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The use of a tea polyphenol dip to extend the shelf life of silver carp (*Hypophthalmicthys molitrix*) during storage in ice

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Abstract

The effects of a tea polyphenols (TP) dip treatment on quality changes of silver carp (*Hypophthalmicthys molitrix*) during iced storage were examined over a period of 35 days. TP (0.2%, w/v) solution was used for the dip treatment. The control and the treated fish samples were analysed periodically for microbiological (total viable count), chemical (pH, TVB-N, TBA, *K*-value), and sensory characteristics. The results indicated that the effect of the TP dip treatment on the fish samples was to enable the good quality characteristics to be retained for longer and to extend the shelf life during the iced storage.

Keywords: Crap; Tea polyphenols; Iced storage; Quality

1. Introduction

Carp, as one of the freshwater fish species, has been one of the most widely cultured species all over the world due to its fast growth rate, easy cultivation, high feed efficiency ratio as well as high nutritional value (Tokur, Ozkütük, Atici, Ozyurt, & Caner Ozyurt, 2006). In China, *Hypophthalmicthys molitrix* species, named silver carp, is extensively cultured. Statistical data show that 10,600,000 tons were caught in China in 2005 corresponding to 26.3% of the harvesting of all fish species (Anonymous, 2006). However, fish are perishable food commodities, which generally spoil faster than do other muscle foods. Deterioration of fish mainly occurs as a result of bacteriological activity leading to loss of quality and subsequent spoilage (Liston, 1980). The spoilage of fish is a complex process in which physical, chemical and microbiological mechanisms are implicated. Iced storage is, therefore, an efficient way of reducing the rate of the deterioration of fish and also of extending the shelf life of fish. Many fish species have been examined during storage on ice (Karungi, Byaruhanga, & Muyonga, 2004; Namulema, Muyonga, & Kaaya, 1999; Ryder, Fletcher, Stec, & Seelye, 1993). However, the quality of fish muscle will also deteriorate during iced storage as the fish muscle is abundant in proteins and unsaturated fatty acid. Endogenous proteases, which are able to hydrolyze different proteins in the fish muscle, are important early in the deterioration process (Cepeda, Chou, Bracho, & Haard, 1990), so taking some measures to delay the decline of fish quality and extending the preservation life of fish during ice storage is worthwhile.

Tea polyphenols (TP), which play an important role in protein precipitation and enzyme inhibition (Cartriona, Cai, Russell, & Haslam, 1988; Shi, He, & Haslam, 1994), have beneficial anti-bacterial and anti-oxidative activities, which demonstrates potential for their use as the preservatives and the anti-oxidants in food industry especially in the field of the preservation of manufactured meat. However, there have been few studies on the use of TP dip to extend the shelf life of silver carp (*H. molitrix*) during storage in ice. The aim of this study was to investigate the effect of a TP dip treatment on the quality changes of silver crap during iced storage.

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2. Materials and methods

2.1. Preparation of the fish samples and storage conditions

The fresh silver carp samples, varying from 200 to 300 g in weight, were procured from the Yangtze River. After being gutted and washed, the fish samples were given a dip treatment in 0.2% (w/v) TP (≥95%, Sigma) solution (4 °C)(lot I) and in distilled water (4 °C) (lot II) as a control, respectively for 90 min and then drained well. After that, they were individually packed in plastic trays and air proofed with polyvinyl dichloride (PVDC), then all the packs were iced with flake ice in the ratio (1:1) fish:ice in an insulated box and were kept in refrigerator maintained at −3 °C for 35 days. Fish samples were taken randomly every seven days for analysing for microbiological, chemical, and sensory parameters.

2.2. Bacteriological analysis

Twenty five grams of fish portions was aseptically weighed and homogenized with 225 ml sterile 0.85% normal saline for 1 min. The homogenized sample was serially diluted using 9 ml sterile saline for bacteriological analysis. Total viable counts (TVC) were determined in plate count agar by the spread plate method (AOAC, 2002).

2.3. Chemical analysis

2.3.1. Determination of pH

A 10 g sample of the fish flesh was homogenized in 100 ml of distilled water and the mixture was filtered. The pH of filtrate was measured using a digital pH meter (Cyberscan PC 510 UK).

