



Shelf life of refrigerated silver carp, *Hypophthalmichthys molitrix*, fillets treated with chitosan film and coating incorporated with ginger extract

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ABSTRACT

Quality maintaining, packaging and coating are of particular importance in food industry. Hence, the present study aimed to examine the effects of chitosan and ginger extract on the fillets shelf life. The experiment was conducted on six treatments, including control, coated with chitosan solution, coated with ginger extract, coated with chitosan solution + ginger extract, packaged with chitosan film and packaged with chitosan film + ginger extract. The samples were stored in a refrigerator for 12 days and examined during the storage period for the spoilage factors (PV, TBA, TVN, TVC and PTC). The results indicated that the application of ginger extract alone or in combination with chitosan had a significant effect on increasing the quality of fish fillets ($P < 0.05$). In all treatments, the optimal result was related to the treatment packaged with chitosan film + ginger extract, which resulted in maintaining the desired fillet quality for 12 days.

Key words: Coating, Chitosan, Ginger, Storage, Silver carp.

INTRODUCTION

Fish are highly susceptible to oxidation because of their unsaturated fatty acids, which is why maintaining their quality and storage is of particular importance. The lipid oxidation occurs in food due to the presence of various types of reactions and thus the formation of free radicals, hydroperoxides and other spoilage agents (Gómez-Estaca *et al.* 2010). The volatile compounds resulting from the breakdown of the oxidation reaction and the hydrolytic reaction of lipids (hydroperoxides, aldehydes, ketones, fatty acids, etc.) cause changes in odor, taste, color, texture, nutritional value and in general the quality and non-acceptance for consumers of these important food sources (Ozyurt *et al.* 2007). Packaging and coating are of particular importance to maintain the quality of foods. Due to the growth of synthetic polymer production, the bulk of environmental pollution is associated with synthetic plastics known as white pollution. Hence, it has become widespread to use films and coatings that produce less pollution and are biodegradable (No *et al.* 2007; Ghasemnejad *et al.* 2010). Among the various polymers utilized for food coatings, the chitosan has attracted much attention in recent years, and has proven its capability in medicine, agriculture, food industry and chemical products. Chitosan is a modified cationic carbohydrate obtained from deacetylation of chitin. The chitin is found in the main composition of external skeleton of arthropods such as insects, crabs, shrimps, lobsters, and cell walls of a particular type of algae. It is the most abundant polysaccharide in nature, behind cellulose (Cao *et al.* 2013; Anraku *et al.* 2018). The chitosan is known as a non-toxic, biocompatible, bioactive and biodegradable compound with antimicrobial and antifungal properties reported in numerous studies (Ma *et al.* 2016). It has the ability to form gels and films due to high molecular weight and solubility in acidic solutions. The chitosan can be applied in food packaging, especially as a coating and edible film, due to the film formation and the unique nature of increasing viscosity upon dehydration. Some applied properties including antioxidant, antimicrobial, and impermeability to oxygen have been reported for chitosan films (Sathivel 2005). There is great tendency to use new types of natural antimicrobial compounds such as spices and herbs to preserve food. Extracts from plants and spices are rich sources of bioactive compounds such as terpenoids and phenolic acids (Youdim & Deans 2000). The foods coated with edible films possessing

antimicrobial and antioxidant properties added by natural agents are highly desirable. The use of plant essential oils and extracts, instead of chemical preservatives, attenuates concerns on complications of these substances. The composition, structure and functional groups of essential oils and extracts play an important role in their antimicrobial activity (Holley & Patel 2005).

The ginger can be used as a natural antioxidant due to volatile and non-volatile antioxidant compounds in various parts, especially rhizomes. The ginger extract contains polyphenol compounds, the most important of which is 6-Gingerol and its derivatives (Zhang *et al.* 2015; Nile & Park 2015; Jelled *et al.* 2015). Some of the volatile compounds of the ginger rhizome are camphene, gamma terpinene and terpinen-4-ol. Its non-volatile antioxidants, which are also phenolic compounds, include Gingerols, Shogaols, Zingerone, Paradol (Jelled *et al.* 2015). Despite the unique properties of the ginger, limited studies have been performed on its antioxidant and antimicrobial activity. Because of the absence of any study to compare the coating with chitosan solution containing the ginger extract and packaging with chitosan film, the aim of the present study was to investigate the effect of the chitosan and the ginger extract alone and in combination with each other on the shelf-life of refrigerated silver carp fillet.

