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Effects of chitosan coating on quality and shelf life of silver carp during frozen storage

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ABSTRACT

The effects of chitosan coating on quality and shelf life of silver carp during frozen storage were investigated. Fish samples were treated with aqueous solution of 2% chitosan, and then stored at −3 °C for 30 days. The control and the treated fish samples were analyzed periodically for microbiological (total viable count), chemical (pH, TBA, TVB-N, *K*-value), and sensory characteristics. The results indicated that the effect of chitosan coating on fish samples was to retain their good quality characteristics and extend the shelf life during frozen storage, which was supported by the results of microbiological, chemical, and sensory evaluation analyses.

1. Introduction

Carp is one of the most widely cultured and traded species all over the world due to its fast growth rate, easy cultivation, high feed efficiency ratio and high nutritional value. In China, the *Hypophthalmicthys molitrix* species, named silver carp, is extensively cultured. Statistical data show that 11,900,000 tons were caught in China in 2006 corresponding to 22.9% of the harvesting of all fish species (Anonymous, 2007). However, fish are perishable foods, which generally spoil faster than do other muscle foods. Frozen storage is a general preservation method, used to control or decrease biochemical changes in fish that occur during storage. Nevertheless, frozen storage does not completely inhibit microbial and chemical reactions that lead to quality deterioration of fish as fish muscle is abundant in proteins and unsaturated fatty acids (Vidya Sagar Reddy & Srikar, 1996). To retain the good quality characteristics for longer, and extend shelf life during frozen storage of fish, preservatives, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have been widely used. At the same time, in order to avoid the use of synthetic preservatives, which do more harm than good, numerous studies are currently focused on using natural ingredients to enhance fish quality and shelf life.

Chitosan [β-(1, 4)-2-amino-2-deoxy-D-glucopyranose], which is mainly made from crustacean shells, is the second most abundant natural polymer in nature after cellulose (Shahidi, Arachchi, & Jeon, 1999). Due to its non-toxic nature, antibacterial and anti-oxidative activity, film-forming property, biocompatibility and biodegradability, chitosan has attracted much attention as a natural food additive (Majeti & Kumar, 2000). Several authors have reported that chitosan has been used in foods, as a clarifying agent in apple juice (Boguslawski, Bunzeit, & Knorr, 1990), and antimicrobial and antioxidant in muscle foods (Darmadji & Izumimoto, 1994; Gómez-Estaca, Montero, Giménez, & Gómez-Guillén, 2007; Kim & Thomas, 2007). Furthermore, chitosan also has potential for food packaging, especially as edible films and coatings (Subramaniam, Quan, Jiaqi, & Witoon, 2007; Tual, Espuche, Escoubes, & Domard, 2000). However, research on the retention of the good quality characteristics for long periods and the extension of shell life during frozen storage of fish by chitosan coating is still lacking. Therefore, the objective of this study was to evaluate the effect of chitosan coating on the quality and shelf life of carp during frozen storage.

2. Materials and methods

2.1. Chitosan solution preparation

Chitosan, with a molecular weight of 1.6×10^5 and 85% degree of deacetylation, was purchased from Sigma Company(Saint Louis, MO, USA). To prepare 2% w/w chitosan solution in glacial acetic acid, 20 g chitosan were mixed with 900 ml of distilled water and stirred for 10 min, and then 10 ml of glacial acetic acid were added to the mixture which was then stirred for 2 h, and the solution was made up to 1000 ml with distilled water.

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2.2. Sample preparation

Fresh silver carp (*H. molitrix*), varying from 200 g to 300 g in weight, were procured from the Yangtze river. After being gutted and washed, fish samples were given a dip treatment in 2% chitosan solution (lot I) and in 1% glacial acetic acid (lot II) as a control, respectively for 120 min and then well drained. After that, they were individually packed in plastic trays and airproofed with polyvinyl dichloride (PVDC); then all the packs were kept in a refrigerator maintained at −3 °C for 30 days (Duun & Rustad, 2008; Gallart-Jornet, Rustad, Barat, Fito, & Escriche, 2007). Fish samples were taken randomly every 5 days for microbiological, chemical and sensory evaluation.