2.3.2. Determination of total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) value was estimated by the microdiffusion method (Goulas & Kontominas, 2005). The microdiffusion method was determined by distillation after the addition of MgO to homogenized fish samples. The distillate was collected in a flask containing a 3% aqueous solution of boric acid and a mixed indicator produced from dissolution of 0.1 g of methyl red and 0.1 g of methylene blue to 100 ml of ethanol. Afterward, the boric acid solution was titrated with a 0.1 N hydrochloric acid solution. The TVB-N value was determined according to the consumption of hydrochloric acid.

2.3.3. Determination of 2-thiobarbituric acid (TBA) value

The 2-thiobarbituric acid (TBA) value was determined colorimetrically by the method of Porkony and Dieffenbancher (Kirk & Sawyer, 1991). The method is based on the spectrophotometric quantitation of the pink complex formed after reaction of one molecule of malondialdehyde (MDA) with two molecules of 2-thiobarbituric acid (TBA).

2.3.4. Determination of K-value

A 2 g sample of the fish flesh was homogenized with 5 ml of cold perchloric acid solution (10 g/100 ml), and centrifuged at 1500*g* for 15 min. The pH of supernatant was adjusted to 6.5–6.8 using 1 mol/l NaOH. The white precipitates were removed by centrifugation (1500*g*, 15 min) for loading to high performance liquid chromatography (HPLC). The supernatants were made up to 10 ml with deionized water, filtered through a 0.2 μm membrane filter and analysed for ATP and its related compounds using HPLC (Agilent1100 series, USA) equipped with CAPCEL, pak ODS C18 column (4.0 × 100mm, 3 μm). The sample (5 μl) was injected at a flow rate at 1.2 ml/min, and the peak was detected at 254 nm. The amounts of each ATP and its related compounds were determined and calculated based on the standard ATP, ADP, AMP, IMP, inosine (HxR) and hypoxanthine (Hx) (Choia, Linb, Tomlinsonb, & Parkb, 2007). *K*-values were defined by the following equation (Saito, Arai, & Matsuyoshi, 1959):

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

2.4. Sensory assessment

The sensory quality of fish sample was evaluated by seven member trained panelists from the laboratory staff. Panelists scored for colour, odour, flavour, general acceptability and texture, using a nine-point hedonic scale (1, dislike extremely to 9, like extremely). A sensory score of four was taken as the borderline of acceptability (Amerine, Pongborn, & Roescler, 1965).

2.5. Statistical analysis

Experiments were replicated twice on different occasions with different fish samples. All analyses were run in triplicate for each replicate. All data were subjected to analysis of variance (ANOVA). The last significant difference (LSD) procedure was used to test for difference between means (significance was defined at $p < 0.05$).

3. Results and discussion

3.1. Bacteriological analysis

Variations in the value of total viable counts (TVC) during the iced storage are presented in Fig. 1. Initial TVC in fish samples was 3.1$\log_{10}$ CFU/g. TVC of lot II samples rose continuously and reached about 9.5$\log_{10}$ CFU/g on the 35th day during the iced storage. The increasing of TVC in fish flesh during iced storage has been demonstrated by Leung, Huang, and Harrison (1992), Lyon and Reddmann (2000). TVC of lot I samples was found to be the same as lot II during the first seven days in the process of the iced storage, but later was observed to increase

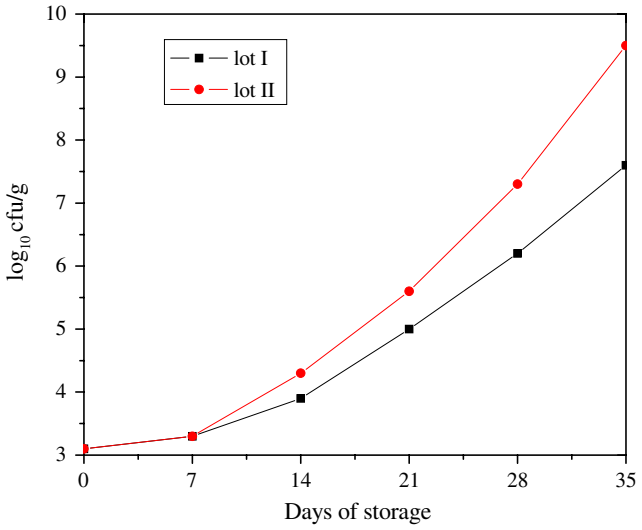

Fig. 1. Changes in total viable counts (TVC) of fish samples during iced storage.

slower than in lot II and reached 7.6$\log_{10}$ CFU/g on the 35th day of the iced storage. The significant reduction in TVC observed in lot I samples can be attributed to the inhibitory effect of TP on spoilage bacteria. The result of the treatment also indicated that dip treatment with 0.2% TP was equally effective in inhibiting spoilage bacteria growth and extending the iced storage life of fish samples to 35 days compared to 28 days for dip treatment with distilled water.