MATERIALS AND METHODS

Chemical

Chitosan (sigma-Aldrich), glacial acetic acid (Merck, Germany), ginger rhizome, chloroform, 1-butanol, Sodium hydroxide (NaOH), hydrochloric acid, sodium chloride, alcohol and glycerol were purchased from Sigma-Aldrich (Dt. Louis, MO, USA). Chemicals (solvents and reactants) employed through the study were reagent grade.

Ginger extraction

According to Cao *et al.* (2013) with some modification, 100 g of chopped ginger root was mixed into 600 ml of distilled water and shaken for 48 h at 25 °C. After filtrations with Watman No. 1 filter paper, the residue was re-extracted with an additional 400 ml of distilled water for additional 30 min and then filtered and stored at 4 °C before use.

Preparation of coating solutions

Chitosan solution (2% w/v) was prepared according to Fan *et al.* (2009), with some modifications: 20 g chitosan were mixed with 900 mL distilled water and stirred for 10 min. Then, 10 mL glacial acetic acid and 1 mL glycerol were added to the mixture, stirred for 2 h, and reached to 1000 ml.

To prepare chitosan solution incorporating ginger, 10 mL ginger extract were added in chitosan solution, then stirring for 30 min (Cao *et al.* 2013).

Preparation of film casting

The chitosan/ginger solution were cast in simple cube molds from Teflon, and then dried for 72 h at room temperature to prepare films. Dried films were then peeled and stored in a desiccator containing saturated magnesium nitrate solution at 25°C and 51.90% relative humidity until evaluation (Hosseini *et al.* 2009).

Preparation of fish samples

Silver carp (40 kg) with the mean weight of 1000 ± 100 g were purchased from the fish market and transferred to the Guilan University laboratory in the presence of ice. After washing, eviscerating and heading, the fillets were prepared, rinsed again and stored in ice until examining. The fillets (200 ± 50 g) were divided into six groups to determine the effect of different types of packaging on the fish shelf-life.

The first group was non-coated (control), the second group was immersed in 2% chitosan solution for 5 min. The third group was immersed in 10% ginger extract for 5 min. The fourth group was first immersed in 10% ginger extract for 5 min, then in 2% chitosan solution for 5 min. The fifth group was packaged with chitosan film and the last one was packaged with chitosan film containing 10% ginger extract. After preparing the treatments, all fillets were packaged in polyethylene plastics and stored in the refrigerator (4°C) for 12 days. During this period (with four-day intervals), the parameters assessing the fillet quality and the effects of the films and coating were studied according to the following methods.

Antimicrobial activity of chitosan films

The antimicrobial activity of the films was qualitatively evaluated following an agar diffusion assay. Spread plates were inoculated with 100 µL of bacteria (*Listeria monocytogenes* and *Escherichia coli*) overnight grown (10^8

CFU /mL⁻¹). The films were aseptically cut into 6 mm diameter discs and placed on plates containing MHA (Mueller-Hinton Agar). The plates were incubated at 30 °C for 24 h, then; diameter of the growth inhibition zones was measured. The tests were carried out in triplicate for each formulation (Gómez-Estaca *et al.* 2010).

Microbiological assays

For microbial evaluation, 5 g of fillet samples was mixed and homogenized with 45 mL peptone-physiological saline solution (0.1% peptone + 0.85% NaCl) and different dilutions were prepared. 1 mL of each diluted sample was used for cultivating bacteria in plate count agar culture. The inoculated plates were incubated for 2 days at 37 °C for total viable counts, and 7 days at 10°C for psychrotrophic bacterial counts. All counts were expressed as log₁₀ CFU g⁻¹ and performed in duplicate (Gómez-Estaca *et al.* 2010).

Measurement of lipid oxidation

For measurement of lipid oxidation, peroxide value (PV) was determined in the lipid extract according to the method described by Egan *et al.* (1997). Results are expressed as mEq oxygen kg⁻¹ lipids.