2.3. Microbial analysis

Total viable counts (TVC) were determined in plate count agar by the spread plate method (AOAC, 2002).

2.4. Chemical analysis

2.4.1. Determination of pH

A 10 g sample of the fish muscle was homogenized in100 ml of distilled water and the mixture was filtered. The pH of filtrate was measured using a digital pH meter (Cyberscan PC 510 UK).

2.4.2. Determination of 2-thiobarbituric acid (TBA)

2-Thiobarbituric acid (TBA) value was determined colorimetrically by the method of Porkony and Dieffenbancher as described by Kirk and Sawyer (1991).

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

Total volatile base 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.

2.4.4. Determination of K-value

K-value was estimated as described by Choia, Linb, Tomlinsonb, and Parkb (2008) and Fan, Chi, and Zhang (2008).

2.5. Sensory evaluation

The sensory quality of fish sample was evaluated by a seven member trained panel from the laboratory staff. Panellists scored for sensory characteristics, such as colour, odour, flavour, general acceptability and texture, using a nine-point hedonic scale (1, dislike extremely to 9, like extremely).

2.6. Data analysis

Experiments were replicated twice on different occasions with different fish samples. All analyses were run in triplicate for each replicate ($n = 2 \times 3$). All data were subjected to analysis of variance (ANOVA). The least 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. Microbial analysis

The initial total viable count (TVC) of fish sample was 2.9 $\log_{10}$ CFU/g, and the low initial TVC indicated very good fish quality. Changes in TVC of fish sample during the frozen storage are shown in Fig. 1. TVC of chitosan-coated fish sample (lot I) was found to be the same as that of the control sample (lot II) during the first 5 days of the frozen storage, but later, was observed to be increasing more slowly than that in lot II and reached 6.9 $\log_{10}$ CFU/g on the 30th day of the frozen storage. It did not exceed the maximal permissible limit of 7.0 $\log_{10}$ CFU/g for the bacterial count in fish (ICMSF, 1986), while the TVC of lot II sample reached about 7.1 $\log_{10}$ CFU/g on the 25th day during the frozen storage. The result of the contrast indicated that 2% chitosan solution coating was equally effective for extending the −3 °C storage life of the fish sample to 30 days compared with 25 days for the control sample, and the significant reduction in TVC observed in the lot I sample can be attributed to the inhibitory effect of chitosan on spoilage bacteria.

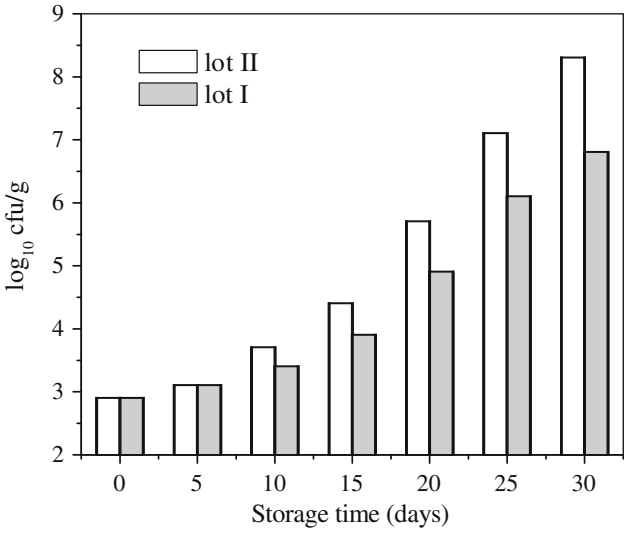

Fig. 1. Changes in TVC of fish sample during frozen storage.

