Effects of Chitosan Coating Containing Antioxidant of Bamboo Leaves on Qualitative Properties and Shelf Life of Silver Carp during Chilled Storage

Fan WENJIAO[1], Zhang YONGKUI[2], Diao PAN[2] and Yi YUWEN[1]

[1]Department of Food Science, Sichuan Tourism University, Chengdu, P.R. China;
[2]School of Chemical Engineering, Sichuan University, Chengdu P.R. China

Abstract

Wenjiao F., Yongkui Z., Pan D., Yuwen Y. (2013): **Effects of chitosan coating containing antioxidant of bamboo leaves on qualitative properties and shelf life of silver carp during chilled storage**. Czech J. Food Sci., **31**: 451–456.

The effect of chitosan coating containing antioxidant of bamboo leaves (AOB) on the shelf life extension of silver carp (*Hypophthalmicthys molitrix*) was evaluated at refrigerated temperature (4 ± 1°C). Microbiological changes (total viable count – TVC), physicochemical changes (water loss, pH, total volatile nitrogen – TVB-N, trimethylamine – TMA-N, and 2-thiobarbituric acid – TBA), and sensory changes were determined during chilled storage. The results indicated that the coating treatments could effectively retard the water loss, inhibit the growth of total viable counts, reduce chemical spoilage, which reflected itself in TVB-N, pH, TMA-N, and TBA, and increase the overall sensory quality of silver carp in comparison with the control sample. The study suggests that chitosan coating containing AOB can be a promising candidate for extending the shelf life of silver carp during chilled storage.

Keywords: *Hypophthalmicthys molitrix*; edible coatings; shelf life extension

Silver carp (*Hypophthalmicthys molitrix*) is one of the most economically important freshwater-cultured fish species in eastern countries for its fast growth rate, easy cultivation, high feed efficiency ratio and high nutritional value. In India, as regards the fresh water fishes, silver carp is the most important species contributing about 67.7% of the total fish production (Siddaiah *et al*. 2001). It is also a major freshwater aquaculture species in China. The statistics show that 31 900 000 t of silver carp were caught in China in 2011, corresponding to 32.7% of the harvest of all fish species (Fishery Bureau of Department of Agriculture of China 2012). However, silver carp is an easily perishable product because of its high water activity, presence of autolytic enzymes and relatively high quantities of volatile basic nitrogen as well as free amino acids (Xu *et al*. 2010). Therefore, taking some measures to delay the loss of silver carp quality and extending the storage life is worthwhile.

The use of edible coatings could be a beneficial method of extending the preservation life of fish products, since they function as a barrier against oxygen, carbon dioxide, and possess suitable mechanical properties at low relative humidity (Pereira de Abreu *et al*. 2012). Chitosan [β-(1,4)-2-amino-2-deoxy-D-glucopyranose], which is mainly made from crustacean shells, is a well-known film-forming biopolymer with strong anti-bacterial and anti-oxidative activities (Kim & Thomas 2007; Aider 2010). It has been widely applied to fish products (Fan *et al*. 2009; Ojagh *et al*. 2010; Siripatrawan & Noipha 2012). The developments of hydrophilic edible coatings incorporating natural preservatives with antibacterial and antioxidant activities that prolong the shelf life

Supported by Scientific Research Fund of Sichuan Provincial Education Department of China, Project No. 13CZ0020.

of food products are valuable (Krkić *et al*. 2013; Li *et al*. 2013a; Rubilar *et al*. 2013). The antioxidant of bamboo leaves, abbreviated to antioxidant of bamboo leaves AOB, is a kind of polyphenol-rich extract from bamboo leaves of the *Phyllostachys* Sieb. et Zucc, which not only blocks the chain reaction of spontaneous oxidation of lipids, but also chelates transition metals, acting as a primary and secondary antioxidant synchronously, and it can be used in edible oils, meat products, aquatic products, and other foods (Hu *et al*. 2000). The main functional components in the antioxidant of bamboo leaves are flavonoids, phenolic acids, and lactones (Lu *et al*. 2006).

However, there have been few studies on the use of chitosan coating containing AOB for the preservation of quality and extension of the shelf life of fish during chilled storage. Therefore, the objective of the present study was to examine the potential effects of chitosan coating containing AOB on the quality and shelf life of silver carp during chilled storage. The examination was based on the microbiological changes, physicochemical changes, and sensory changes after 21 days of storage.