The thiobarbituric acid (TBA) value was determined colorimetrically by the method of Porkony and Dieffenbacher as described by Kirk & Sawyer (1991). A portion (200 mg) of sample was weighed into 25 mL volumetric flask. An aliquot (1 mL) of 1-butanol was added to dissolve the sample. The mixture was made to volume with 1-butanol and mixed. A portion (5 mL) of the mixture was pipetted into a dry stopped test tube and 5 mL of TBA reagent (prepared by dissolving 200 mg TBA into 100 mL 1-butanol, filtered, stored at 4°C) were added. The test tubes were stoppered, vortexed and placed in water bath at 95°C for 120 min, then cooled. Absorbance (As) was measured at 530 nm against water blank. A reagent blank was run and absorbance (Ab) recorded. TBA value (mg of malonaldehyde equivalents/kg of tissue) was obtained by the following formula:

$$\text{TBA} = [50 \times (\text{As} - \text{Ab})] / 200$$

Total volatile basic nitrogen (TVB-N)

The total volatile basic nitrogen (TVB-N) value was evaluated by the AOAC method (1995) and was expressed in mg nitrogen kg⁻¹ sample.

Statistical analyses

Differences between factors and levels were evaluated using the analysis of variance (ANOVA) and Duncan's multiple range tests ($p < 0.05$). SPSS software (SPSS 17.0 for Windows, SPSS Inc., Chicago, IL, USA) was used for data analyses.

RESULTS AND DISCUSSION

Chemical analysis

The lipid oxidation is one of the main causes of meat spoilage and its degradation, which reduces the nutritional value and produces toxic compounds (Ozyurt *et al.* 2007). The lipid oxidation generates hydroperoxide resulting in the colour loss and unpleasant odor due to the reaction with other molecules. Therefore, the peroxide level measurement can provide results about the freshness and usability of meat. Since the peroxides are compounds without flavour and odour, they cannot be detected by consumers. As shown in Fig. 1, the peroxide level in all treatments was increased during storage ($P < 0.05$). In the control, treatments coated with chitosan solution and those coated with chitosan film, this level reached its highest level on the 8th day of storage ($P < 0.05$), followed by a decreasing trend.

In other treatments, the peroxide level continued to increase until the 12th day ($p < 0.05$). In the treatment packaged with chitosan + ginger film, this level was less than 5 mEq until the 8th day, indicating the excellent maintenance of the fillet quality. The PV of all the samples was below the proposed acceptable level of 10–20 mEq peroxide kg⁻¹ in fat fish (Huss 1995). The produced peroxide is unstable during the oxidation process and is converted to a number of other compounds, including aldehydes, alcohols and ketones. So that, the peroxide measurements alone are inadequate to evaluate spoilage and employing other methods seems to be necessary. The second stage of oxidation begins with the appearance of carbonyl compounds. One of synthesized aldehydes, called malondialdehyde (MDA), can be measured with TBA index. The TBA test is widely used to evaluate the meat

quality (Sun *et al.* 2018; Rostamzad *et al.* 2011). As shown in Fig. 2, the TBA level in the fish fillet was very low ($0.03 \text{ mg MDA kg}^{-1}$) at the beginning of the storage period, indicating the freshness of the fish used.

During the storage period, the TBA level in all samples showed an increasing trend due to the formation of aldehydes resulting from the breakdown of peroxides. There was a significant difference between the treatments ($p < 0.05$).