3.2. Chemical analysis

3.2.1. pH

Variations in values of pH during the frozen storage are depicted in Fig. 2. The initial pH of the fish sample was found to be

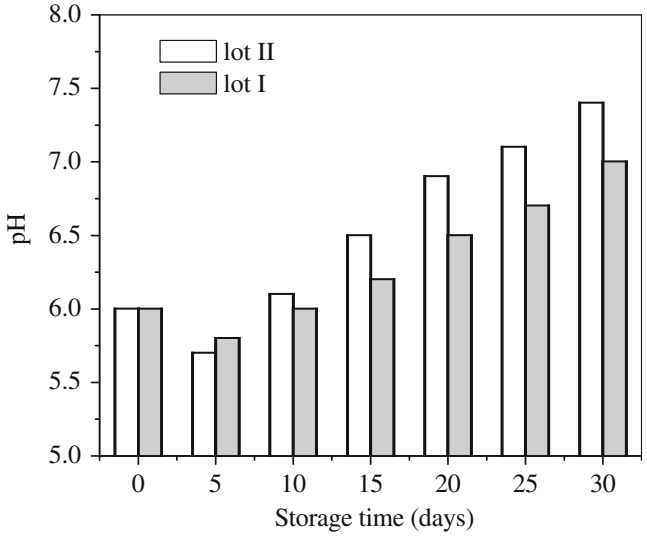

Fig. 2. Changes in pH of fish sample during frozen storage.

6.0. In all fish samples, the values of pH decreased initially and then increased. Similar observations were made by Alasalvar et al. (2001) and Manju, Jose, Srinivasa Gopal, Ravishankar, and Lalitha (2007). The initial pH decrease may be attributed to the dissolution of CO_2 in the fish sample, while the increase of pH was postulated to be due to an increase in volatile bases produced, e.g. ammonia and trimethylamine, by either endogenous or microbial enzymes (Manat, Soottawat, Wonnop, & Cameron, 2005; Ruiz-Capillas & Moral, 2001). The data revealed that variations in values of pH in the lot I sample have the same trend as have those in lot II, except that pH increased slowly during the frozen storage. It is concluded that the lower pH of lot I can enhance microbial inhibition and contributes to the extending of the preservation of fish samples by inhibiting the activity of the endogenous proteases (by chitosan).

3.2.2. 2-Thiobarbituric acid (TBA)

2-Thiobarbituric acid (TBA) is an index of lipid oxidation. Changes in TBA value are shown in Fig. 3. TBA value of the lot II fish sample was significant higher than the corresponding value of the lot I sample during the frozen storage. This observation was indicative of the fact that chitosan clearly inhibited lipid oxidation in fish flesh. According to Connell (1990), TBA values of 1–2 mg MDA/kg of fish flesh are usually regarded as the limit beyond which fish will normally develop an objectionable odour. In this study, the initial TBA value of the lot II fish sample was 0.38 mg MDA/kg and this value increased to 1.41 mg MDA/kg after 10 days of the storage period. After 15 days, this value reached 2.32 mg MDA/kg, which exceeded the maximal permissible limit of 2 mg MDA/kg in the fish muscle. However, the final TBA value of lot I, which was within the limit value, was 0.83 mg MDA/kg after 30 days of frozen storage. The data revealed that the lot I sample, which was dipped with chitosan, indicated preservation of fish flesh by inhibiting the oxidation of lipid.