MATERIAL AND METHODS

Natural antioxidants and chemicals. Food grade chitosan (MW 4.9×10^5, degree of deacetylation 90%, viscosity 75 cps) was procured from Leshan KeLiXin Chemical Co., Ltd. (Sichuan, China). AOB samples (Food grade) containing 35 ± 3% total falconoid and 12 ± 1% phenolic acid were obtained from Ya'an Natural Additive Co., Ltd. (Sichuan, China).

Fish 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, the fish samples were given a dip treatment in 0.2% (w/v) AOB aqueous solution for 10 min and in distilled water as a control, and then were drained for 15 minutes. After that, they were individually coated by immersing in 1% (w/v) chitosan aqueous solution for 10 min, after which they fish samples were removed and permitted to drain for 30 minutes. Subsequently they were packed in air-proof polyethylene pouches and stored at 4 ± 1°C for the following quality assessment. The fish samples were taken randomly every 3 days for the microbiological, physicochemical, and sensory evaluation. Each analysis was repeated three times with three fish and the averages were used to estimate the overall quality of fish (Li *et al*. 2013b).

Microbiological analysis. Total viable counts (TVC) were determined in plate count agar by the spread plate method (AOAC 2002). Microbial counts were presented as colony forming units (CFU)/g fish sample.

Physico-chemical analysis. The water loss was determined as described by Lu *et al*. (2009). The percentage of water loss relative to the initial weight was calculated by weighing the samples every 3 days in triplicate. Fish muscle (10 g) was homogenised thoroughly with 100 ml of distilled water and the homogenate was used for pH determination. The pH of filtrate was measured using a digital pH meter (Cyberscan PC 510; Eutech, Chicago, UK). Total volatile base nitrogen (TVB-N) value was estimated by the micro diffusion method (Goulas & Kontominas 2005). Trimethylamine (TMA-N) was determined using the procedure described by Malle and Poumeyrol (1989). TVB-N and TMA-N contents were expressed as TVB-N or TMA-N per 100 g of fish muscle. The 2-thiobarbituric acid (TBA) value was determined colorimetrically by the method of Pokorny and Dieffenbancher as described by Kirk and Sawyer (1991). Sensory analysis was evaluated as described by Fan *et al*. (2009). The sensory quality of the fish sample was evaluated by a seven member trained panel from the laboratory staff. The panelists 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). A sensory score of 4 was taken as the limit of acceptability.

Data analysis. The 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 the analysis of variance (ANOVA). The least significant difference (*LSD*) procedure was used to test the difference between means. Significance level was set at 5%.

RESULTS AND DISCUSSION

Microbiological analysis

The variations in the value of TVC during chilled storage are presented in Figure 1. The initial number of bacteria in the silver carp mus-

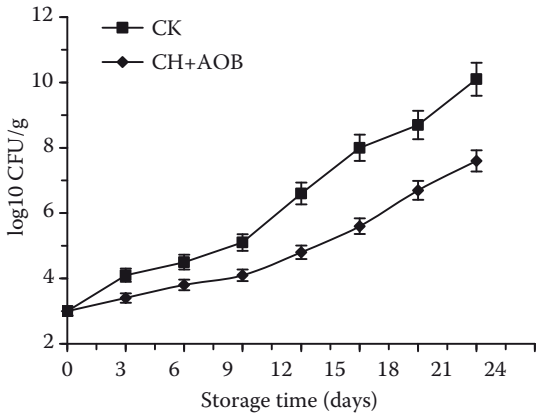

Figure1. Changes in total viable counts (TVC) of silver carp during chilled storage

CK – control; CH+AOB – chitosan coating with antioxidant of bamboo leaves

cle was 3.0 $\log_{10}$ CFU/g, which indicated a good quality of the fish used in this experiment. Figure 1 shows that TVC of all samples increased with the storage time, and that the value of the control sample (CK) increased faster than that of the sample with chitosan coating containing AOB (CH+AOB). After 12 days of storage, the TVC of CK sample increased quickly and reached 8.0 $\log_{10}$ CFU/g on day 15, which exceeded the maximum acceptable level of 7.0 $\log_{10}$ CFU/g for freshwater fish (ICMSF 1986), while CH+AOB sample reached about 6.8 $\log_{10}$ CFU/g on day 18, which was still within the maximal permissible limit for the bacterial count in fish. The results showed that the microbiological growth was significantly influenced by the chitosan coating containing AOB. The significant difference in TVC observed between CK and CH+AOB samples may be due to the fact that the coating, which is rich in AOB and acts as an antimicrobial agent and barrier against oxygen transfer as well, leads to the inhibition of the bacteria growth.

