J Food Sci Technol (October 2015) 52(10):6182–6193
DOI 10.1007/s13197-014-1706-y

ORIGINAL ARTICLE

# Shelf-life extension of refrigerated sea bass slices wrapped with fish protein isolate/fish skin gelatin-ZnO nanocomposite film incorporated with basil leaf essential oil

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Revised: 18 November 2014 / Accepted: 29 December 2014 / Published online: 14 January 2015
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**Abstract** Microbiological, chemical and sensory changes of sea bass slices wrapped with fish protein isolate (FPI)/fish skin gelatin (FSG) films incorporated with 3 % ZnO nanoparticles (ZnONP) (w/w, based on protein content) and 100 % basil leaf essential oil (BEO) (w/w, based on protein content) during storage of 12 days at 4 °C were investigated. Sea bass slices wrapped with FPI/FSG-ZnONP-BEO film had the lowest growth of psychrophilic bacteria, lactic acid bacteria and spoilage microorganisms including *Pseudomonas* , $H_2S$-producing bacteria and *Enterobacteriaceae* throughout storage of 12 days in comparison with those wrapped with FPI/FSG-BEO, FPI/FSG-ZnONP, FPI/FSG film, polypropylene film (PP film) and the control (without wrapping), respectively ($P<0.05$). Lowered increases in pH, total volatile base, peroxide value and TBARS value were found in FPI/FSG-ZnO-BEO film wrapped samples, compared with others ($P<0.05$). Sensory evaluation revealed that shelf-life of sea bass slices was longest for samples wrapped with FPI/FSG-ZnONP-BEO film (12 days), as compared to the control (6 days) ($P<0.05$).

**Keywords** Bionanocomposite · Fish protein isolate · Fish skin gelatin · Basil essential oil · ZnO nanoparticles · Active food packaging

## Introduction

Generally, fish and fish products have been known to have a high nutritional value. They are rich in protein, polyunsaturated fatty acids (PUFA) and essential minerals (Simopoulos 2002). Ready-to-cook fish have become increasingly popular. Due to the rapid microbial growth and lipid oxidation, such perishable products have the limited shelf-life (Masniyom et al. 2002). With increasing demand for high quality ready-to-cook fish with the extended shelf-life, several innovative techniques for maintaining quality and safety of products have been developed (Maftoonazad and Badii 2009). To satisfy these requirements, the uses of conventional chemical additives in food formulation have been decreased. The alternative use of novel natural additives has gained the increasing attention (Sánchez-González et al. 2011).

Biopolymer films are excellent vehicles for incorporating a wide variety of additives, such as antimicrobials, antioxidants, antifungal agents, colours, and other nutrients (Rhim and Ng 2007). Active biopolymer packaging systems, based on the incorporation of antimicrobial or antioxidative substances in biodegradable food packaging materials could delay microbial spoilage of food, control undesirable changes and extend shelf-life of food products (Emiroğlu et al. 2010). The uses of essential oils (EOs) as sources of antimicrobials and antioxidants have long been acknowledged and the biopolymer packaging industry has recently paid more attention to their applications as natural antimicrobials and antioxidants for smart packaging (Holley and Patel 2005). Chitosan coating incorporated with cinnamon essential oil was reported to extend the shelf-life of rainbow trout during storage at 4 °C for 16 days, mainly by retarding the microbial growth and lowering the lipid oxidation (Ojagh et al. 2010). *In-vitro* studies have revealed significant antimicrobial and antioxidant effects of essential oil from basil leaves, which are abundant and have low market value with great potential for use in food preservation (Hussain et al. 2008; Suppakul et al. 2003). Additionally, essential oils are hydrophobic in nature and the incorporation of essential oils could improve the water vapour

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barrier property and impart flexibility of protein films (Tongnuanchan et al. 2013). The use of essential oil exhibited the enhanced antimicrobial activity, when combined together with various nanoparticles (Allahverdiyev et al. 2011). ZnONP is currently listed as a generally recognised as safe (GRAS) material by the Food and Drug Administration (21CFR182.8991) and has previously shown strong antimicrobial activity against food borne pathogens and spoilage bacteria (Espitia et al. 2013).

Sea bass (*Lates calcalifer*) is very popular in tropical areas of South-East Asia (Masniyom et al. 2002). It is commonly sold as whole fish or as fillets. However, microbial spoilage and lipid oxidation shorten the shelf-life of sea bass slices (Masniyom et al. 2002). Based on the organoleptic property, the shelf-life of sea bass (*Dicentrarchus labrax*) was found to be 16 days in ice, 4 days in boxes without ice and 8 days when wrapped with cling film or aluminium foil and stored at 4 °C (Ozogul et al. 2005). Recently, FPI/FSG films have been prepared and showed the improved mechanical and water vapour barrier properties, compared with films prepared from single material (Arfat et al. 2014). To widen the application as the active packaging, the incorporation with ZnONP and BEO having antimicrobial and antioxidative activities could be a promising means. Nevertheless, there is no information on the use of FPI/FSG film containing ZnONP and essential oil for the shelf-life extension of fish slices. Therefore, the objective of this investigation was to study the impact of FPI/FSG-ZnO nanocomposite film incorporated with BEO on the shelf-life extension of sea bass slices stored at 4 °C.

## Materials and methods

### Chemicals

Zinc oxide nanoparticles (ZnONP) (particle size: 20–40 nm, specific surface area: 26.22 $m^2$/g) were purchased from Nano materials technology Co. Ltd. (Bangkok, Thailand). Commercial fish skin gelatin (FSG) from tilapia (~240 bloom) was obtained from Lapi Gelatine S.p.A (Empoli, Italy). Basil (*Ocimum basilicum*) leaf essential oil (BEO) was purchased from Botanicessence essential oils (Suanlung, BKK, Thailand).

### Collection and preparation of fish sample

Fresh yellow stripe trevally (*Selaroides leptolepis*) with an average weight of 90–100 g/fish were purchased from a local market in Hat Yai, Songkhla province, Thailand. Fish were kept in ice with a fish/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University within 30 min. Upon the arrival, fish were immediately washed, filleted, and minced to uniformity using a Model HC 5000 mincer (Microfluidics, Massachusetts, USA) with a hole diameter of 0.5 cm. Fresh sea bass (*Lates calcarifer*) were purchased from a local market in Hat Yai, Songkhla, Thailand and transported in ice with fish/ice ratio of 1:2 (w/w) to the laboratory within 30 min. Sea bass were filleted and sliced to a thickness of 1.5 cm.