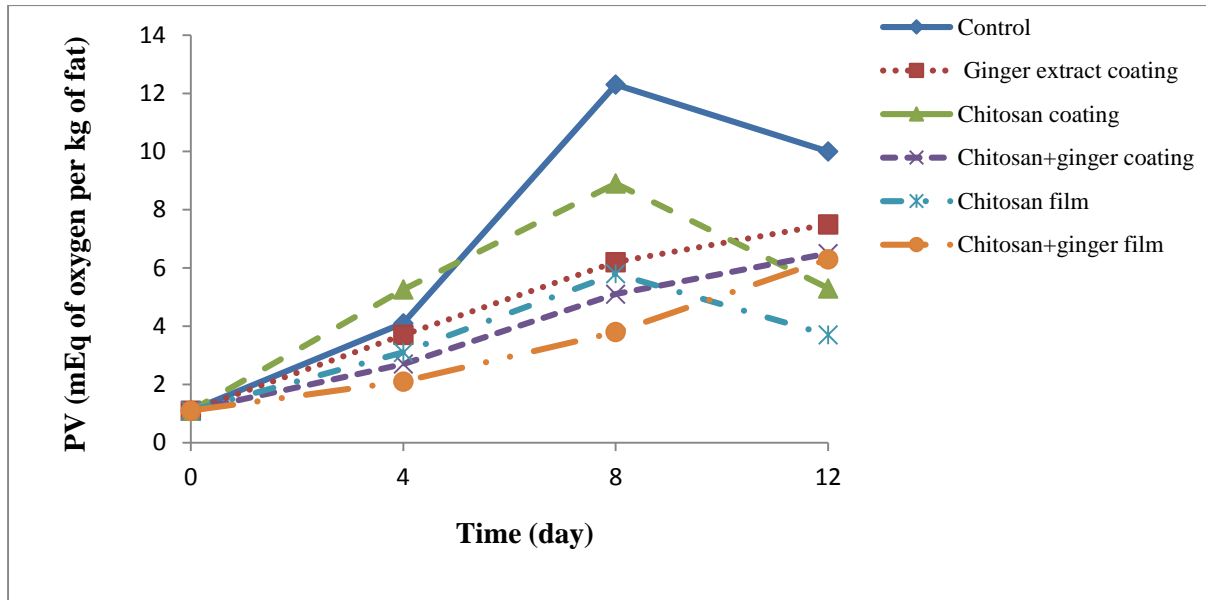


Fig. 1. Alterations in peroxide value of silver carp fillets covered with chitosan film and coating incorporated with ginger extract during refrigerated storage (4°C).

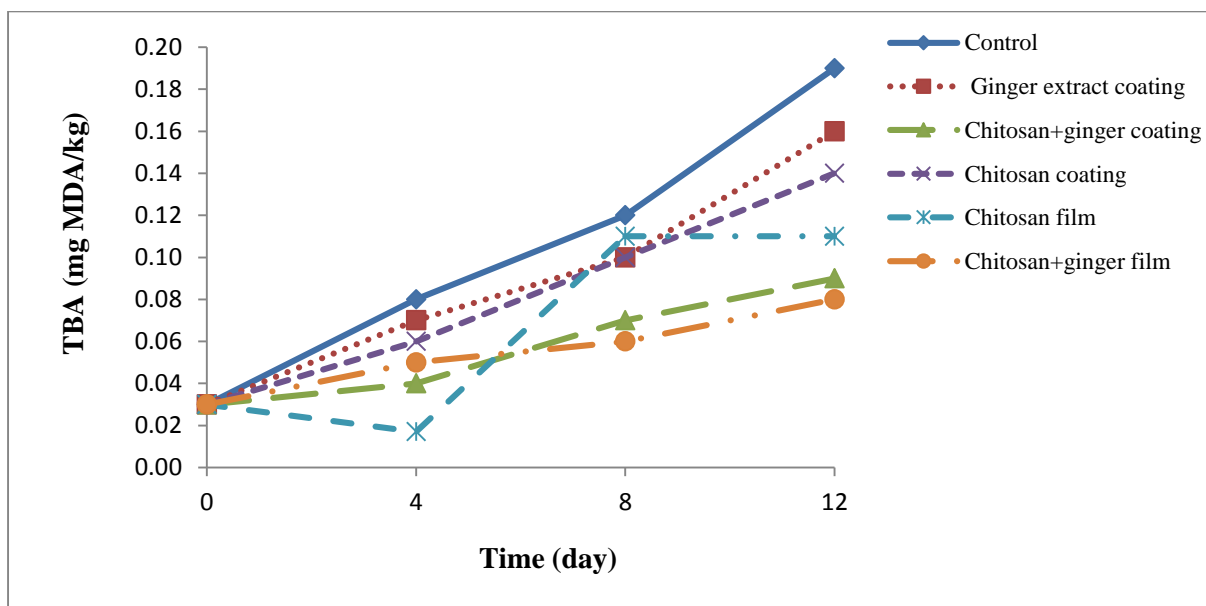


Fig. 2. Alterations in TBA value of silver carp fillets covered with chitosan film and coating incorporated with ginger extract during refrigerated storage (4°C).

This increase was the highest in the control group and the lowest in the treatment packaged with chitosan + ginger extract film ($p < 0.05$). $1\text{-}2 \text{ mg malonaldehyde kg}^{-1}$ in fish muscle is usually taken as the limit of acceptability (Lakshmanan 2000). In the present study, TBA values for treated samples were much lower than the proposed limit at the end of storage. Among chitosan solution-coated treatments, the chitosan + ginger treatment had the greatest effect on reducing TBA production. In all treatments, the lowest increase in TBA level was related to the fillets packaged with chitosan film + ginger extract, probably due to better prevention of the lipid oxidation by the

films produced. Other authors have suggested the inhibitory properties of chitosan film in combination with different types of extracts (Holley & Patel 2005; Gomes *et al.* 2017). They pointed out that the chelating ability of metallic ions or the combination of chitosan with lipid is attributed to its antioxidant activity. The antioxidant mechanism of chitosan can be explained by the activity of initial amine groups of the chitosan. These active agents form a stable microsphere using volatile aldehydes resulting from the breakdown of fats during oxidation. The chelating capacity of the metal ions is another characteristic of chitosan that introduces it as a potent and natural antioxidant to prevent the lipid oxidation in foods and their subsequent increased shelf-life (Mohan *et al.* 2012; Anraku *et al.* 2018).

