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Characteristics of a biodegradable protein based films from Silver carp *(Hypophthalmichthys molitrix)* and their application in Silver carp fillets

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Abstract

In this study, fish myofibrillar protein (FMP) films of Silver carp *(Hypophthalmichthys molitrix)* were produced and used to wrap silver crap fillets as biodegradable packages. In order to do that, myofibrillar proteins were extracted from fish fillets and protein based films were prepared and their physical, mechanical and optical properties, were investigated. Results showed that some properties of FMP films such as water vapor permeability (WVP), film solubility, tensile strength (TS) and elongation at break (EAB) were better than many of other edible films that were reported by other researchers. After producing FMP films, fish fillets were wrapped by them and stored at 4°C. Polyethylene bags were used as control to be compared with FMP films. According to the results of 16 days storage, lipid oxidation of fillets which were wrapped in FMP films was significantly lower than samples which were packed in polyethylene packages ($p \leq 0.05$); Also fillets which were packed in FMP films, had lower microbial flora and TVN-B values in compare with control ($p \leq 0.05$). Finally, we suggest biodegradable FMP films, alone or incorporated with antioxidants as proper packages for wrapping foods such as fish fillets.

Introduction

Packaging is used to protect food from secondary contaminations after processing and during storage to maintain the food product quality. Many materials are employed for preparation of packages but the majority of them are made of plastics. Synthetic plastic packages have come into wide-spread use thanks to its good mechanical properties and effectiveness as a barrier to oxygen and water. However, synthetic films represent a serious ecological problem due to their non-biodegradability. As a consequence, in recent years, packaging research has focused more on biodegradable and/or edible films made from natural polymers. Such polymers may be protein, lipid or polysaccharide-based and their chemical nature determines the physical properties of the resulting films. Among these materials, proteins from different sources have been extensively employed because of their relative abundance, film-forming ability and nutri- tional qualities (Pires *et al*., 2011). Proteins biopolymers are capable of forming the film and their properties can be varied with source of it. Myofibrillar and sarcoplasmic proteins from fish muscle have been widely used as film forming material (Tongnuanchan *et al.*, 2011). Fish processing, particularly production of fish fillets and fish proteins, generates a substantial amount of by-products with protein content similar to that of fish muscle. Thus, the recovery of these proteins and their utilization to prepare biodegradable films represents a valuable alternative for the upgrading of by-products from fish processing industry (Pires *et al*., 2011). Transparent and flexible edible films were also made from blue marlin myofibrillar proteins (Shahidi, 1994). The properties of films produced from surimi of threadfin bream (Prodpran and Benjakul, 2005) and bigeye snapper (Chinabhark *et al*., 2007) were also evaluated (Pires *et al.*, 2011). The important functional characteristics of edible films and coatings are to retard the migration of moisture, oxygen, carbon dioxide, microbes or solutes, as well as to prevent collapse of products (Artharn *et al*., 2007) Thus, the main objectives of this study were to prepare biodegradable-edible films from Myofibrillar proteins of Silver carp which is not commonly used as food and investigation on some physical, mechanical and optical properties of them and then using these films to wrap fish fillets.

Material and Methods

Chemicals

Acetic acid, chloroform, 1-butanol, Sodium hydroxide (NaOH), hydrochloric acid (HCl), sodium chloride (NaCl), alcohol and glycerol were purchased from Sigma (St. Louis, MO, USA). Chemicals (solvents and reactants) employed through the study were reagent grade (Sigma, USA).

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Preparation of fish myofibrillar protein (FMP)

Fresh Silver carp *(Hypophthalmichthys molitrix)* was captured and kept on ice (1h) until delivery to the laboratory. Upon the arrival, washed with tap water, filleted and manually chopped. The film-forming solution was prepared according to the method of Limpan *et al.* (2010). Briefly, the fish mince was mixed with three volumes of cold distilled water and homogenized at 13,000 rpm for 2 min, followed by filtering through a layer of nylon cloth. The mince was mixed with five volumes of 50 mM NaCl for 5 min and filtrated through a layer of nylon cloth. The washing process was repeated twice. Then, washed mince obtained were stored on ice until used for film preparation.