Determination of pH and water loss

The variations in the pH values during the chilled storage are shown in Figure 2. In all fish samples, the values of pH initially decreased and then increased. 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 *et al*. 2005). Similar observations were made in other fish species during chilled storage by Manju *et al*. (2007) and Li *et al*. (2013b). The data from Figure 2 also revealed that the pH of CH+AOB sample was lower than that of CK, thus the sample of CH+AOB could effectively delay the enzyme activity.

The water loss of silver carp during chilled storage is depicted in Figure 2. The pattern of water loss for CH+AOB sample was similar but situated lower than that of CK sample. Pham and Willix (1984) found that the desiccated surface layer developed during cold storage produces a further resistance to the mass transfer in the case of biological substances, and Lu *et al*. (2009) also reported that the reduction in dehydration of snakehead is attributed mainly to the fact that the alginate-calcium coatings act as water vapour barriers during the entire storage period. Therefore, the significantly lower water loss that occurred in the sample of CH+AOB

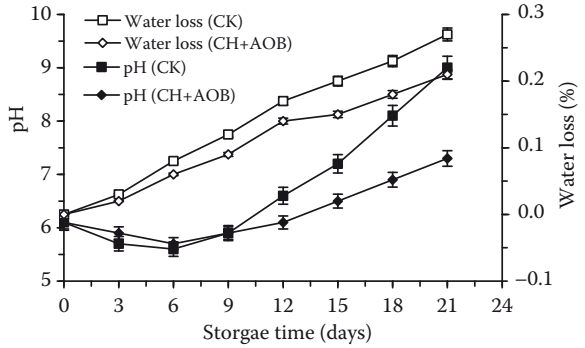

Figure 2. Changes in pH and weight loss of silver carp during chilled storage

CK – control; CH+AOB – chitosan coating with antioxidant of bamboo leaves

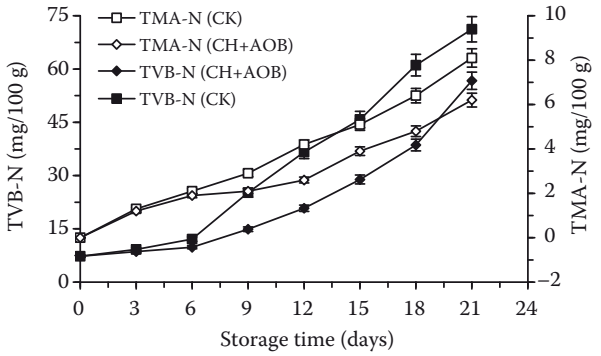

Figure 3. Changes in TVB-N and TMA-N of silver carp during chilled storage

CK – control; CH+AOB – chitosan coating with antioxidant of bamboo leaves

can be attributed to the chitosan coating containing AOB which acted effectively as a water vapour barrier during the entire storage period.

Determination of TVB-N and TMA-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 fish deterioration. Özyurt *et al.* (2009) proposed that the quality of fish products regarding TVB-N values can be classified as follows: "high quality" up to 25 mg/100 g, "good quality" up to 30 mg/100 g, "limit of acceptability" up to 35 mg/100 g, and "spoilt" above 35 mg/100 g. The changes in TVB-N values in the fish samples during chilled storage are shown in Figure 3. The results show that the values increased progressively from the initial values of 7.3 mg/100 g to the final values of 71.2 and 56.7 mg/100 g for the CK and CH+AOB samples, respectively. The data also show that CH+AOB sample maintained a significantly lower TVB-N value than that of the CK. This finding may be attributed to either a faster reduction of bacterial population or a decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds (or both) (Banks *et al.* 1980). When considering the TVB-N value level of 35 mg per 100 g of fresh fish as the acceptable limit, the shelf life of the CK and CH+AOB treated samples were about 12 and nearly 18 days, respectively.