### Preparation of fish protein isolate

Prior to the isolation of fish protein, the prepared yellow stripe trevally mince was subjected to washing as per the method of Toyohara et al. (1990) with slight modifications. Fish mince was homogenised with 5 volumes of cold 0.05 M NaCl (2–4 °C) at a speed of 13,000 rpm for 2 min, using an IKA Labortechnik homogeniser (Selangor, Malaysia). The washed mince was filtered through two layers of cheese-cloth. The washing process was repeated twice. Washed mince obtained was stored on ice until used. Washed mince was added with cold distilled water at the ratio of 1:9 (w/v), followed by homogenisation for 1 min at a speed of 13,000 rpm. The pH of homogenate was then adjusted to 11 using 2 M NaOH. The resulting mixture was centrifuged at 10,000×g for 20 min at 4 °C using a refrigerated centrifuge (Avanti-JE Centrifuge, Beckman 163 Coulter Inc., Fullerton, CA, USA). The supernatant was collected and the pH was adjusted to 5.5 using 2 M HCl. The precipitate was then filtered through 4 layers of cheese-cloth. The retentate was dewatered by centrifugation at 12,000×g for 20 min at 4 °C. The final pH of the sample was adjusted to pH 7.0 using 2 M NaOH. The sample was referred to as "fish protein isolate; FPI". FPI was used for film preparation.

### Preparation of fish protein isolate/fish skin gelatin film containing ZnONP and BEO

Firstly, film-forming solution was prepared according to the method of Chinabhark et al. (2007). FPI was added with 3 volumes of distilled water and homogenised at 13,000 rpm for 1 min using a homogeniser. Subsequently, the pH of the mixture was adjusted to 3 using 1 N HCl to solubilise the protein. The obtained solution was filtered through 2 layers of cheese-cloth to remove undissolved debris. The protein concentration of the filtrate determined by the Kjeldahl method (AOAC 2000) was adjusted to 3 % (w/v). Glycerol at 30 % (w/w) of protein was used as a plasticiser. The mixture was stirred gently for 30 min at room temperature and was used for preparing blend FFS.

Prior to blending, FSG powder was dissolved in distilled water to obtain the protein concentration of 3 % (w/v). The pH of the mixture was adjusted to 3 using 1 N HCl. The solution was heated at 70 °C for 30 min and cooled at room temperature (28–30 °C) for 20 min. Glycerol at concentrations of 30 % (w/w) of protein content was used as a plasticiser. Thereafter,

both FPI and FSG solutions were mixed at a ratio of 1:1 (v/v). The obtained solution was added without and with 3 % ZnONP (w/w, protein content) in droplets. Before addition of ZnONP, ZnONP was suspended in distilled water and homogenised for 1 min at 5,000 rpm. The obtained FPI/FSG/ZnONP suspension was stirred for 5 min and then homogenised for 30 s at the speed of 5,000 rpm.

BEO previously mixed with Tween 20 at 25 % (w/w, based on essential oil) was added to FPI/FSG solution or FPI/FSG/ZnONP suspension at 100 % (w/w, protein content). Final volume was made up to 100 ml using distilled water previously adjusted to pH 3. To obtain the uniform distribution of BEO in suspensions, the mixtures were homogenised with three passes using a high pressure homogeniser (Microfluidizer M-110EH, Microfluidics Corp., Newton, MA, USA) with an operating pressure of 1,500 bars. Suspensions were gently stirred for 30 min at room temperature and were referred to as 'film-forming suspension' (FFS).

Prior to casting, all FFS samples were degassed for 10 min using the sonicating bath (Elmasonic S 30 H, Singen, Germany). To prepare the film, 4 g of FFS was cast onto a rimmed silicone resin plate (5×5 $cm^2$), air-blown for 12 h at 25 °C, followed by drying in an environmental chamber (Binder GmbH, Tuttlingen, Germany) at 25±0.5 °C and 50±5 % relative humidity (RH) for 24 h. Dried film samples were manually peeled off and subjected to analyses.

### Effect of FPI/FSG-ZnO nanocomposite film incorporated with BEO on quality changes of sea bass slices

Fish slices (4×4 $cm^2$) were wrapped with films (5×5 $cm^2$), in which all sides were completely covered. Films used included PP film, FPI/FSG film, FPI/FSG-ZnONP film, FPI/FSG-BEO film and FPI/FSG-ZnONP-BEO film. Subsequently, the samples were placed in polystyrene trays (9×7 $cm^2$), wrapped with an extensible polypropylene film (Thickness # 11 μm, MMP Corporation Ltd., Bangkok, Thailand) prior to storage at 4 °C for 12 days. Control samples were prepared in the same manner except that the slices were not covered with any film. During storage, the samples were randomly taken every 2 days for analyses.

### Microbiological Analyses

Microbiological analyses were performed using the spread plate method (Sallam 2007). Samples were collected aseptically and used as the composite sample. The sample (25 g) was placed in a Stomacher bag containing 225 ml of 0.85 % saline solution. After mixing for 1 min in a Stomacher blender (Stomacher M400, Seward Ltd., Worthington, England), further serial dilutions were made from the homogenate using 0.85 % saline solution as the diluent. Appropriate dilutions were used for analysis of the total viable count (TVC), psychrophilic bacteria and hydrogen sulphide ($H_2S$) producing bacteria counts as described by Sallam (2007). The total viable and psychrophilic bacteria counts were determined on plate count agar (HiMedia, Mumbai, India) containing 1 % NaCl after incubation at 35 °C for 3 days and 4 °C for 10 days, respectively. $H_2S$-producing bacteria were enumerated from black colonies grown on sugar iron agar (HiMedia, Mumbai, India) after incubation at 25 °C for 3 days. For *Pseudomonas* count, it was examined on Pseudomonas Isolation Agar (Difco Laboratories, BD, Sparks, MD, USA) after 48 h of incubation at 25 °C (Escudero-Gilete et al. 2014). The enterobacterial count was determined using eosin methylene blue agar (EMB) (HiMedia, Mumbai, India) and the incubation was conducted at 37 °C for 24 h following the method of Ahmad et al. (2012). Lactic acid bacteria were enumerated on a double-layered plates of deMann-Rogosa Sharpe medium (MRS) agar (HiMedia, Mumbai, India) after 72 h of incubation at 37 °C (Asahara et al. 2001).

### Chemical analyses

TVB content was determined following the method of Conway and Byrne (1933) and expressed as mg N/100 g sample. pH measurement was performed as per the method of López-Caballero et al. (2007). The pH of homogenagte (2:10, w/v) was determined using a pH-meter (Sartorious North America, Edgewood, NY, USA). Peroxide value (PV) was determined according to the method of Richards and Hultin (2002) and expressed as mg cumene hydroperoxide/kg sample. The thiobarbituric acid reactive substances (TBARS) were examined as described by Buege and Aust (1978). TBARS value were calculated and expressed as mg malonaldehyde/kg sample.

### Sensory evaluation

Sensory evaluation of sea bass slices was undertaken by a panel consisting of 8 trained panelists. The sensory evaluation was based on a five-point-scale to determine: texture (5, firm; 1, very soft); colour (5, no discoloration; 1, extreme discoloration); odour (5, extremely desirable; 1, extremely unacceptable/off-odours); and overall quality (5, extremely desirable; 1, extremely unacceptable) of the samples. For shelf-life prediction, the rejection was claimed when the sensory score was below 4.0 (Ojagh et al. 2010).