Preparation of film-forming solutions (FFS) and film casting

To prepare FMP–FFS, washed mince was added with distilled water to obtain the final protein concentrations of 2% (w/v). The mixture was homogenized at 13,000 rpm for 1 min using (Wiggen Hauser D-500, Germany) homogenizer. Glycerol was then added at 50% (w/w) of protein content. The mixture was stirred gently for 30 min at room temperature. The pH of the mixture was adjusted to 3 using 1N HCl, to solubilize the protein. The solution was filtered through a layer of nylon cloth to remove undissolved debris Limpan *et al.* (2010).

Film casting and drying

The film-forming solution (4 g) was cast onto a rimmed silicone plate (50×50 mm) and drying at 25°C and 50% relative humidity (RH) for 24 h in an environmental chamber (WTB Binder, Tuttlingen, Germany). The resulting films were manually peeled off and used for analyses.

Determination of FMP film properties

Film thickness

A manual digital micrometer (0.001 mm, Mitutoyo, Mizonokuchi, Japan) was used to measure thickness of the FMP films. Average values of ten measurements in the different regions of each sample were calculated and used in water vapor permeability and tensile properties calculations.

Mechanical properties

Prior to testing the mechanical properties, films were conditioned for 48 h at 50 ± 5% relative humidity (RH) at 25°C. Tensile strength (TS) and elongation at break (EAB %) of the film samples were determined according to ASTM standard method D882–02 (ASTM, 2002). The film samples were cut in rectangular specimens (2.5 × 10 cm). Initial grip separation was set at 50 mm, and cross-head speed was set at 50 mm/min. This test was repeated five times for each specimen to confirm its repeatability.

Fourier transfer infrared spectra (FTIR)

Fourier transform infrared (FTIR) spectra were collected in transmission mode by using a Bruker (EQUI-NOX 55, Ettlingen, Germany) FTIR spectrophotometer with DTGS detector (16 scans) in the range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}.

Surface color measurement

Color properties of films were measured using a color meter. Measurements are expressed as L^* (lightness), a^* (red/green), and b^* (yellow/blue). The parameters were determined by placing film samples on a standard plate (L^* = 94.63, a^* = -0.88, and b^* = 0.65). Color difference (ΔE) and whiteness index (WI) were calculated with respect to standard plate parameters by using following Equations (3) and (4), respectively.

$$E=\sqrt{[(a^*)^2+(b^*)^2+(L^*)^2]} \quad (4)$$

$$WI=100-\sqrt{[(100-L^*)2+a^{*2}+b^{*2}]} \quad (5)$$

Light transmission and film opacity

Transmission and opacity of the FMP film were evaluated according to the method of Tunc and Duman (2010). The films were cut into rectangular specimens and placed in the spectrophotometer cell. An empty compartment was used as a reference in the measurements. The light barrier properties of the film samples were measured by scanning the samples at wavelengths between 200 and 800 nm using a UV spectrophotometer. Three replicates of each film were tested. The transparency was calculated using the following equation:

$$Opacity = Abs600/X \quad (2)$$

Where Abs600 is the value of absorbance at 600 nm and X is the film thickness (mm).

Water vapor permeability (WVP)

The films' water vapor permeability (WVP) was determined gravimetrically according to the ASTM E96-92 method (ASTM, 1995; Abdollahi *et al*., 2012) Glass permeation cups with internal diameter of 30 mm containing distilled water were covered with films and then placed in a desiccator. It was maintained at 20°C and 1.5% RH (28.044 Pa water vapor pressure) with silica gel, and air in the desiccators was stirred.