Furthermore, TMA-N is also used as an index in evaluating the freshness of fish products (Figure 3). The changes in TMA-N values in fish samples followed a similar pattern of TVB-N during chilled storage, whereas a significant increase was observed during chilled storage. Bindu *et al.* (2013) also observed an increase of TMA during ice storage of the treated Indian white prawn (*Fenneropenaeus indicus*) during chilled storage. According to Connell (1990), a level of 5 mg TMA-N/100 g of fish flesh or higher is usually regarded as spoilage indicator, the shelf life of the CK and CH+AOB having been about 14 and nearly 19 days, respectively.

Determination of TBA

2-Thiobarbituric acid (TBA) is widely used as an indicator of the degree of lipid oxidation, which can lead to off-flavour, colour and odour changes, and contribute to the texture deterioration in the fish products. According to Connell (1990), a TBA value of 2 mg MDA/kg is regarded as the acceptability limit. The initial TBA value of the fish samples was 0.36 mg MDA/kg and this value increased to 4.23 and 2.34 mg MDA/kg at the end of storage for the CK and CH+AOB, respectively (Figure 4). The increase in TBA value during the chilled storage may be attributed to the partial dehydration of fish and to the increased oxidation of unsaturated fatty acids (Mendes *et al.* 2008). In the CH+AOB sample, chitosan coating used directly on the surface of fish might act as a barrier between silver carp meat and its surroundings, thus lowering the diffusion of oxygen to the fish meat surface. Besides, dipping silver carp in AOB

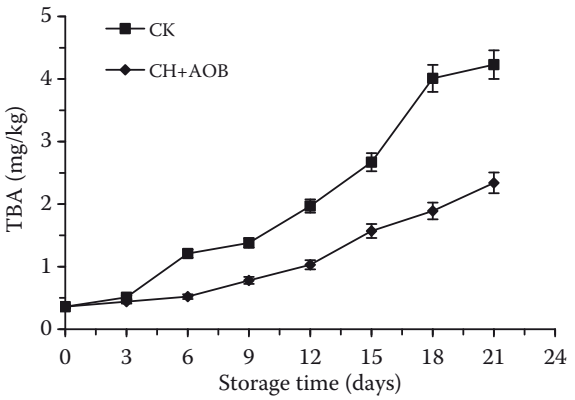

Figure 4. Changes in TBA of silver carp during chilled storage

CK – control; CH+AOB – chitosan coating with antioxidant of bamboo leavesB

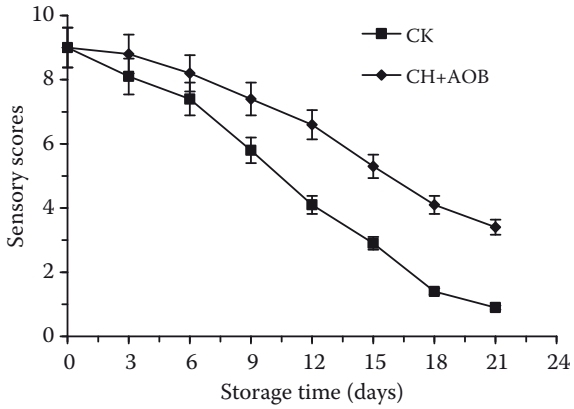

Figure 5. Changes in sensory scores of silver carp during chilled storage

CK – control; CH+AOB – chitosan coating with antioxidant of bamboo leaves

Table 1. Shelf life of silver carp during chilled storage under CK and CH+AOB treatments

Treatment	Shelf-life (days)				
	TVC[1,6]	TVB-N[2,6]	TMA-N[3,6]	TBA[4,6]	sensory scores[5,6]
CK	13–14	12–13	14–15	12–13	12–13
CH+AOB	18–19	17–18	18–19	18–19	18–19

[1]based on a TVC limit value of 7.0 $\log_{10}$ CFU/g (ICMSF 1986); [2]based on a TVB-N limit value of 35 mg/100g (Özyurt *et al.* 2009); [3]based on a TMA-N limit value of 5.0 mg/100 g (Connell 1990); [4]based on a TBA limit value of 2 mg/kg (Connell 1990); [5]based on acceptance sensory score of 4 (Fan *et al.* 2009); [6]data obtained from Figures 1 and 3–5, respectively

before coating could significantly reduce its lipid oxidation and extend its shelf life.

