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Shelf-life extension of crucian carp (*Carassius auratus*) using natural preservatives during chilled storage

Tingting Li [a,b], Jianrong Li [a,c,*], Wenzhong Hu [b], Xuguang Zhang [a], Xuepeng Li [c], Jin Zhao [a,d]

[a] College of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou 310035, China
[b] College of Life Science, Dalian Nationalities University, Dalian 116600, China
[c] Bohai University, Jinzhou 121013, China
[d] Zhejiang Marine Food Processing Quality Control Techniques and Instrumentation Engineering Laboratory, College of Life Science, China Jiliang University, Hangzhou 310018, China

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ABSTRACT

The effect of the natural preservatives, tea polyphenols and rosemary extract, on microbiological [total viable count (TVC)], chemical [pH, total volatile base nitrogen (TVB-N), *K*-value and thiobarbituric acid (TBA) values], texture and sensory changes of air-packaged whole crucian carp (*Carassius auratus*) stored at 4 ± 1 °C was investigated for 20 days. The shelf-life of crucian carp was found to be 7–8 days for untreated group (control), 13–14 days for tea polyphenols group and 15–16 days for rosemary extract treated group according to sensory assessment results, for which the corresponding microbiological assessment also showed an increased shelf-life. Meanwhile, the increases of pH, TVB-N, *K*-value and TBA values were significantly delayed in both treated groups of samples compared to the control group. Thus, either tea polyphenols or rosemary extract could be used as potential preservatives to extend the shelf-life of crucian carp during chilled storage.

1. Introduction

Crucian carp (*Carassius auratus*) is one of the most economically important freshwater-cultured fish species. It is popular in China due to its fast growth rate, palatability, and the nutritional quality of its flesh (Zeng, Huang, Li, & Huang, 2001). In China, production of crucian carp reached nearly 2,000,000 tons in 2009 (Fishery Bureau of Department of Agriculture of China., 2011). However, fish are usually more perishable than other muscle foods, and a considerable number of fish are spoiled due to lack of good preservation.

Crucian carp is an easily perishable product because of its relatively high quantities of volatile basic nitrogen as well as free amino acids, high water activity, and presence of autolytic enzymes (Duan, Jiang, Cherian, & Zhao, 2010). Xiao, Kang, and Xin (2007) reported the freshness variation of crucian carp (*Carassius auratus*) during chilled storage, while Yue, Shen, and Ou (2002) studied the effect of coatings with different molecular weight chitosan on crucian carp quality.

The spoilage of fish is a complicated process in which microbiology, physical and chemical changes interact. Activities of the fish's own enzymes and chemical reactions are usually responsible for the initial loss of fish freshness, whereas the metabolic activities of microorganisms are involved in the whole spoilage (Sallam, Ahmed, Elgazzar, & Eldaly, 2007). The inherent quality of seafood makes it more susceptible to food-borne hazards. Therefore, effective methods for extending shelf-life and improving quality of fresh crucian carp are necessary.

The application of good manufacturing practices (GMP), good hygienic practices (GHP), and hazard analysis of critical control point (HACCP) is essential in the production, distribution, storage and retailing of refrigerated foods. Because of consumer desire for fresh chilled foods with extended shelf-life, numerous studies have been directed toward using diverse preservation strategies to preserve or prolong the shelf-life of fresh foods including fishery products to ensure product safety (Sallam, 2007).

Natural preservatives, such as tea polyphenols and rosemary extract, have been widely used in the food industry because of their good preservative effect (Li et al., 2012; Ozogul et al., 2010; Fan, Chi, & Zhang, 2008; Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007; Tironi, Tomás, & Añón, 2009; Yi, Zhu, Fu, & Li, 2010). The preservative effect of tea polyphenols and rosemary extract is mainly due to the inhibition of some enzymes' activities, as well as the free radical scavenging ability and therefore prevention of lipid oxidation (Bubonja-Sonje, Giacometti, & Abram, 2011; Fan et al., 2008). In addition, their antimicrobial properties are also important (Georgantelis et al., 2007; Yi et al., 2010). Limited data, however, are available with regard to the application of tea polyphenols and rosemary extract for extension of the shelf-life of crucian carp. Thus, the objective of the present

* Corresponding author at: College of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou 310035, China. Tel./fax: +86 416 3400008.
E-mail addresses: tingting780612@yahoo.com (T. Li), lijianrong@mail.zjgsu.edu.cn (J. Li).

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study was to evaluate the potential effects of tea polyphenols and rosemary extract as antioxidants on the quality and shelf-life of crucian carp during chilled storage.

2. Materials and methods

2.1. Fish sample preparation

Fifty live commercial-sized crucian carp with an average weight of 360 ± 10 g were purchased from Dalian Aquatic Market in September (Dalian, Liaoning province, China). They were transferred to the Food Processing Laboratory of Dalian Nationalities University within 0.5 h and kept alive before being processed. The fish were killed by slurry ice and kept whole and ungutted at 0 °C before use.

2.2. Natural antioxidants and chemicals

Tea polyphenols was purchased from Zhejiang University Tea Scientific Co., Ltd. (purity ⩾ 98%, Hangzhou, Zhejiang province, China). Rosemary extract containing 22 ± 4% phenolic diterpenes (carnosic acid, carnosol, rosmarinic acid, as stated by the manufacturer) was bought from Guizhou Red Star Development Duyun Luyou Co., Ltd. (Duyun, Guizhou province, China). The two extracts were packed in polyethylene bags and stored at 4 ± 1 °C before using.