Weight loss of the cups was measured at intervals of 1 h for 8 h, which was considered equal with the transferred water through the film and adsorbed by the desiccant. The slope of weight loss vs. time was obtained by linear regression. The WVP was then calculated as follows:

$$WVP = WVTR \times L/\Delta P \quad (1)$$

Where WVTR is the measured water vapor transmission rate ($g/m^{-2}\ s^{-1}$) through the film, L is the mean film thickness (m), and ΔP is the partial water vapor pressure difference (Pa) across the two sides of the film. For each type of film, WVP measurements were replicated three times.

Total soluble matter of films in water

The solubility of the films in water was determined as described by Tunc *et al.,* 2007. The cut samples in rectangular (4 cm × 4 cm) form were dried at 105°C for 24 h to measure initial dry matter of the films (W_i). After 24 h agitation (250 rpm) in 50 mL distilled water at 25°C, the samples were filtered through Whatman No. 1 filter paper. Then, the filter papers were dried at 105°C for 24 h to achieve final dry weight (W_f) and the film solubility (%) was calculated using Equation.

$$\text{Solubility in water (\%)} = [(W_i - W_f)/W_i] \times 100 \quad (3)$$

W_i is Initial dry weight and W_f is Final dry weight of samples.

Water sorption kinetics

The water sorption kinetic of FMP films was evaluated by determining their water sorption according to the method explained by Lavorgna *et al*. (2010). The samples were cut into small pieces (2×2 cm), desiccated overnight and weighed to determine their dry mass. The weighed samples were placed in closed beakers containing 30 mL of water (pH=7) and stored at T=25 °C. The kinetic of swelling was evaluated by periodically measuring the weight increment of the samples. The films' wet surface was gently blotted with a tissue before weighing with a balance accurate to 0.0001 g. The weighing was continued until equilibrium state. The procedure was repeated three times for each sample to confirm the repeatability. The water gain of each sample was calculated as follows:

$$\text{Water Gain (\%)} = \frac{(\text{Weight of wet film} - \text{Weight of dry film}) \times 100}{\text{Weight of dry film}} \quad (6)$$

Fish fillets preparation for storage

Fresh Silver carp *(Hypophthalmichthys molitrix)* was captured and kept on ice (1 h) till delivery to the laboratory. Then, the fish were gutted, dressed and filleted manually weighing 300-350 g. Then fillets were divided into two groups. First group was left untreated and directly packaged traditionally in polyethylene bags (Control samples; BC treatment). Samples of the second group were packaged with FMP and then packed in polyethylene bags (FMP treatment). Then, samples were kept in refrigerator at 4°C for 16 days. Samplings were carried out from the fresh fish (initial material) and then during storage (at 4^{th}, 8^{th}, 12^{th} and 16^{th} days). For each treatment (BC and FMP), three different fish batches (totally 24 batches of fillets) were considered and examined individually.

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 index (TBA-i) (mg malondialdehyde kg^{-1} flesh muscle) was determined in a 5% trichloracetic acid extract according to the method of Kirk and Sawyer (1991).

Total volatile basic nitrogen (TVB-N) content

Total volatile basic nitrogen (TVB-N) determinations were carried out in triplicate over the storage period using the method of Antonacopoulos and Vyncke (1989). Analyses were performed at least in triplicate, and results were expressed as mg TVB-N/100 g muscle.

Antibacterial activity of FMP film

For microbial evaluations, 5 g of fillet samples (from sterilized under part tissue) was mixed and homogenized with 45 ml physiologic serum solution and different dilutions of it were prepared. 1ml of each diluted sample was used for cultivating bacteria in plate count agar culture. Cultivated samples were kept at 37°C for 48 h in order to find total bacterial count. Colonies were counted at the end of each period.