In addition to the items mentioned about chitosan, notably, the ginger extract has always been used as a natural antioxidant due to volatile and non-volatile antioxidant compounds. Some of the most important volatile compounds in the ginger rhizome include camphene, gamma terpinene, phenolic compounds are gingerols, shogaols, zingerone and paradol (Jelled *et al.* 2015).

Another indicator for determining the freshness of fish is total volatile nitrogen (TVN) encompassing a wide range of volatile compounds such as ammonia, methylamine, dimethylamine and trimethylamine, which are produced by microbial activity (Rodríguez *et al.* 2008). In general, the TVB-N level alterations depend on the species, genus, place of catch, season and age of the fish (Kilinc & Cakli 2005). Microbial metabolism of amino acids in fish leads to the accumulation of ammonium, monoethylamine, diethylamine, triethylamine and other volatile bases, all of which cause unpleasant taste in fish (Duan *et al.* 2010).

As shown in Fig. 3, at the beginning of the storage period, the TVB-N level of the fillets was very low i.e. about 4.5 mg N/100 g meat, which increased over time in all treatments and there was a significant difference between the treatments ($P < 0.05$).

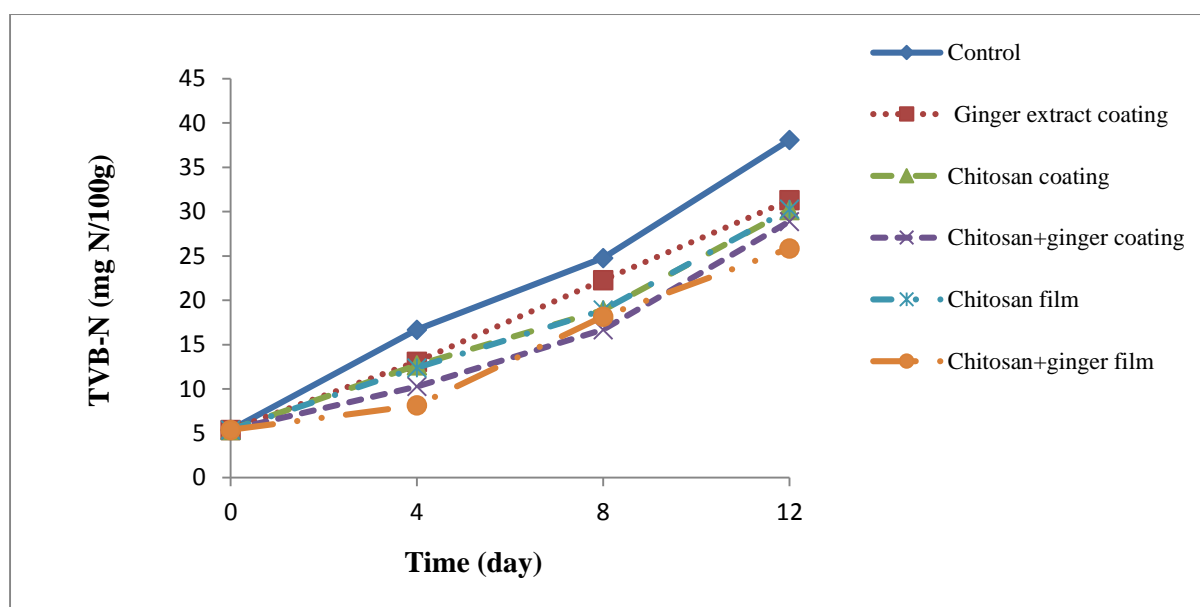


Fig. 3. Alterations in TVB-N value of silver carp fillets covered by chitosan film and coating incorporated with ginger extract during refrigerated storage (4°C).