### Statistical analyses

Experiments were performed in triplicate ($n=3$) and a completely randomised design (CRD) was used. Data were presented as means±standard deviation and $P<0.05$ was considered significant. Analysis of variance (ANOVA) was performed and the mean comparisons were done by Duncan's

multiple range tests (Steel and Torrie 1980). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for Windows, SPSS Inc., Chicago, IL, USA).

## Results and discussion

### Changes in microbial loads

Total viable count (TVC) of sea bass slices wrapped without and with films (PP, FPI/FSG, FPI/FSG-ZnONP, FPI/FSG-BEO and FPI/FSG-ZnONP-BEO) during storage at 4 °C for 12 days is shown in Fig. 1a. TVC of all samples at day 0 was around 3.7 log CFU/g, indicating the presence of some microorganisms, probably contaminated during slice preparation, packaging, etc. TVC of the control sample (without wrapping) increased during the first 4 days of storage, with an approximate value of 6.8 log CFU/g at day 4 and this load became higher and reached 8.9 log CFU/g at day 12. This exceeded the value of 7 log cfu/g, which is considered as the upper acceptability limit for fresh water and marine species as defined by ICMSF (2002). In addition, the control had the higher increase in TVC, compared with others, throughout the storage of 12 days ($P<0.05$). For PP and FPI/FSG film wrapped samples, slightly lower TVC was observed, compared with that of control samples during refrigerated storage ($P<0.05$). For FPI/FSG-ZnONP, FPI/FSG-BEO and FPI/FSG-ZnONP-BEO-film wrapped samples, TVC increased gradually and reached the value of 7.3, 6.6 and 5.9 log CFU/g at day 12, respectively. Thus, FPI/FSG-ZnONP, FPI/FSG-BEO and FPI/FSG-ZnONP-BEO films were able to retard the microbial growth of sea bass slices. Amongst all films, FPI/FSG-ZnONP-BEO had the highest inhibitory effect on microbial growth. Based on microbial assessment, the shelf-life of sea bass slice was found to be 4 days in air (without wrapping), 5 days with PP film, 6 days with FPI/FSG film and 10 days with FPI/FSG-ZnONP. Nevertheless, the shelf-life of sea bass was extended to 12 days when the sample was wrapped with FPI/FSG–BEO and FPI/FSG-ZnONP-BEO. The delay in microbial spoilage of sea bass slices was plausibly due to the combined antimicrobial effect of BEO and ZnONP distributed throughout the films matrix. After 12 days of storage, FPI/FSG-ZnONP-BEO film wrapped samples had the lower TVC by approximately 3 log CFU/g, as compared to the control samples ($P<0.05$). Generally, the total number of microorganisms varies enormously and ranges from $10^2$ to $10^7$ CFU/cm$^2$ in fish (Liston 1980). Antimicrobial activity of ZnONP and the mechanism of inhibition against the microorganisms have been demonstrated (Espitia et al. 2013; Zhang et al. 2010). The release of $Zn^{2+}$ ions from the powder could penetrate through the cell wall of microorganism and react with interior components that finally affect the viability of cells. ZnONP has been known to mediate the generation of hydrogen peroxide ($H_2O_2$), a powerful oxidising agent causing damage to the cell membrane of bacteria (Tayel et al. 2011). Emamifar et al. (2010) confirmed that active packaging based on low-density polyethylene (LDPE) containing ZnONP prolonged the shelf-life of fresh orange juice up to 28 days by reducing the rate of microbial growth. Microbial inhibition by film containing BEO could be attributed to its hydrophobic nature. BEO and its main components, especially linalool, an oxygenated monoterpenoid, could penetrate through the cell wall of a bacterium and attack on the phospholipid bilayer present in cell membranes (Hussain et al. 2008). This causes the increased permeability and leakage of cytoplasm, or BOE could interact with enzymes located on the

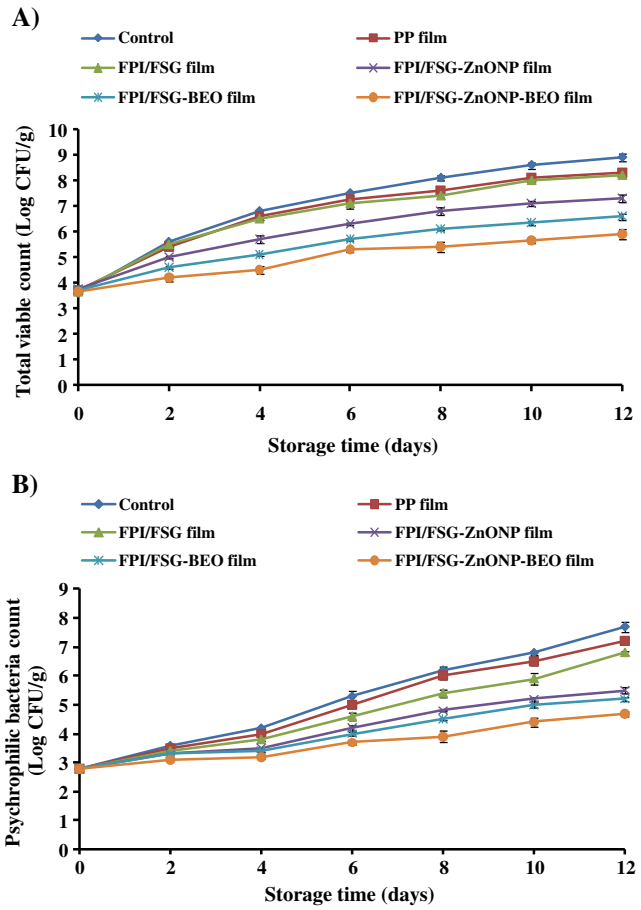


**Fig. 1** Total viable count (**a**) and psychrophilic bacterial count (**b**) of sea bass slices wrapped without and with films (PP film, FPI/FSG film, FPI/FSG-ZnONP film, FPI/FSG-BEO film and FPI/FSG-ZnONP-BEO film) during storage at 4 °C for 12 days. Control: unwrapped samples, PP film: samples wrapped with polypropylene film, FPI/FSG film: samples wrapped with fish protein isolate/fish skin gelatin film, FPI/FSG-ZnONP film: samples wrapped with fish protein isolate/fish skin gelatin film incorporated with zinc oxide nanoparticles, FPI/FSG-BEO film: samples wrapped with fish protein isolate/fish skin gelatin film incorporated with basil leaf essential oil, FPI/FSG-ZnONP-BEO film: samples wrapped with fish protein isolate/fish skin gelatin film incorporated with zinc oxide nanoparticles and basil leaf essential oil. Bars represent the standard deviation ($n=3$)

cell wall (Emiroğlu et al. 2010). Linalool has the potential to act as either a protein denaturing agent or as a solvent dehydrating agent, contributing to its antimicrobial activity (Emiroğlu et al. 2010). Films incorporated with essential oils could retard the microbial growth in rainbow trout (Mexis et al. 2009) and bologna slices (Zivanovic et al. 2005).