Sensory analyses

Sensory analysis was conducted by a taste panel consisting of five to seven panelists, according to the guidelines presented in Table 1 (DOCE, 1989; Rostamzad *et al*., 2011), four categories were ranked: highest quality (E), good quality (A), fair quality (B) and poor quality (C). Sensory assessment of the

Table 1. Thickness, tensile strength (TS), elongation at break (EAB %), opacity, moisture content (%), Film solubility, water vapor permeability and Surface colour parameters (L^*, a^*, b^* and ΔE) of fish myofibril protein (FMP) film

Fish Myofibril Protein film (FMP)	Thickness (mm)	Tensile strength (Mpa)	Elongation at break (%)	Opacity (600nm)	Film solubility (%)	Water Sorption	water vapor permeability (g/m 2 .Pa.s)× 10^{-10}	L*	a*	b*	ΔE	WI
	0.06±0.01	9.16±.58	35.86±5.3	1.196±0.76	19.1±1.3	101±12.11	2.95±.34	86.1±3	0.4±0.1	3.9±0.3	86.18±0 .98	94.58±1 .23

Colour difference (ΔE) and Whiteness index (WI)

fish fillets included the following parameters: flesh appearance, rancid odor and flesh consistency. In order to do that, fish fillets were thawed and then analyzed in the same session. The fish fillets were served to the panel members in individual bags in which they had been kept in refrigerator and they were scored individually. Sensory analyses were carried out at 0, 4, 8, 12 and 16 days after storage. Each evaluation was performed in triplicate.

Statistical analysis

Difference between factors and levels was evaluated by the analysis of variance (ANOVA) and Duncan's multiple range tests. SPSS statistic program (SPSS 17.0 for Windows, SPSS Inc., Chicago, IL, USA) was used for data analysis.

Results and Discussion

Mechanical properties

The mechanical properties of the FMP films are presented in Table 1. Tensile strength (TS) and elongation at break (EAB %) of the FMP film were 9.16 MPa and 39.86%, respectively. The result revealed that washing process could remove low-molecular-weight proteins such as sarcoplasmic proteins, leading to the increased concentration of myofibrillar proteins that play an essential role in the formation of strong film network (Artharn *et al.*, 2007). Films containing low-molecular-weight proteins have higher chain mobility of the matrix. Film formation generally takes place through the development of a three dimensional network of protein molecules by ionic, hydrogen, hydrophobic, and disulfide bonds (Gounga e*t al.*, 2007). Myosin heavy chain (MHC) and actin were found to be the major proteins in all muscles which were used. In general, MHC is the dominant protein in fish muscle (Shiku *et al*., 2003). The TS of film in this study was higher than that found in FMP films from bigeye snapper (Shiku *et al.*, 2004) washed mince obtained from fresh round scad (Artharn *et al*., 2007), Nile tilapia protein based film for both preheating conditions (Garcia and Sobral., 2005), Alaska pollack surimi film (Shiku *et al*., 2004), hake protein edible films (Pires *et al*., 2013) and whey protein films (Fang *et al*., 2002; Di Pierro *et al*., 2006; Sothornvit *et al*., 2009).