At the end of the storage period (12th day), the highest TVB-N level was observed in the control groups (38.1 mg N/100 g meat), while the lowest level was reported in the fillets coated by chitosan film containing extract (23.87 mg /100 g meat) ($P < 0.05$). 25-30 mg N/100 g meat has been suggested as the maximum acceptable TVB-N level in fish meats (Shakila *et al.* 2005). In the present study, at the 12th day of storage, the TVB-N in all treatments was lower than this level, while in control group TVB-N value was higher than the limit. Among the treatments, the lowest TVB-N level was observed in the treatment packaged with chitosan + ginger extract film which may be due to the presence of antibacterial compounds in the ginger extract and chitosan, leading to reduced bacterial growth or decreased capacity in oxidative deamination; a phenomenon reported by other authors (Stoilova *et al.* 2007; Noori *et al.* 2018; Si *et al.* 2018) as well. Chitosan reduces the activity of internal proteases, thereby

reducing the production of TVB-N such as ammonia and trimethylamine, resulting from the microbial or endogenous enzymes of the fish (Fan *et al.* 2009).

Microbial analyses

Antimicrobial activity of films

The results from the antimicrobial activity of produced films (chitosan and chitosan + ginger extract) are presented in Table 1. As shown in this table, the chitosan film was unable to prevent the growth of both *E. coli* and *L. monocytogenes* bacteria on the film disc, but the film containing ginger extract showed a good inhibitory effect on the both bacteria, indicating the antibacterial activity of the ginger extract, which is in line with those reported by other authors. These studies have also pointed out the antibacterial properties of ginger (Jiang *et al.* 2006; Abo-Esa, 2008).

Table 1. Qualitative antimicrobial activity of chitosan films

Film	Bacteria	
	<i>L. monocytogenes</i>	<i>E.coli</i>
Chitosan	-	-
Chitosan + Ginger	++	++

++: totally inhibited; +: slightly inhibited; -: no inhibited (Gómez-Estaca *et al.* 2010).

Assessment of microbial spoilage

The presence of bacteria is one of the important reasons for spoiling and reducing the quality of fish fillets during the storage period, because the fish meat contains suitable compounds for the growth of microbes. Hence, the estimate of total viable count (TVC) is commonly used as an acceptance index in standards and criteria. The International Committee on Food Microbiology and Hygiene (ICMFC, 1986) has reported the TVC limit of 7 \log_{10} CFU g^{-1} in raw fish. In general, the level of microbial spoilage in fish and its products will vary depending on the microbial flora, ambient temperature, climatic condition, storage setting and packaging (Guillerm-Regost *et al.* 2006). Several parameters such as manipulation during filleting, contamination of the equipment and hygiene of people are involved in determining the initial level of the bacterial load in the fillet.

In the present study, the TVC level in the fillets at the beginning of the experiment was 3.34 \log_{10} CFU g^{-1} indicating the quality of the specimens and observing hygiene standards during filleting and packaging (lower than 4 \log_{10} CFU g^{-1}).

The total number of bacterial count of fillets stored in the refrigerator is shown in Fig. 4. The results of comparing the means showed that the level of bacterial load in all treatments was significantly increased during the storage period ($p < 0.05$).