The increases in psychrophilic bacterial count in all samples were noticeable with increasing storage time up to day 12 ($P<0.05$) (Fig. 1b). During the storage, the lowest psychrophilic bacterial count was obtained in the samples wrapped with FPI/FSG-ZnO-BEO film, followed by those wrapped with FPI/FSG-BEO film, FPI/FSG-ZnONP film, FPI/FSG film and PP film, respectively. The control sample showed the highest psychrophilic bacterial count during the storage ($P<0.05$). At the end of storage (day 12), psychrophilic bacterial count of the control, those wrapped with PP film, FPI/FSG film, FPI/FSG-ZnONP film, FPI/FSG-BEO film, and FPI/FSG-ZnONP-BEO film was 7.7, 7.2, 6.8, 5.5, 5.2 and 4.7 log CFU/g, respectively. The result indicated the combined antimicrobial activity of ZnONP and BEO toward psychrophilic bacteria in sea bass slices during storage at 4 °C for 12 days.

*Pseudomonas* (Fig. 2a) in sea bass slices increased over the entire storage period. *Pseudomonas* are generally recognised to dominate in the meat system and contribute to spoilage. This can be attributed to their ability to degrade glucose and amino acids even under refrigeration conditions (Ercolini et al. 2006). *Pseudomonas* count of all samples at day 0 was around 3.3 log CFU/g. *Pseudomonas* count of the control sample increased during the storage, with an approximate value of 8.3 log CFU/g at day 12 ($P<0.05$). FPI/FSG-ZnONP, FPI/FSG-BEO and FPI/FSG-ZnONP-BEO films had a profound effect on the inhibition of *Pseudomonas* growth. For FPI/FSG-ZnONP, FPI/FSG-BEO and FPI/FSG-ZnONP-BEO film wrapped samples, *Pseudomonas* increased gradually ($P<0.05$) and reached the value of 6.6, 6.3 and 5.8 log CFU/g at day 12, respectively. Amongst all samples, those wrapped with FPI/FSG-ZnONP-BEO film had the lowest *Pseudomonas* count. After 12 of storage, the sample had the lower *Pseudomonas* count than the control by 2.6 log CFU/g. This was due to the combined antimicrobial effect of ZnONP and BEO distributed throughout the films matrix. Both ZnONP and BEO showed the strong antimicrobial activity against *Pseudomonas* (Suppakul et al. 2003). ZnONP could potentially be used as an effective antibacterial agent to protect food related bacteria including *Pseudomonas* (Tayel et al. 2011). As shown in Fig. 2a, PP and FPI/FSG films also inhibited the growth of *Pseudomonas* to some extent. *Pseudomonas* are strictly aerobic microorganisms and are unable to survive in the limited oxygen or absence of oxygen.

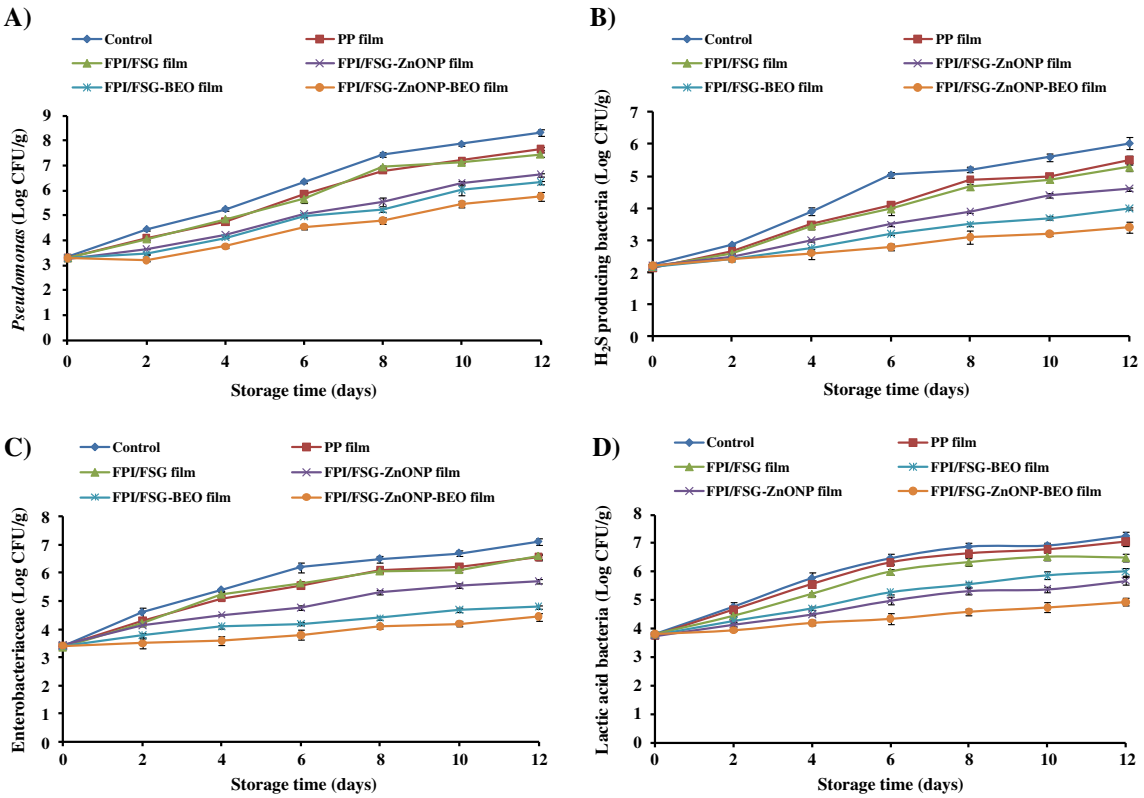


**Fig. 2** *Pseudomonas* (**a**), $H_2S$-producing bacteria (**b**), *Enterobacteriaceae* (**c**) and Lactic acid bacteria (**d**) counts of sea bass slices wrapped without and with films (PP film, FPI/FSG film, FPI/FSG-ZnONP film, FPI/FSG-BEO film and FPI/FSG-ZnONP-BEO film) during storage at 4 °C for 12 days. Key: see the caption for Fig. 1. Bars represent the standard deviation ($n=3$)