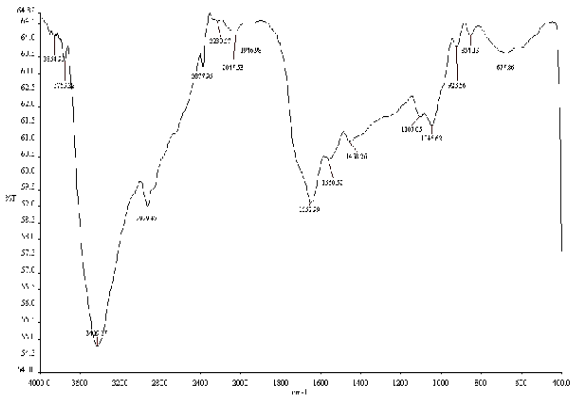

Figure. 1. FTIR spectra of FMP film

Fourier transform infrared (FTIR) analysis of FMP film

Fourier transform infrared spectra of protein-based films were studied to understand the interaction between functional groups of protein, as presented in Figure. 1. The major peak between 3500 and 3000 cm^{-1} correspond to the stretching vibration of free hydroxyl and to the asymmetric and symmetric stretching of the N–H bonds in the amino group (Siripatrawan and Harte, 2010). The bands appearing between 2750 and 3000 cm^{-1} in the spectrum of protein film occur because of stretching vibrations of the C–H bond in $–CH_2$ (m = 2929 cm^{-1}) group (Paluszkiewicz *et al*., 2010). In this experiment, the peak at 1652 cm^{-1} was an indication of contribution of the α-helical structure to protein conformation (Ojagh *et al*., 2011). The α-helix structures existing in this sample was attributed to protein unfolding. Film solution is known to cause protein unfolding and re-association of subunits from dissociated oligomers, inducing changes in both secondary and tertiary structures (Lullien-Pellerin and Balny, 2002). The peak situated around 1046 cm^{-1} in all spectra might be related to the glycerol added as a plasticizer (Bergo and Sobral, 2007).

Table 2. % Transmittance versus wavelength for fish myofibrillar protein (FMP) film

Wave length (nm)	200	250	300	350	400	450	500	550	600	650	700	750	800
%Transmittance	0.31	0.35	0.44	44.66	56.23	63.09	79.43	89.12	89.12	89.25	90.15	90.15	90.15

Color of film

L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) values of films from FMP are shown in Table 1. Color is an important characteristic of biodegradable films because it could affect consumer acceptance in potential food applications. In this study, high a^* and b^* values and low L^* value were observed for FMP films which were prepared under acidic condition. This result revealed that an acidic condition could induce the formation of yellowish pigment, especially via Maillard reaction. Acidic condition induced the degradation of myofibrillar proteins, leading to the availability of free amino group for browning reaction (Prodpran and Benjakul, 2005; Chinabhark *et al.*, 2007).

Light transmission and film opacity

Transparency values of FMP film, in UV-vis range is presented in Table 2. FMP film was excellent barrier of light transmission in UV range. Abortion of MP film 600 nm was 0.079. Opacity is an established measurement of the transparency of a film. A higher value of opacity means lesser transparency (Pereda *et al*., 2011). Film transparency is a relevant property of film since it has a direct impact on the appearance of the packaged product. Light transmission of FMP film was low and this result suggested that films could prevent the lipid oxidation induced by UV light in a food system. The result was in agreement with Shiku *et al*. (2003) and hamaguchi *et al*. (2007) who reported that protein films are considered to have very good UV barrier properties, owing to their high content of aromatic amino acids that absorb UV light. Surimi films (Shiku *et al*., 2004), blue marlin myofibrillar protein films (Shiku *et al*., 2003; Hamaguchi *et al*., 2007) and whey protein isolate films (Gounga *et al.*, 2007) had excellent barrier property for UV light owing to their high content of aromatic amino acids that absorb UV light (Hamaguchi *et al*., 2007).

Water vapor permeability

As it is shown in table 1, the Water vapor permeability (WVP) of the FMP film was 2.95 (g/msPa) 10^{-10}. Protein films which are derived from fish muscles are usually poor in water vapor barrier properties (Hamaguchi *et al*., 2007). Furthermore, the polar proteins or amino acids, especially from sarcoplasmic proteins such as heme proteins, were leached out more effectively. As a consequence, water vapor barrier property of protein film which were extracted from myofibllar protein was improved. This results revealed that higher polarity leads to more leach out due to washing the mince triple or more times and as a consequence, non-polar components go denser in tissue. Constituents with lowered polarity could absorb less water from the surrounding atmosphere (Morillon *et al*., 2002) Water vapor permeation through a hydrophilic film depends on both diffusivity and solubility of moisture in the film matrix. An increase in the inter chain spacing due to inclusion of glycerol molecules between the polymer chains may promote water vapor diffusion through the film. Additionally, more pronounced protein cross-linking of the film with more rigidity and denser structure might retard diffusion of water through the films (Artharn *et al*., 2007). FMP films in this study might have dense protein network with the low polarity. As a result, it could be resistant to water molecule transfer through the film (Limpan *et al.*, 2010). Myofibrillar proteins contain of high amount of polar ionic amino acids such as aspartic acid, glutamic acid, arginine and lysine (Shahidi, 1994; Paschoalick *et al.*, 2003).

