al.

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