Changes in $H_2S$-producing bacterial count of sea bass slices during 12 days of refrigerated storage are illustrated in Fig. 2b. Counts of $H_2S$-producing bacteria, including *Shewanella putrefaciens* have been used as spoilage indicators of seafood products (Stamatis and Arkoudelos 2007). *S. putrefaciens* produces very intense and unpleasant off-odours associated with $H_2S$ formation and reduces TMAO to TMA (Sivertsvik et al. 2002). The lowest $H_2S$-producing bacterial count was found in samples wrapped with FPI/FSG-ZnONP-BEO film as compared to others during 12 days of storage ($P<0.05$). Therefore, ZnONP and BEO incorporated into FPI/FSG film could retard the growth of spoilage bacteria, which were able to produce $H_2S$. At the end of storage, the control (sea bass without any wrapping) showed $H_2S$-producing bacterial counts of 6.02 log CFU/g, whilst the samples wrapped with PP film, FPI/FSG film, FPI/FSG-ZnONP film, FPI/FSG-BEO film and FPI/FSG-ZnONP-BEO film had the counts of 5.5, 5.2, 4.6, 4.0 and 3.4 log CFU/g, respectively. Specific spoilage organisms such as $H_2S$-producing bacteria are mostly predominant during the spoilage of fish and fish products, producing very intense and unpleasant off-odours and rejection (Sivertsvik et al. 2002). Tayel et al. (2011) reported that nanosized ZnO suspensions are active in inhibiting the growth of $H_2S$-producing bacteria. Oregano essential oil inhibited growth of $H_2S$-producing bacteria in rainbow trout stored at 4 °C (Mexis et al. 2009). For the sea bass slices, gelatin film and gelatin film incorporated with lemon grass essential oil inhibited the growth of $H_2S$-producing bacteria during 12 days of refrigerated storage and had bacterial counts of 3.6 and 2.5 log CFU/g, respectively, as compared to control (4.2 log CFU/g) (Ahmad et al. 2012). Inhibition of $H_2S$-producing bacteria growth of sea bass slice was observed when wrapped with PP film and FPI/FSG film. It was suggested that $H_2S$-producing bacteria might proliferate at lower level when the surface of the sample had the lower oxygen level (Ahmad et al. 2012).

With respect to *Enterobacteriaceae* (Fig. 2c), considered as a hygiene indicator (Zeitoun et al. 1994), the initial count at day 0 was 3.4 log CFU/g, but reached 7.1 log CFU/g in the control samples at day 12. PP film and FPI/FSG film showed a significant impact on the inhibition of *Enterobacteriaceae* and the counts of 6.5 and 6.6 log CFU/g were obtained at the end of storage (day 12), for samples wrapped with PP and FPI/FSG films, respectively. Aerobic microorganisms might have the retarded growth when the surface of slice samples was covered by PP and FPI/FSG film. PP and protein based film were reported to have the excellent oxygen barrier property (Chiou et al. 2008; Tihminlioglu et al. 2010). Nevertheless, FPI/FSG-ZnONP film, FPI/FSG-BEO film and FPI/FSG-ZnONP-BEO film wrapped samples had the lower counts by 1.4, 2.3 and 2.6 log CFU/g, as compared to the control, at day 12 of storage. The lowest count observed in FPI/FSG-ZnONP-BEO film wrapped samples was more likely associated with inhibitory action of ZnONP and BEO against the spoilage bacteria ($P<0.05$). The combined use of ZnONP and BEO was therefore able to inhibit the growth of *Enterobacteriaceae* effectively during the storage as their population remained below 5 log CFU/g. Tassou et al. (1996) reported that treatment of fresh sea bream fillets with a mixture of olive oil, lemon and essential oil (oregano) reduced the final *Enterobacteriaceae* counts by approximately 2.5 log CFU/g, compared to the control.

Lactic acid bacteria (LAB) (Fig. 2d) are facultative anaerobic bacteria that can grow under both anaerobic and aerobic conditions and constitute a substantial part of the natural microflora of stored food products under anaerobic conditions (Mastromatteo et al. 2009). The initial LAB count was 3.8 log CFU/g in sea bass slices at day 0. LAB count gradually increased when storage time increased ($P<0.05$). LAB increased to the levels of 7.2, 7.0, 6.5, 5.7, 6.0 and 4.9 log CFU/g at the end of storage (day 12) for the control, PP, FPI/FSG, FPI/FSG-ZnONP, FPI/FSG-BEO and FPI/FSG-ZnONP-BEO film wrapped samples, respectively (Fig. 2d). The use of the BEO was slightly less effective than ZnONP. The limited action of BEO as compared to ZnONP was possibly attributed to the high tolerance of LAB against the action of essential oils (Holley and Patel 2005). Although LAB is the most resistant among the Gram-positive bacteria towards the antimicrobial action of essential oils, thyme oil along with modified atmosphere packaging (MAP) was able to retard their growth (Kostaki et al. 2009). The combined use of ZnONP and BEO was the most effective for retardation of LAB growth during the storage as their population remained below 5 log CFU/g at the end of refrigerated storage. LDPE nanocomposite packaging containing Ag and ZnO caused the reduction of *Lactobacillus plantarum* growth in orange juice (Emamifar et al. 2011). Ahmad et al. (2012) reported a reduction of 1.4 log CFU/g in LAB populations in the sea bass slices wrapped with gelatin film incorporated with lemon grass essential oil. Chouliara et al. (2007) also found a reduction of 1.1 log CFU/g in LAB for chopped chicken meat after 6 days of storage with the addition of 0.1 % oregano essential oil. It was noted that FPI/FSG film also showed the slight antimicrobial activity. This was possibly owing to the antimicrobial peptides present in fish protein isolate and fish skin gelatin. Gómez-Guillén et al. (2010) reported that gelatin peptides showed antimicrobial activity toward several microorganisms.

## Changes in chemical compositions

### *Total volatile base content*

TVB content of sea bass slices wrapped without and with films (PP, FPI/FSG, FPI/FSG-ZnONP, FPI/FSG-BEO and FPI/FSG-ZnONP-BEO) during storage at 4 °C for 12 days