Sensory analysis

The results of the sensory analysis of silver carp samples are given in Figure 5. Sensory scores showed a significant decline in the CK and CH+AOB treatments during storage. It is well known that the fish spoilage gives rise to the subsequent development of strong fishy, rancid, and putrid odours. In this paper, the sensory score of 4 was taken as the limit of acceptability (Fan *et al*. 2009). The CK sample was unacceptable after 12 days, while CH+AOB sample was still in good and acceptable condition on the 18th day of storage. From all the data presented, it can be concluded that the treating with the AOB-chitosan coating could retain good quality characteristics and extend the shelf life of sliver carp by 6–7 days, compared to the control samples. These conclusions are well consistent with the results of the microbiological and chemical quality analyses.

Shelf life discussion

Shelf life of fish products is defined as the storage time until spoilage. The point of spoilage may be defined by a certain maximum acceptable level of the microbiological and physico-chemical indicators and sensory requirements. Table 1 summarises the data obtained from Figures 1 and 3–5 on the shelf life of the CK and CH+AOB samples based on the microbiological, physic-chemical, and sensory analyses. As the results show, the shelf life values of the CK and CH+AOB samples obtained from the maximum acceptable levels of TVC, TVB-N, TMA-N, TBA, and sensory scores are in good correlation. Moreover, the shelf life of the CK treated silver carp samples was approximately 12–14 days, while that for the CH+AOB treated samples was 17–19 days.

CONCLUSION

The results of the present study indicate that chitosan coating with AOB treatment could effectively retard the water loss, inhibit the growth of total viable counts, reduce chemical spoilage (reflected in TVB-N, pH, TMA-N, and TBA), preserve the sensory quality of silver carp, and extend the shelf life of silver carp by 5–7 days in comparison with the control group. Therefore, chitosan coating containing AOB can be a promising candidate for extending the shelf life of fresh fish products during chilled storage.

References

Aider M. (2010): Chitosan application for active bio-based films production and potential in the food industry: review. LWT-Food Science and Technology, **43**: 837–842.

AOAC (2002): Official Methods of Analysis. 17th Ed. Association of Official Analytical Chemists, Washington.

Banks H., Nickelson R., Finne G. (1980): Shelf life studies on carbon dioxide packaged finfish from Gulf of Mexico. Journal of Food Science, **45**: 157–162.

Bindu J., Ginson J., Kamalakantha C.K., Asha K.K., Srinivasa Gopal T.K. (2013): Physico-chemical changes in high pressure treated Indian white prawn (*Fenneropenaeus indicus*) during chill storage. Innovative Food Science and Emerging Technologies, **17**: 37–42.

Connell J.J. (1990): Methods of assessing and selecting for quality. In: Control of Fish Quality. 3rd Ed. Springer, Berlin.

Fan W.J., Sun J.X., Chen Y.C., Qiu J., Zhang Y., Chi Y.L. (2009): Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. Food Chemistry, **115**: 66–70.

Fishery Bureau of Department of Agriculture of China (2012): Fishery Yearbook in China in 2011. Chinese Agriculture Press, Beijing.

Goulas A.E., Kontominas M.G. (2005): Effect of salting and smoking-method on the keeping quality of chub mackerel (*Scomber japonicus*): Biochemical and sensory attributes. Food Chemistry, **93**: 511–520.

Hu C., Zhang Y., David D.K. (2000): Evaluation of antioxidant and prooxidant activities of bamboo *Phyllostachys nigra* var. *henonis* leaf extract *in vitro*. Journal of Agriculture and Food Chemistry, **48**: 3170–3176.

ICMSF (1986): International commission on microbiological specifications for foods. Sampling plans for fish and shellfish. In: ICMSF (ed.): Microorganisms in Foods 2. Sampling for Microbiological Analysis: Principles and Scientific Applications. 2nd Ed. International Commission on Microbiological Specifications for Foods. University of Toronto Press, Toronto.

Kim K.W., Thomas R.L. (2007): Antioxidative activity of chitosans with varying molecular weights. Food Chemistry, **101**: 308–313.