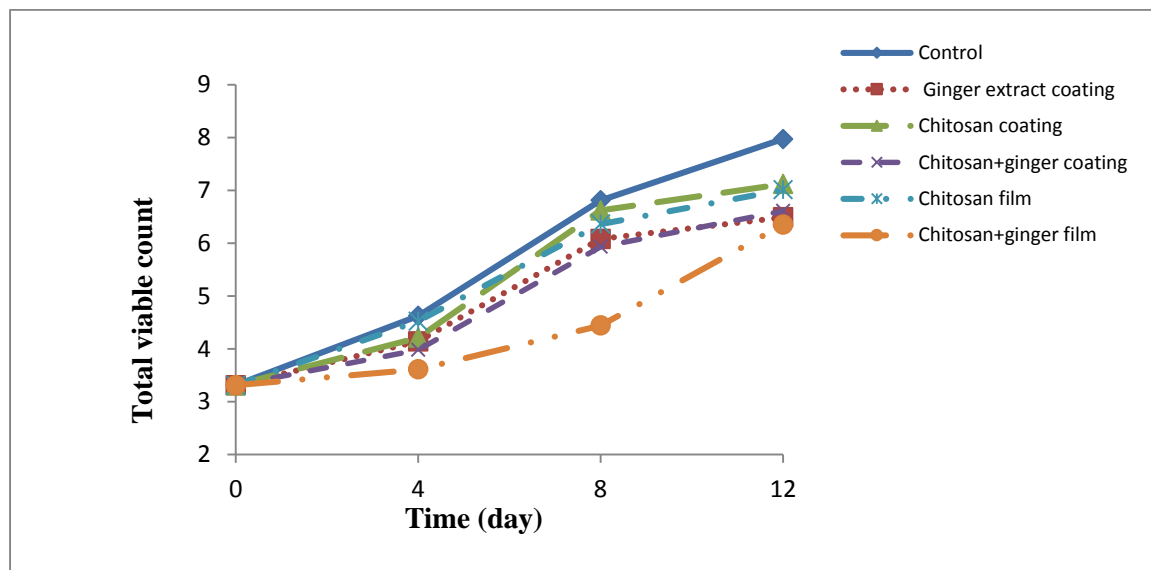


Fig. 4. Alterations in total viable count of silver carp fillets covered by chitosan film and coating incorporated with ginger extract during refrigerated storage (4°C).

The increased TVC value in the control group was higher than the others, reaching from $3.34 \log_{10} \text{CFU g}^{-1}$ at the beginning of the experiment to 7.97 at the 12th day. Among the treatments, the TVC value in the samples packaged with chitosan + ginger extract film during storage was significantly lower than the control ($p < 0.05$) while increased up to $6.35 \log_{10} \text{CFU g}^{-1}$ at the 12th day which may be due to the antimicrobial effect of ginger extract and chitosan. In fact, the antimicrobial effect of ginger extract along with the beneficial effects of coatings on preventing oxygen penetration has led to this event. Similarly, Pires *et al.* (2013) also reported that the use of edible coatings, along with some plant essential oils and extracts, prevented the increase of TVC in the specimens. Psychrotrophic count (PTC) value was $3.53 \log_{10} \text{CFU g}^{-1}$ at the beginning of the period and increased faster than TVC during the storage time. So that, the TVC values in the control group and the treatment coated by chitosan film on the 8th day were higher than the limit ($7 \log_{10} \text{CFU g}^{-1}$) of consumption. This phenomenon indicates that the psychrophilic bacteria were predominant bacterial flora. The results of this study were consistent with the findings of other authors (Duan *et al.* 2010; Mohan *et al.* 2012) concerning to the PTC in other species during storage in the cold condition. In the present study, chitosan + ginger extract film had the best effect on the storage of fish fillets. So that the TVC and PTC values of chitosan + ginger extract film treatment were significantly lower than the other treatments ($p < 0.05$). It was also found that the treatments of chitosan + ginger extract coating and film at the 12th day were able to hold the PTC level below the allowance (6.98 and $63.71 \log_{10} \text{CFU g}^{-1}$ respectively); hence, the fillets were usable. It is maybe due to the antibacterial properties of chitosan and ginger extract, which has also been reported by the other authors (Noori *et al.* 2018; Soni *et al.* 2018).