Film solubility

Solubility of protein-based films is shown in Table 1. Water solubility (WS) is considered as an indicator of the resistance of film samples to water, which is an important factor for food packages due to high water activity and probability of contamination in presence of water (Bourtoom and Chinnan, 2005). Generally, higher water solubility would indicate lower water resistance. Table .1 shows WS of FMP film was 19.1%. This result was significantly lower ($P<0.05$) than what other researchers reported about protein-based film from round scad (Decapterus maruadsi) with 45% water solubility (Artharn *et al.*, 2007). This is because sarcoplasmic proteins had been mostly removed during the washing process and the remaining myofibrils proteins were mainly soluble in salt solution. As the cross-linking became intensive, glycerol was imbibed to a lesser extent in the film

Table 3. Change in PV, TBA, TVB-N and TVC value of Silver carp fillets during storage at 4°C

	Time(days) / Treatment	0	4	8	12	16
PV (mg oxygen kg^{-1} lipids)	Blank	1.32±0.4	3.44±0.6	12.71±0.54	4.24±0.42	5.76±0.78
	FMP	1.32±0.4	3.10±0.79	12.22±0.92	3.71±0.64	4.32±0.63
TBA (mg malonaldehid)	Blank	0.01±0.05	0.02±0.09	0.1±0.07	0.15±0.06	0.24±0.04
	FMP	0.01±0.05	0.03±0.08	0.09±0.04	0.13±0.09	0.21±0.08
TVB-N (mg/100g)	Blank	6.31±0.9	15.31±0.20	28.18±1.1	45.32±1.8	65.12±1.3
	FMP	6.31±0.9	14.5±0.12	23.23±1.4	42.13±1.6	64.31±1.9
TVC (log UFC/g)	Blank	3.43±0.61	4.94±0.40	6.91±0.42	7.62±0.44	8.73±0.43
	FMP	3.43±0.61	4.51±0.63	6.55±0.53	7.43±0.67	8.62±0.55

Table 4. Changes in sensory parameters during storage of Silver carp fillets pretreated under different conditions

Storage time (days)	Flesh appearance		Rancid odor		Flesh consistency	
	Control	FMP	Control	FMP	Control	FMP
0	E	E	E	E	E	E
4	A	A	A	A	A	A
8	B	A	B	A	B	A
12	C	B	C	B	C	B
16	C	C	C	C	C	C

Freshness categories: E (excellent), A (good), B (fair) and C (poor).
*All fish were category E for all attributes initially.

network. The cross-linked proteins in film network were insoluble, whereas almost all of the glycerol was released (Orliac *et al.*, 2002; Artharn *et al.*, 2007). FMP films were mostly stabilized by various bonds, including intermolecular disulfide covalent bonds and this result to lower solubility (Chinabhark *et al.*, 2007). Due to higher degraded protein molecules at pH=3, Maillard reaction was more favorable, leading to the formation of strong protein cross-links stabilized by covalent bond. However, glycerol which was used as a plasticizer could be leached out. Glycerol is hydrophilic plasticizer added into film-forming solution and could enhance film solubility in water. Cuq *et al.* (1997) studied the effect of plasticizer concentration on the properties of films from fish myofibrillar protein. The solubility of these films was increased by increasing plasticizer content. A linear relationship between water soluble dry matter content and hydrophilic plasticizer content in the film was observed. Similar result was found for glutenin-rich films by other researches (Hernandez-Munoz *et al.*, 2004; Artharn *et al.*, 2007; Cao *et al.*, 2008; Limpan *et al.*, 2010).