Krkić N., Šojić B., Lazić V., Petrović L., Mandić A., Sedej I., Tomović V. (2013): Lipid oxidative changes in chitosan-oregano coated traditional dry fermented sausage Petrovská klobása. Meat Science, **93**: 767–770.

Kirk R.S., Sawyer R. (1991): Pearson's Composition and Analysis of Foods. 9th Ed. Longman Scientific and Technical, Harlow.

Li T.T., Li J.R, Hu W.Z., Li X.P. (2013a): Quality enhancement in refrigerated red drum (*Sciaenops ocellatus*) fillets using chitosan coatings containing natural preservatives. Food Chemistry, **138**: 821–826.

Li T.T., Li J.R., Hu W.Z., Zhang X.G., Li X.P., Zhao J. (2013b): Shelf-life extension of crucian carp (*Carassius auratus*) using natural preservatives during chilled storage. Food Chemistry, **135**: 140–145.

Lu B.Y., Wu X.Q., Shi J.Y., Dong Y.J., Zhang Y. (2006): Toxicology and safety of antioxidant of bamboo leaves: Part 2. Development toxicity test in rats with antioxidant of bamboo leaves. Food and Chemical Toxicology, **44**: 1739–1743.

Lu F., Liu D. H., Ye X.Q., Wei Y., Liu F. (2009): Alginate-calcium coating incorporating nisin and EDTA maintains the quality of fresh northern snakehead (*Channa argus*) fillets stored at 4°C. Journal of the Science of Food and Agriculture, **89**: 848–854.

Malle P., Poumeyrol M. (1989): A new chemical criterion for the quality control of fish: trim ethylamine/total volatile basic nitrogen (%). Journal of Food Protection, **52**: 419–423.

Manat C., Soottawat B., Wonnop V., Cameron F. (2005): Changes of pigments and colour in sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) muscle during iced storage. Food Chemistry, **93**: 607–617.

Manju S., Jose L., Srinivasa Gopal T.K., Ravishankar C.N., Lalitha K.V. (2007): Effects of sodium acetate dip treatment and vacuum-packaging on chemical, microbiological, textural and sensory changes of pearlspot (*Etroplus suratensis*) during chill storage. Food Chemistry, **102**: 27–35.

Mendes R., Pestana C., Gonçalves A. (2008): The effects of soluble gas stabilization on the quality of packed sardine fillets (*Sardina pilchardus*) stored in air, VP and MAP. International Journal of Food Science and Technology, **43**: 2000–2009.

Ojagh S. M., Masoud R., Razavi S. H., Hosseini S.M.H. (2010): Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chemistry, **120**: 193–198.

Özyurt G., Kuley E., Özkütük S., Özogul F. (2009): Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) during storage in ice. Food Chemistry, **114**: 505–510.

Pereira de Abreu D.A., Maroto J., Villalba Rodríguez K., Cruz J.M. (2012): Antioxidants from barley husks impregnated in films of low-density polyethylene and their effect over lipid deterioration of frozen cod (*Gadus morhua*). Journal of the Science of Food and Agriculture, **92**: 427–432.

Pham Q.T., Willix J. (1984): A model for food desiccation in frozen storage. Journal of Food Science, **49**: 1275–1294.

Rubilar J.F. Cruz R.M.S., Silva H.D., Vicente A.A., Khmelinskii I., Vieira M.C. (2013): Physico-mechanical properties of chitosan films with carvacrol and grape seed extract. Journal of Food Engineering, **115**: 466–474.

Siddaiah D., Vidya Sagar Reddy G., Raju C.V., Chandrasekhar T.C. (2001): Changes in lipids, proteins and kamaboko forming ability of silver carp *(Hypophthalmichthys molitrix)* mince during frozen storage. Food Research International, **34**: 47–53.

Siripatrawan U., Noipha S. (2012): Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages. Food Hydrocolloids, **27**: 102–108.

Xu Y.S., Xia W.S., Yang F., Nie X.H. (2010): Physical and chemical changes of silver carp sausages during fermentation with *Pediococcus pentosaceus.* Food Chemistry, **122**: 633–637.

Received for publication March 30, 2013
Accepted after corrections May 2, 2013

Corresponding author:

Prof Fan Wenjiao, Sichuan Tourism University, Department of Food Science, Chengdu 610100, P.R. China;
E-mail: wenjiaolc@163.com