3.2.3. Total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N), which is mainly composed of ammonia and primary, secondary and tertiary amines, is widely used as an indicator of meat deterioration. Its increase is related to the activity of spoilage bacteria and endogenous enzymes (Kyrana, Lougovois, & Valsamis, 1997; Vareltzis, Koufidis, Gavriilidou, Papavergou, & Vasiliadou, 1997). According to Connell (1990), a level of 35–40 mg TVB-N/100 g of fish flesh is usually regarded as spoiled. Changes in TVB-N value are shown in Fig. 4. The initial TVB-N value was 7.3 mg/100 g and it increased progressively with time of frozen storage for both fish samples. The final TVB-N values of both fish samples did not exceed the upper acceptability limit after 30 days of frozen storage. The data also showed that TVB-N increase was significantly lower in the lot I sample than in lot II. After 30 days of frozen storage, TVB-N contents increased from an initial value to 18.8 mg/100 g in lot I to 30.2 mg/100 g in lot II. This fact was indicative of either a faster reduction of bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds (or both) due to the effect of chitosan in the fish samples.

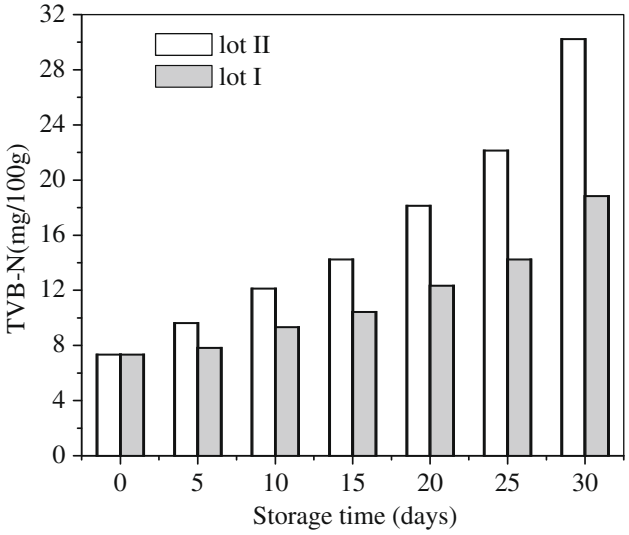

Fig. 4. Changes in TVB-N values of fish sample during frozen storage.

3.2.4. K-value

The *K*-value, which is the index of the degradation of ATP, is used as the most effective indicator for testing 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). Changes in *K*-value during the frozen storage are shown in Fig. 5. Initial *K*-value in fish samples were 4.5%.

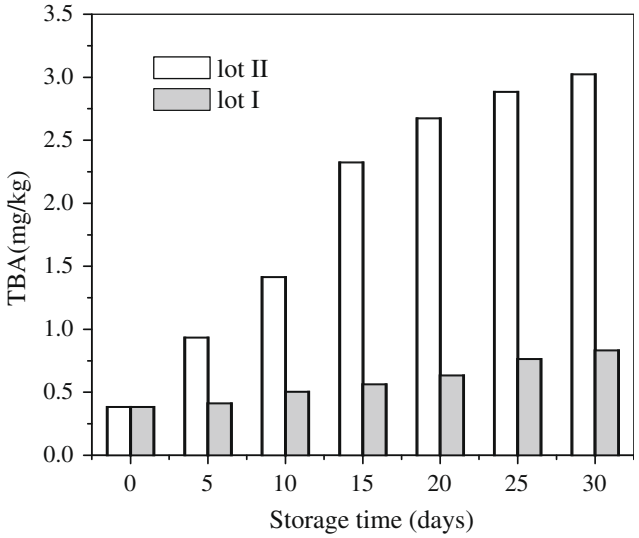

Fig. 3. Changes in TBA values of fish sample during frozen storage.

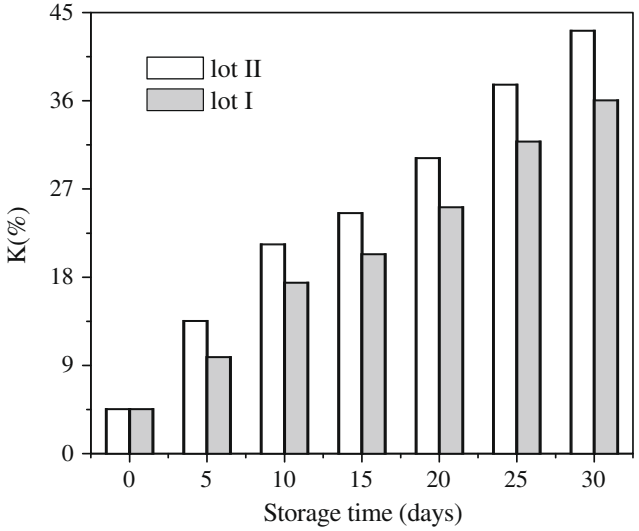

Fig. 5. Changes in *K*-values of fish sample during frozen storage.