Water sorption kinetics

Water sorption kinetics is a means to characterize the water absorption of the film, which in turn is transmitted to the product inside. Knowledge of the swelling is also important for predicting stability and quality changes during packaging and storage of food product (Siripatrawan and Harte, 2010). Swelling results of FMP films are displayed in table 1. As it is shown in table 1, this low water sorption is also most likely to be due to the formation of the cross-link network.

Lipid oxidation in Silver carp fillets during storage

The primary oxidation products (PV) and Secondary lipid oxidation products (TBA) of the fillet samples increased during storage, but packing with FMP film led to lower PV and TBA during storage, compared to the control (Table 3). A marked increase in samples at the 8th days of storage was significant difference ($p \leq 0.05$) were obtained at 4th and 12th days of storage. Secondary lipid oxidation products, as reported by the TBA-i, presented low values at the beginning of the study (Table 3) and increased during storage, especially when PV values were decreased after the 8th day of storage. A significant increase in Thiobarbituric acid TBA-i value was observed for control samples ($p \leq 0.05$) compared with the FMP treatment. After 16 days of storage, the PV and TBA values of fillets which were packed with FMP film were lower than control. These results show that FMP is a better barrier against lipid oxidation. Briefly, according our results, FMP films prevented

lipid oxidation which could be induced by UV light; as a result packing fillets with FMP can delay lipid oxidation during storage.

TVB-N value in Silver carp during storage

Results of TVB-N value changes of fillets which were stored at 4 ± 1°C are given in table 3. TVB-N is well documented as an index of the quality of fresh fish because its increase corresponds to bacterial spoilage. The concentration of TVB in freshly caught fish is typically between 5 and 20 mg TVB-N/100 g, whereas levels of 30–35 mg/100 g flesh are generally regarded as the limit of acceptability for fish stored on ice; as it is shown in table 4, values were ranged from 5.13 to 49 mg/100 g. TVB-N levels of fillets were low at the beginning of storage since all products were fresh, but increased significantly ($p \leq 0.05$) during 16 days storage period. Also TVB-N values of the fillets which were packed in 12th and 16th days of storage were significantly different ($p \leq 0.05$) Though sensory values showed 12 days of storage life, according to chemical results, fillets lost its consumable properties after 12 days when the TVB-N value reached 19.2 mg/100 g. In the same research, TVB-N values were measured in whole and filleted rainbow trout stored in ice for 18 days. The results of their study indicated that the shelf-life of whole ungutted and filleted trout stored on ice were 1–16 and 10–12 days, respectively (Ko¨se *et al*., 2006).

Microbiological analysis of Silver carp packed with the FMP film

The populations of colonies in fish fillets which were packed with polyethylene bags and FMP films were determined during storage at 4°C (Tables 3). As it is shown, initial populations on fillets were 3.59±.46 log CFU/g and they were increased during storage. After 16 days of storage, the population of colony in the control was 9.71 log CFU/g, whereas the population of the bacteria in the fillets packed with the FMP film was 9.5 log CFU/g ($P \leq 0.05$). It appears that FMP film had a greater inhibitory effect on population of the bacteria, and packing with them can be an effective treatment for reducing microbial populations in fish fillets during storage. However, it should be noted that the degree of reduction in microbial counts is marginal, resulting in about 0.21 log CFU/g.

In the end, flesh consistency assessment showed a better score at 8th days of storage for FMP packaging treatment samples, while at the end of the storage time no differences were obtained among the two kinds of the samples (table 4). Sensory analyses of attributes considered indicate that FMP film can slow down quality loss during frozen storage.

Conclusion

In this study, we prepared fish myofibrillar protein (FMP) films with proper mechanical properties and water vapor permeability. Results showed that these films could delay photo oxidation of fish fillets due to inhibiting light diffusion in compare with polyethylene films. Also, according to microbial analysis, FMP films were effective on preventing bacteria growth in compare with control. Due to these results, using these edible biodegradable films is suggested.

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