is shown in Fig. 3a. TVB content of all samples at day 0 was approximately 13 mg N/100 g, indicating the good quality of the fresh samples. As the storage time increased, a continuous increase in TVB content was observed in all samples ($P<0.05$), but the increasing rate varied amongst samples wrapped with different films. A rapid increase in TVB content was noticed in the control, which reached a value of 33.24 mg N/100 g muscle at day 12. However, the gradual increase in TVB content was observed throughout the storage of 12 days for PP, FPI/FSG, FPI/FSG-ZnONP, FPI/FSG-BEO and FPI/FSG-ZnONP-BEO wrapped samples, reaching a value of 28.45, 25.87, 20.12, 18.27 and 16.44 mg N/100 g at day 12, respectively. Since TVB is produced mainly by bacterial decomposition of fish flesh, the higher values of total viable counts in the control indicated the higher spoilage. The lower TVB values of wrapped samples, especially with FPI/FSG-ZnONP-BEO film, could be attributed to either more rapid reduction at bacterial population or decreased capacity of bacteria for oxidative de-amination of non-protein nitrogen compounds or both (Fan et al. 2008). Silver carp fillets coated with chitosan biopolymer incorporated with nanoclay had the lower TVB-N content as compared to the control (chitosan only) during 12 days of storage (Abdollahi et al. 2013). Harpaz et al. (2003) found that the addition of oregano or thyme oil (0.05 %, v/v) in sea bass maintained the TVB values below the limit (30 mg N/100 g) up to 35 days of storage at 0 to 2 °C. At the end of the storage period (12 days), TVB contents of sea bass slices wrapped with gelatin film and gelatin film incorporated with lemongrass essential oil were 18.34 and 15.84 mg N/100 g, respectively (Ahmad et al. 2012). TVB value in freshly caught fish is typically between 5 and 20 mg N/100 g, and TVB value of 30–35 mg N/100 g has been established as an upper acceptability limit for fresh fish by the European Commission (Commission Decision 95/149/EC 1995). TVB content was in accordance with microbial load (Fig. 1a) in the corresponding samples. Thus, the combined inhibitory effect of ZnONP and BEO in FPI/FSG-ZnONP-BEO films against microbial growth could retard or lower the production of microbial degradation products.

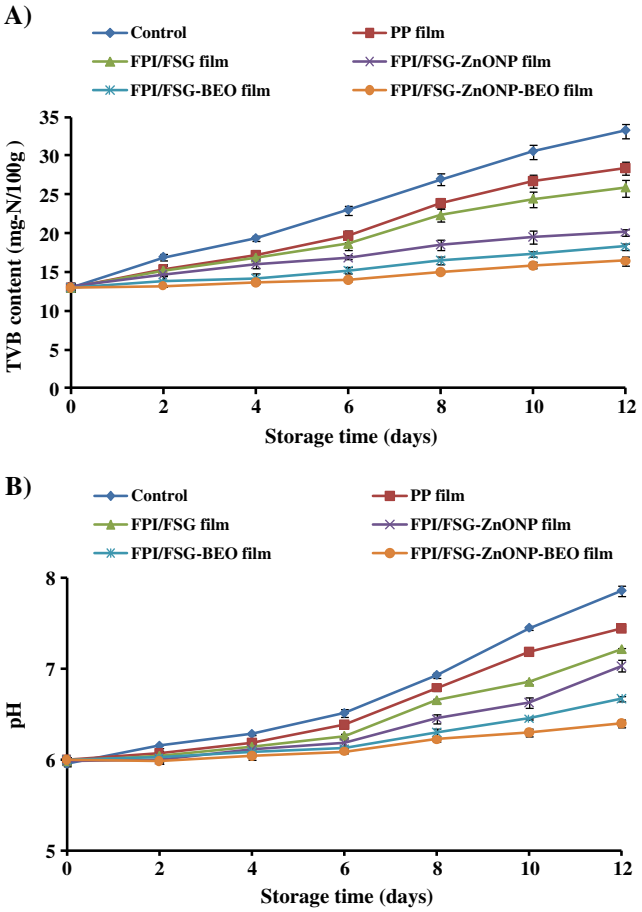


**Fig. 3** TVB content (**a**) and pH (**b**) of sea bass slices wrapped without and with films (PP film, FPI/FSG film, FPI/FSG-ZnONP film, FPI/FSG-BEO film and FPI/FSG-ZnONP-BEO film) during storage at 4 °C for 12 days. Key: see the caption for Fig. 1. Bars represent the standard deviation ($n=3$)

*pH*

pH of sea bass slices wrapped without and with films during storage at 4 °C for 12 days is shown in Fig. 3b. pH of the control increased from 5.95 to 7.86 after storage for 12 days. Generally, pH of all samples slightly increased, when the storage time increased ($P<0.05$). Such a trend was associated with the accumulation of basic compounds such as ammonia and TMA, etc. generated from microbial enzymatic actions (López-Caballero et al. 2007). The pH of refrigerated sea bass muscle was increased to 7.7 after 15 days of storage, most likely due to the production of basic amines (Masniyom et al. 2002). The lower increase in the pH of sea bass slices wrapped with FPI/FSG-ZnONP-BEO film was observed, when compared with other samples ($P<0.05$). The result was in agreement with the lower microbial count and TVB content in FPI/FSG-ZnONP-BEO film wrapped samples. Similar results for sea bass slices wrapped with LEO incorporated gelatin films were reported (Ahmad et al. 2012).

*Peroxide Value (PV)*

Changes in PV of refrigerated sea bass slices wrapped without and with various films are presented in Fig. 4a. PV of all samples increased during the early stage of storage and reached a maximum by day 8. Subsequently, it decreased until the end of the storage period. PV shows the amount of oxidised substances, which are usually hydroperoxides and are the primary products of autoxidation (Yanishlieva and Marinova 2001). The PV increase in the early stages shows lipid oxidation and formation of hydroperoxides with a rate higher than that of their decomposition. Its decrease after reaching the maximum value is related to hydroperoxide

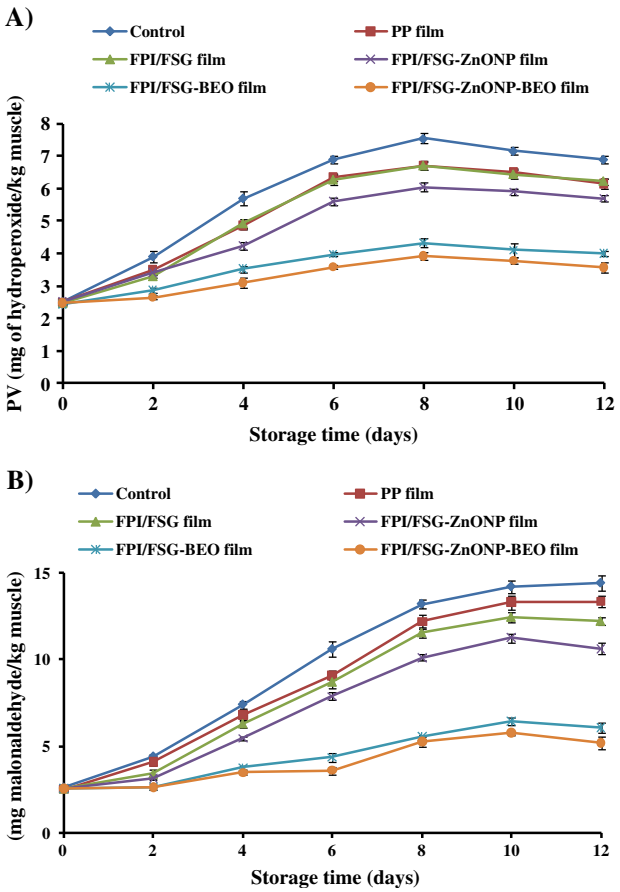


**Fig. 4** Peroxide value (**a**) and TBARS value (**b**) of sea bass slices wrapped without and with films (PP film, FPI/FSG film, FPI/FSG-ZnONP film, FPI/FSG-BEO film and FPI/FSG-ZnONP-BEO film) during storage at 4 °c for 12 days. Key: see the caption for Fig. 1. Bars represent the standard deviation ($n$=3)