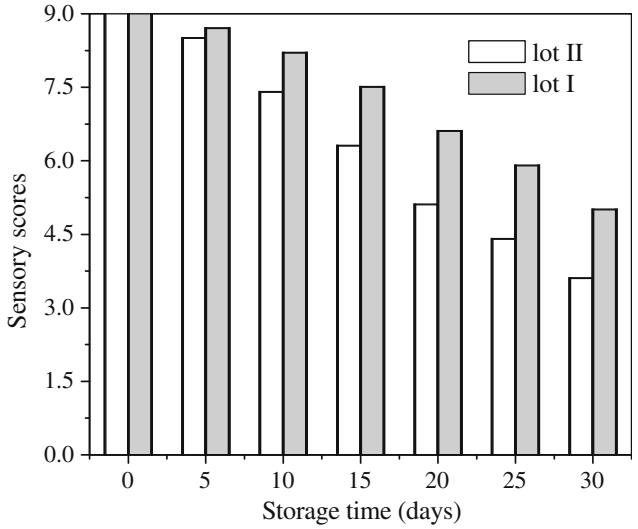

Fig. 6. Changes in sensory scores of fish sample during frozen storage.

K-value of lot II rose continuously and reached about 43.1% on the 30th day of frozen storage. The changes of the *K*-value in lot I show the same trend as do those in lot II except that there were lower *K*-values during the frozen storage. The lower *K*-value in lot I resulted from the decomposition of inosine monophosphate (IMP), as a result of the activity of 5-nucleotidase.Therefore, the relatively lower *K*-value in lot I could be explained by the ability of chitosan to minimize the activity of 5-nucleotidase (Aubourg, Pineiro, Gallardo, & Barros-Velazquez, 2005; Losada, Pineiro, Barros-Velázquez, & Aubourg, 2005; Nejib, Moza Abdallah, Ismail Mohammed, Ann, & Mohammad, 2005). The result also indicated that the dip with 2% chitosan was equally effective in inhibiting the degradation of ATP and extending frozen storage life of fish samples.

3.3. Sensory evaluation

The sensory qualities of fish samples were evaluated in terms of colour, odour, flavour, general acceptability and texture, using a nine-point hedonic scale (1, dislike extremely to 9, like extremely). The fish samples were considered to be acceptable for human consumption until the sensory score reached 4 (Truelstrup Hansen, Gill, & Huss, 1995). The results of the sensory evaluation of samples are given in Fig. 6. Sensory scores showed a significant decline in both lot I and lot II samples with increasing storage period and, also, the lot I sample received a higher score than did the lot II sample. It is well known that fish spoilage gives rise to the subsequent development of strongly fishy, rancid and putrid odours, and fish are then clearly rejected for consumption by any taste panel. Thus, lot II was acceptable up to 25 days while lot I was in good and acceptable condition during the entire 30 days of storage. This may be attributed to chitosan's functional properties, e.g. antioxidant, antimicrobial and oxygen barrier, and this conclusion is supported by the results of chemical quality analyses.

4. Conclusions

The results of microbiological (total viable count), chemical (pH, TBA,TVB-N, *K*-value), and sensory evaluation analyses indicate that chitosan coating on silver carp (*H. molitrix*) can lead to retention of the good quality characteristics and extension of the shelf life during frozen storage.

Acknowledgements

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