3.2. Chemical analysis

3.2.1. pH

The changes in the pH of fish samples during the iced storage are depicted in Fig. 2. The initial pH of the fish samples was 6.2. The pH of the samples in lot II decreased initially and then increased. The initial pH decrease may be attributed to the dissolution of CO_2 in the fish samples. Similar observations were made by Tiffney and Mills (1982), Manju, Jose, Srinivasa Gopal, Ravishankar, and Lalitha (2007). Several authors have reported a decrease in the pH of fish samples with increase in the concentration of CO_2 in the atmosphere (Lannelongue, Hanna, Finne, Nickelsen, & Vanderzant, 1982; Meekin, Hulse, & Bremner, 1982). The increase of pH may be attributed to the production of volatile basic components, such as ammonia and trimethylamine by fish spoilage bacteria (Hyytia, Hielm, Mokkila, Kinnunen, & Korkeala, 1999; Ruiz-Capillas & Moral, 2001). The changes in the pH of lot I samples have the same trend as lot II except that it increased slowly during the iced storage. It can be concluded that the lower pH of lot I can enhance microbial inhibition and contributes to the extending of the preservation of fish samples for inhibiting the activity of the endogenous proteases by TP.

3.2.2. Total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N), which is mainly composed of ammonia and primary, secondary and tertiary amines (Beatty, 1938), resulted from degradation of proteins and non-protein nitrogenous compounds, which is chiefly caused by microbial activity (Ruiz-Capillas & Moral, 2005). TVB-N is widely used as an indicator for meat deterioration (Olafsdottir et al., 1997). A level of 35–40 mg TVB-N/100 g of fish muscle is usually regarded as spoiled (Lakshmanan, 2000). However, various authors have reported different acceptability levels for different fish species, specific treatments, and processing conditions for TVB-N value: 35–40 mg/100 g (Connell, 1990); 25–30 mg/100 g (Lopez-Caballero, Perez-Mateos, Montero, & Borderias 2000); 25–35 mg/100 g (Ababouch et al., 1996). Changes in TVB-N value are shown in Fig. 3. As the results show, the values increased gradually in all samples during the iced storage. TVB-N contents increased from an initial

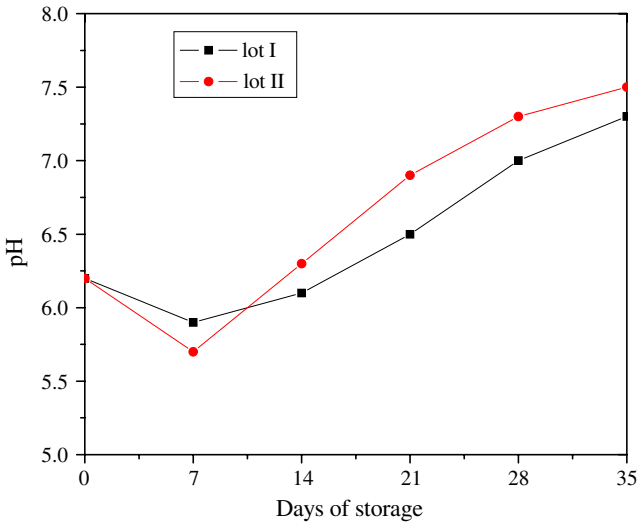

Fig. 2. Changes in pH of fish samples during iced storage.