degradation, producing the secondary lipid peroxidation products (Boselli et al. 2005). During the storage, a higher increase in PV was observed in the control samples as compared to others ($P$<0.05). PP and protein based films have low oxygen permeability, which more likely produced an oxygen-resistant layer on the surface of fish slice and decreased lipid oxidation (Tihminlioglu et al. 2010). Samples wrapped with FPI/FSG-ZnONP film showed the lower PV in comparison with the control, PP and FPI/FSG wrapped samples. It is well known that nanoparticles improve the barrier properties of biopolymers due to its functional filler property and the formation of a tortuous path for the molecule diffusion (Arora and Padua 2010). This more likely decreased the oxygen permeability of FPI/FSG-ZnONP film, compared with FPI/FSG film. Thus, the decrease in PV in the samples wrapped with FPI/FSG-ZnONP was more likely related with their higher oxygen barrier properties. However, the lowest PV during the storage period was observed in the samples wrapped with FPI/FSG-ZnONP-BEO film ($P$<0.05). This could be caused by the combined effect of ZnONP (oxygen barrier) and antioxidant activity of BEO, which was mediated by polyphenols (Suppakul et al. 2003). Phenolic antioxidants do not function as oxygen absorbers; they prevent the formation of fatty acid free radicals, which react with or absorb oxygen in the autoxidation process. This delays the onset of the autoxidative process in fat or oil (Turhan et al. 2009). The results were in agreement with Abdollahi et al. (2013) who reported that silver carp fillets coated with chitosan nanocomposites activated with rosemary essential oil had the lower PV, compared with the samples coated with chitosan and chitosan nanocomposite film.

*TBARS value*

During the storage, a higher increase in TBARS was observed in the control samples, followed by those wrapped with PP, FPI/FSG, FPI/FSG-ZnONP, FPI/FSG-BEO and FPI/FSG-ZnONP-BEO film, respectively ($P$<0.05) (Fig. 4b). TBARS value is an index of lipid oxidation, in which malondialdehyde (MDA) content is measured (Benjakul et al. 2005). Attacks of oxygen against the double bond in fatty acids can cause initiation of free radical chain reactions in lipid oxidation (Abdollahi et al. 2013). Samples wrapped with PP and FPI/FSG films also showed the lower TBARS than the control ($P$<0.05). These films might function as a barrier to oxygen permeability and only a small amount of oxygen could therefore contact with samples. As a result, the lower oxidation rate was obtained. TBARS value in the sample wrapped with FPI/FSG-ZnONP film was lower than those wrapped with PP or FPI/FSG, which might be related to well-known and documented excellent oxygen barrier properties of bionanocomposite films (Arora and Padua 2010). However, sea bass slices wrapped with FPI/FSG-ZnONP-BEO film showed the lowest TBARS value ($P$<0.05). The result suggested that lipid oxidation in sea bass slices could be retarded when film incorporated with BEO was applied, probably due to the antioxidant activity of BEO as well as low oxygen permeability characteristics of film incorporated with nanoparticles and essential oil (Abdollahi et al. 2013). Thus, the incorporation of BEO enhanced the antioxidant property of the resulting film, as evidenced by lower TBARS of samples wrapped with FPI/FSG-BEO and FPI/FSG-ZnONP-BEO films, compared to other films. Tongnuanchan et al. (2013) reported DPPH radical– and ABTS radical–scavenging activities of gelatin films incorporated with BEO. The antioxidant activities of the essential oils have been attributed to the redox properties, ability to scavenge a variety of reactive species such as superoxide, hydroxyl and peroxyl radicals and hypochlorous acid, singlet oxygen quenching, metal ion chelation and decomposition of peroxides (Perumalla and Hettiarachchy 2011). During the refrigerated storage, psychrotrophic bacteria, mainly *Pseudomonas* spp., produce lipase and

phospholipase, causing an increase in free fatty acids (Koka and Weimer 2001). These free fatty acids are highly vulnerable to oxidation and form unstable lipid hydroperoxide. This hydroperoxide is readily decomposed to shorter chain products such as aldehydes, which can be detected as TBARS (Benjakul et al. 2005). Lowered lipid oxidation was in agreement with the lower microbial growth of sea bass slices wrapped with different films (Fig. 1).

**Table 1** Sensory properties of sea bass slices wrapped without and with films (PP film, FPI/FSG film, FPI/FSG-ZnONP film, FPI/FSG-BEO film and FPI/FSG-ZnONP-BEO film) during storage at 4 °C for 12 days