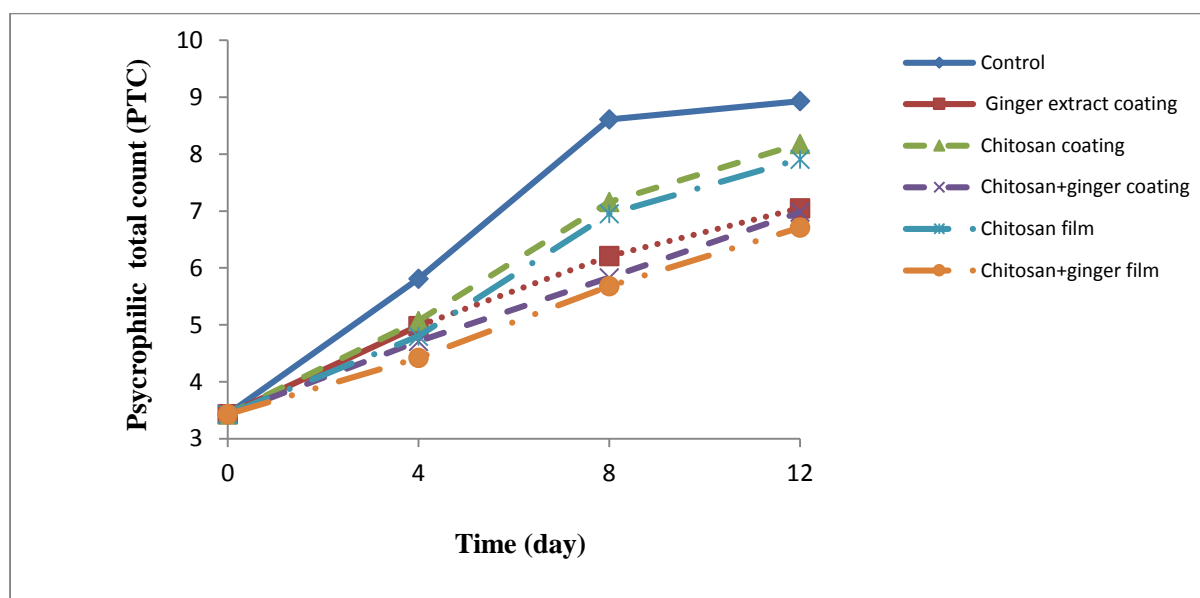


Fig. 5. Alterations in Psychrophilic total count of silver carp fillets covered by chitosan film and coating incorporated with ginger extract during refrigerated storage (4°C).

In addition, a group of authors (Mayachiew & Devahastin 2010; Sánchez-Gonzalez *et al.* 2010) have reported the positive properties of plant compounds on the antimicrobial and antioxidant activities of the chitosan films. Thus, according to our results and in order to benefit from the unique and positive properties of ginger, it is recommended to use this plant in the production of biodegradable films for maintaining a better quality of food products.

CONCLUSION

Given the consumer's demand for high-quality food products and concerns on the use of artificial preservatives as well as environmental problems caused by the accumulation of synthetic polymers, the employing of biodegradable coatings with antioxidant and antibacterial activities can be appropriate alternatives. Therefore, the present study was designed to provide edible coatings with antioxidant and antibacterial properties of the chitosan in combination with the ginger extract. The current comparative study investigated the effect of coating with the chitosan solution and film alone and in combination with the ginger extract.

The spoilage factors (peroxide, TBA, TVN, TVC and PTC) were measured during the storage time (12 days) with four-day intervals.

Among the treatments, the efficiency of the chitosan film containing 10% ginger extract with the lowest levels of peroxide, TBA, TVC and PTC was demonstrated in comparison with other treatments, especially the control. Considering the antioxidant properties of chitosan and ginger extract in this study, it is obvious that better preservation of the quality of fillets is achieved by chitosan + ginger extract together. Therefore, the biodegradable chitosan film containing 10% of ginger extract can meet the consumer's demand for the food free of harmful chemicals.

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تأثیر فیلم و پوشش کیتوزان در ترکیب با عصاره زنجبیل بر ماندگاری فیله‌های ماهی کپور نقره‌ای (*Hypophthalmichthys molitrix*)

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چکیده

حفظ کیفیت و تازگی مواد غذایی از اهمیت ویژه‌ای برخوردار است و با توجه به نگرانی‌های ناشی از مصرف نگهدارنده‌های مصنوعی و همچنین مشکلات زیست محیطی ناشی از تجمع پلیمرهای مصنوعی، استفاده از پوشش‌های زیست تخریب پذیر در حال گسترش است. لذا در تحقیق حاضر تاثیر استفاده از کیتوزان در ترکیب با عصاره زنجبیل بر ماندگاری فیله‌های ماهی فیتوفاگ مورد بررسی قرار گرفت. بدین منظور از شش تیمار استفاده شد که شامل تیمار شاهد (بدون پوشش‌دهی)، پوشش‌دهی با محلول کیتوزان، پوشش‌دهی با محلول عصاره زنجبیل، پوشش‌دهی با محلول کیتوزان در ترکیب با عصاره زنجبیل، پوشش‌دهی با فیلم کیتوزان و پوشش‌دهی با فیلم کیتوزان حاوی عصاره زنجبیل بود. پس از پوشش‌دهی، فیله‌ها به مدت ۱۲ روز در یخچال نگهداری شدند. طی مدت نگهداری (فواصل ۴ روزه) فاکتورهای مرتبط با فساد (TVB-N، TBA، PV) و بار باکتریایی) مورد بررسی قرار گرفتند. نتایج حاکی از این بود که پوشش‌دهی با محلول کیتوزان و عصاره زنجبیل به تنهایی و یا در ترکیب با هم تاثیر معنی‌داری بر حفظ کیفیت فیله‌های ماهی داشت. بهترین تاثیر را در بین تیمارهای اعمال شده، تیمار پوشش‌دهی شده با فیلم کیتوزان حاوی عصاره زنجبیل داشت و کیفیت فیله‌ها تا پایان دوره نگهداری مورد قبول بود. لذا استفاده از پوشش‌ها و فیلم‌های مذکور جهت افزایش ماندگاری فیله ماهی پیشنهاد می‌شود.

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