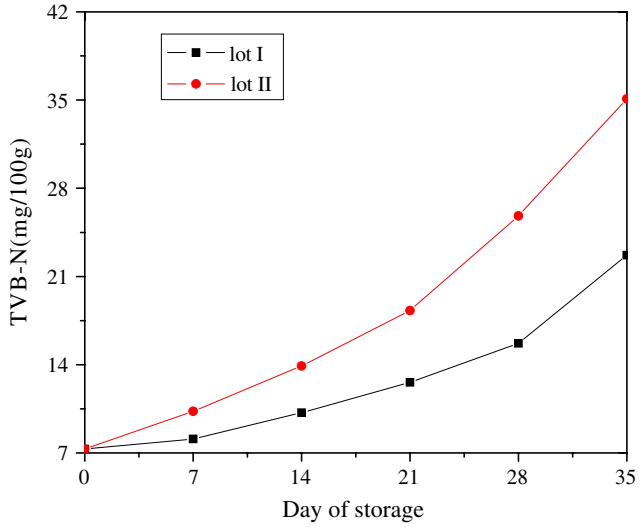

Fig. 3. Changes in TVB-N values of fish samples during iced storage.

value of 7.3 mg/100 g to 35.1 mg/100 g in lot II samples, to 22.7 mg/100 g in lot I. This increase was significantly lower in lot I samples than in lot II. This can be attributed to either a more rapidly reduced bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds or both (Banks, Nickelson, & Finne, 1980), which was due to the effect of TP in fish samples.

3.2.3. 2-thiobarbituric acid (TBA)

2-Thiobarbituric acid (TBA) is widely used as an indicator of degree of lipid oxidation, and the presence of TBA reactive substances is due to the second stage auto-oxidation during which peroxides are oxidized to aldehydes and ketones (Lindsay, 1991). In this study, changes in TBA value are shown in Fig. 4. The initial TBA value of fish samples was 0.37 mg MDA/kg and this value increased to 3.09 mg MDA/kg of fish samples in lot II. The increase in TBA value during the iced storage may be attributed to the partial dehydration of fish and to the increased oxidation of unsaturated fatty acids. This observation of the increase of the TBA value during iced storage is in agreement with results reported by Yanar and Fenercioglu (1998) for fish balls made from carp and by Gelman and Benjamin (1988) for minced pond-bred flesh of silver carp. However, during the iced storage of lot I samples, TBA values were not greatly altered. To the contrary, the TBA values of lot II samples rose continuously and reached 2 mg MDA/kg, which is usually regarded as the limit beyond which the fish will normally develop an objectionable odour and taste (Connell, 1990). After approximately 13 days of iced storage, however, the final TBA values of lot I, which were within the limit value, were 0.85 mg MDA/kg after 35 days of iced storage. The data revealed that lot I samples which were dipped with TP can extend the preservation of fish samples by inhibiting the oxidation of lipids in fish.

3.2.4. K-value

The *K*-value, which is the index of the degradation of ATP, is used as the most effective indicator of the freshness of fish. The rejection levels of the *K*-value observed in the present study are close to the 60% limit set by Ehira (1976) and Ehira and Uchiyama (1974). On the day of sensory rejection, the *K*-values of all the samples exceeded 60%, which indicates good correlation of *K*-values with the sensory scores (Manju, Srinivasa Gopal, Jose, Ravishankar, & Ashok Kumar, 2007). Variations in *K*-value during the iced storage are shown in Fig. 5. Initial *K*-value in fish samples was 4.3%. For freshly caught fish, the initial *K*-value reported was around 5% (Aleman, Kakuda, & Uchiyama, 1982). *K*-value of lot II samples rose continuously and reached about 47.9% on the 35th day during the iced storage. The results of the present study are also in agreement with those of Ozogul, Taylor, Quantick, and Ozogul (2000) who reported an increase in the *K*-value with storage time in vacuum packed Atlantic herring during chill storage. The changes in the *K*-value of lot I have the some trend as in lot II samples except that it exhibited lower *K*-values during the iced storage. It can be concluded that the lower *K*-value of lot I may result from the slower ATP degradation (Sugimoto & Fujiata, 1986) by the effect of TP dip treatment on the fish samples. The result of the data also indicated that dip with 0.2% TP was equally effective in inhibiting the degradation of ATP and extending iced storage life of fish samples.