2.3. Preparation of fish samples

The whole fish were washed under running tap water and divided into three groups. First group was used as the control, second group was immersed in a solution of 2 g of tea polyphenols in 1 L of distiled water (4 ± 1 °C) for 60 min (0.2% TP), and the third was immersed in a solution of 2 g of rosemary extract in 1 L of distiled water (4 ± 1 °C) for 60 min (0.2% R), then the three groups of fish were kept on a plastic net for 2 h to drain at 4 ± 1 °C. After that, they were packed in air-proof polyethylene pouches and stored at 4 ± 1 °C for subsequent quality assessment. Microbiological, chemical, and sensory analyses were performed at 5-day intervals, each analysis was repeated three times with three fish and the averages were used to measure the overall quality of fish.

2.4. Proximate composition analyses

A proximate composition analysis was performed on five fish on day 0 of preservation. Proximate analyses (moisture content, total crude protein, lipid content and ash content) of the fish samples were based on the procedures set by the AOAC (1997).

2.5. Bacteriological analyses

Fish samples were taken aseptically in a vertical laminar-flow cabinet and 10 g were transferred to a stomacher bag; 90 ml of 0.1% peptone water with salt (NaCl, 0.85%, w/v) were added and homogenised for 60 s with a stomacher. From this dilution, other decimal dilutions were obtained and 1 ml of three dilutions was transferred in triplicate to Petri dishes containing 15 ml commercial plate count agar (PCA, Base Bio-Tech, Hangzhou, China). Total viable counts (TVC) were determined by counting the number of colony-forming units after incubation at 25 °C for 48 h.

2.6. Chemical analyses

2.6.1. pH and Total volatile basic nitrogen (TVB-N)

The pH values were measured to illustrate the hygienic standard of fish and other aquatic products according to the GB/T of the Chinese standard (GB/T 5009.45-2003). A 10.0-g sample of fish muscle was mixed with 90 ml of distiled water and the mixture was filtered. After 30 min, the pH of the filtrate was measured using a digital 320 pH metre (Mettler Toledo, Zurich, Switzerland).

Total volatile basic nitrogen (TVB-N) value was estimated by the FOSS. (2002). The microdiffusion method was mensurated by distillation after adding MgO to the homogenised samples. TVB-N values were determined with a Kjeltec 2300 (FOSS, Hiller, Denmark). TVB-*N* values were expressed in mg nitrogen kg^{-1} (mg N kg^{-1}) fish sample.

2.6.2. K-value

ATP and its breakdown products were measured according to the modified method of Özogul, Özden, Özoğul, and Erkan (2010). Five grams of minced fish flesh without skin were extracted with 25 ml of 0.6 M perchloric acid using an Ultra-Turrax (T25 basic; IKA-Werke, Staufen, Germany) for 1 min in an ice bath. The extraction mixture was centrifuged at 1940*g* for 10 min and after that, 10 ml of supernatant were quickly adjusted to pH 6.5–6.8 using 1 M KOH. The neutralised supernatant was allowed to stand for 30 min in an ice bath to precipitate most of the potassium perchlorate, which was then removed by centrifuging at 1940*g* for 10 min. The supernatant solution was made up to 20 ml and then stored at −80 °C until HPLC analysis. The identification of nucleotides, nucleosides and bases was determined and calculated by comparing their retention times with those of commercially obtained standards and by adding or spiking of standards. The *K*-value was defined as the per cent ratio of inosine (HxR) and hypoxanthine (Hx) to the sum of ATP and degradation products as follows:

$$K\% = [(\mathrm{HxR} + \mathrm{Hx})/(\mathrm{ATP} + \mathrm{ADP} + \mathrm{AMP} + \mathrm{IMP} + \mathrm{HxR} + \mathrm{Hx})] \times 100.$$

2.6.3. Thiobarbituric acid reactive substances (TBARS)

The thiobarbituric acid value was determined colorimetrically by the method of Porkony and Dieffenbacher, as described by Kirk and Sawyer (1991). A portion (200 mg) of sample was weighed into a 25-ml volumetric flask. An aliquot (1 ml) of 1-butanol was added to dissolve the sample. The mixture was made to volume and mixed. A portion (5.0 ml) of the mixture was pipetted into a dry stoppered test tube and 5 ml of TBA reagent (prepared by dissolving 200 mg of 2-TBA in 100 ml 1-butanol, filtered, stored at 4 °C for not more than 7 days) were added. The test tubes were stoppered, vortexed and placed in a water bath at 95 °C for 120 min, then cooled. Absorbance (A_s) was measured at 530 nm against water blank. A reagent blank was run and absorbance (A_b) recorded. TBA value (mg of malonaldehyde equivalents/kg of tissue) was obtained by the formula:

$$\mathrm{TBA} = \frac{50 * (A_s - A_b)}{200}$$

2.6.4. Texture measurements

Texture profile analyses (TPA) were carried out according to Sigurgisladottir et al. (1999). The TA.XT texture analyser (Stable Micro Systems Ltd., Godalming, UK) was used. A flat-ended cylinder that simulated the human finger was used. Constant penetration depth of 2.5 mm was applied on the fillets of about 15 mm thickness after testing penetrations in the range of 2–5 mm. This penetration depth was chosen as the maximum distance which could be