3.3. Sensory assessment

The acceptability of fish and fishery products during frozen storage depends on the changes in their sensory attributes. The fish samples were considered to be acceptable for human consumption until the sensory score reached 4 (Amerine et al., 1965). The results of the sensory assessment of samples are given in Fig. 6. The results indicate

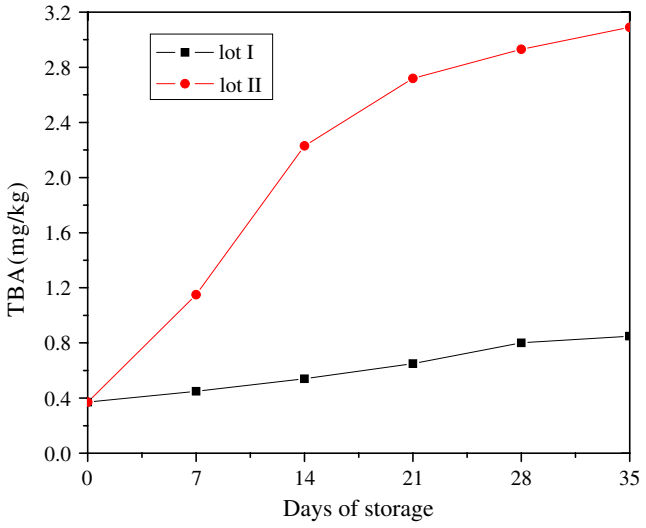

Fig. 4. Changes in TBA values of fish samples during ice storage.

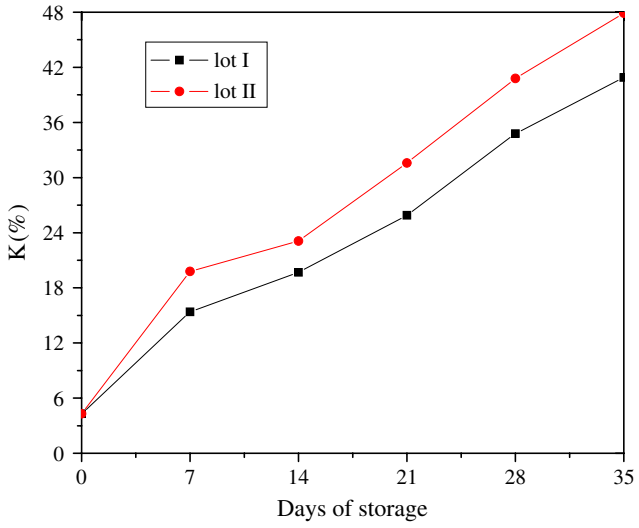

Fig. 5. Changes in *K*-values of fish samples during ice storage.

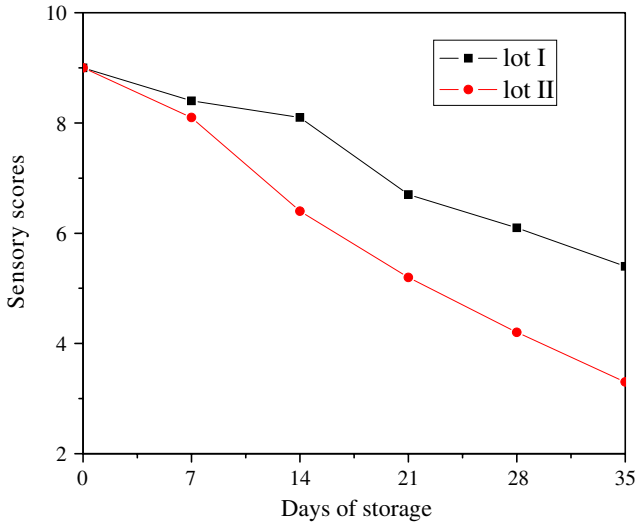

Fig. 6. Changes in Sensory scores of fish samples during ice storage.

that sensory scores showed a significant decline in both lot I and lot II samples with increasing storage period, and the results also indicate that the sensory properties of lot I samples received a higher score than lot II samples. Thus, lot II samples were acceptable up to 28 days and lot I samples were in good and acceptable condition during the whole 35 days iced storage. From the data, we can learn that lot I samples could be retaining their good quality characteristics in terms of sensory assessment. These conclusions were supported by the results for chemical quality analyses.

4. Conclusions

On the basis of the present results, TP dip treatment on silver carp (*H. molitrix*) leads to a retention of the good quality characteristics for longer and an extension of the shelf life during iced storage. The conclusion was supported by the results of microbiological (total viable count), chemical (pH, TVB-N, TBA, *K*-value), and sensory assessment analyses.

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