| Sensory attributes | Treatments | Storage (days) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 | 12 |
| Texture | Control | 5±0.00aA | 5±0.00aA | 4.6±0.14aB | 4.1±0.09cC | 3.66±0.13bD | 3.0±0.13dE | 2.06±0.14eF |
| | PP film | 5±0.00aA | 4.75±0.22aB | 4.7±0.12aB | 4.45±0.11bC | 4.13±0.12aD | 3.53±0.16cE | 2.88±0.09dF |
| | FPI/FSG film | 5±0.00aA | 4.93±0.12aA | 4.76±0.08aAB | 4.56±0.09abB | 4.11±0.26aC | 3.85±0.23bC | 3.25±0.18cD |
| | FPI/FSG-ZnONP film | 5±0.00aA | 4.90±0.10aA | 4.8±0.13aA | 4.55±0.11bB | 4.17±0.21aC | 4.02±0.16abC | 3.62±0.08bD |
| | FPI/FSG-BEO film | 5±0.00aA | 4.85±0.12aAB | 4.7±0.16aB | 4.65±0.11abB | 4.24±0.14aC | 3.98±0.06abD | 3.79±0.13bD |
| | FPI/FSG-ZnONP-BEO film | 5±0.00aA | 4.82±0.16aA | 4.78±0.18aA | 4.76±0.14aA | 4.41±0.18aB | 4.18±0.11aBC | 4.05±0.10aC |
| Odour | Control | 5±0.00aA | 4.75±0.17aB | 4.31±0.12cC | 4.09±0.21cD | 2.06±0.04dE | 1.0±0.00dF | 1.0±0.00eF |
| | PP film | 5±0.00aA | 4.8±0.12aA | 4.43±0.12bcB | 4.22±0.17bcB | 3.63±0.31cC | 2.97±0.21cD | 2.48±0.18dE |
| | FPI/FSG film | 4.8±0.16aA | 4.61±0.10aAB | 4.50±0.08abB | 4.16±0.09bcC | 3.78±0.18bcD | 3.05±0.22cE | 2.67±0.15dF |
| | FPI/FSG-ZnONP film | 4.93±0.12aA | 4.65±0.09aB | 4.58±0.1abB | 4.37±0.14abC | 4.08±0.16abD | 3.82±0.12bE | 3.41±0.08cF |
| | FPI/FSG-BEO film | 4.8±0.21aA | 4.70±0.1aA | 4.58±0.03abAB | 4.38±0.11abB | 4.14±0.15aC | 3.96±0.14abCD | 3.74±0.13bD |
| | FPI/FSG-ZnONP-BEO film | 4.8±0.16aA | 4.75±0.09aA | 4.65±0.11aAB | 4.50±0.08aBC | 4.37±0.11aC | 4.19±0.07aD | 4.05±0.04aD |
| Colour | Control | 5±0.00aA | 4.65±0.13bB | 4.40±0.14cC | 4.1±0.04cD | 3.66±0.04eE | 2.40±0.18dF | 1.76±0.17fG |
| | PP film | 4.9±0.10aA | 4.8±0.08abA | 4.5±0.12abcB | 4.35±0.12bB | 3.90±0.12dC | 3.33±0.12cD | 2.78±0.13eE |
| | FPI/FSG film | 5.0±0.00aA | 4.8±0.05abB | 4.45±0.08bcC | 4.30±0.08bD | 4.1±0.08cdE | 3.45±0.08cF | 3.05±0.09dG |
| | FPI/FSG-ZnONP film | 4.9±0.10aA | 4.85±0.09abA | 4.6±0.14abcB | 4.55±0.14aB | 4.27±0.14bcC | 3.92±0.14bD | 3.42±0.16cE |
| | FPI/FSG-BEO film | 4.9±0.10aA | 4.8±0.16abAB | 4.65±0.12abB | 4.6±0.06aB | 4.34±0.13bC | 4.13±0.16abC | 3.81±0.15bD |
| | FPI/FSG-ZnONP-BEO film | 5.0±0.00aA | 4.9±0.07aA | 4.7±0.07aB | 4.6±0.13aB | 4.55±0.11aB | 4.30±0.12aC | 4.10±0.11aD |
| Overall | Control | 5±0.00aA | 4.6±0.12bB | 4.30±0.10cC | 4.05±0.04cD | 3.16±0.05dE | 1.85±0.15dF | 1.0±0.00dG |
| | PP film | 5±0.00aA | 4.70±0.09abB | 4.40±0.06bcC | 4.15±0.12cD | 3.53±0.12cE | 2.75±0.10cF | 2.18±0.18cG |
| | FPI/FSG film | 4.9±0.15aA | 4.65±0.08abB | 4.40±0.08bcC | 4.06±0.08cD | 3.48±0.06cE | 2.68±0.12cF | 2.17±0.15cG |
| | FPI/FSG-ZnONP film | 4.9±0.10aA | 4.8±0.10aA | 4.50±0.12bB | 4.37±0.12bB | 4.09±0.14bC | 3.72±0.16bD | 3.41±0.10bE |
| | FPI/FSG-BEO film | 5.0±0.00aA | 4.8±0.08aB | 4.50±0.10bC | 4.40±0.08bC | 4.21±0.10abD | 4.08±0.06aD | 3.86±0.10aE |
| | FPI/FSG-ZnONP-BEO film | 4.9±0.10aA | 4.8±0.11aAB | 4.70±0.08aBC | 4.60±0.10aC | 4.37±0.09aD | 4.19±0.08aE | 4.03±0.05aF |

Different lowercase letters in the same column within the same sensory attribute indicate significant differences ($P<0.05$)

Different uppercase letters in the same row indicate significant differences ($P<0.05$)

Control: unwrapped samples, PP film: samples wrapped with Polypropylene film, FPI/FSG film: samples wrapped with fish protein isolate/fish skin gelatin film, FPI/FSG-ZnONP film: samples wrapped with fish protein isolate/fish skin gelatin film incorporated with zinc oxide nanoparticles, FPI/FSG-BEO film: samples wrapped with fish protein isolate/fish skin gelatin film incorporated with basil leaf essential oil, FPI/FSG-ZnONP-BEO film: samples wrapped with fish protein isolate/fish skin gelatin film incorporated with zinc oxide nanoparticles and basil leaf essential oil

Sensory evaluation

Sensory properties of sea bass slices wrapped without (control) and with different films during refrigerated storage are given in Table 1. The fish samples were considered to be acceptable for human consumption until the sensory score reached 4 (Ojagh et al. 2010). The score for texture, odor, color and overall quality decreased ($P<0.05$) over the storage of 12 days. Texture, odour, colour and overall quality of control samples were given 'unacceptable' scores by the 8th day. Due to high microbial growth and lipid oxidation, the control samples (unwrapped) of sea bass slices showed spoilage as evidenced by the formation of slime and off-odour with discolouration after 6 days storage. However, the samples wrapped with the films especially FPI/FSG-ZnO-BEO possessed the higher scores than others. The score below critical score of 4 (overall quality) was obtained at day 10 and 12 for sea bass slices wrapped with FPI/FSG-ZnONP and FPI/FSG-BEO, respectively. However, the score higher than 4 was found for the sample wrapped with FPI/FSG-ZnONP-BEO after 12 days of storage. Based on sensory analysis, the samples wrapped with FPI/FSG-ZnO-BEO film had the longer shelf-life by 6 days, as compared to the control. The antioxidant, antimicrobial and gas barrier effects of films, especially FPI/FSG-ZnO-BEO, could minimise the oxidative effects, thereby prolonging the shelf-life, whilst maintaining quality. Adding ZnONP and BEO to FPI/FSG film provided the beneficial effects on colour, odour and overall quality of the refrigerated sea bass slices over a period of 12 days. The results were in good agreement with those reported by Mahmoud et al. (2004) for carp treated with carvacrol–thymol solution (1 %, v/v) stored at 5 °C. An extension of 8 days based on odour and taste evaluation was reported for the carp treated with the essential oil mixture. Mejlholm and Dalgaard (2002) also reported that the use of oregano oil (0.05 %, v/w) extended the shelf-life of cod from 11–12 days to 21–26 days. It is noteworthy that the presence of BEO in FPI/FSG-BEO and FPI/FSG-ZnO-BEO did not show the detrimental effect on odour of sea bass slices.

## Conclusion

The active nanocomposite films based on FPI/FSG incorporated with ZnONP and BEO could retard microbial growth and lipid oxidation in refrigerated sea bass slices more effectively than PP, FPI/FSG, FPI/FSG-ZnONP and FPI/FSG-BEO films. The observed shelf-life of sea bass were found to be 4 days in air (without wrapping), 5 days with PP film, 6 days with FPI/FSG film and 10 days with FPI/FSG-ZnONP. FPI/FSG-BEO or FPI/FSG-ZnONP-BEO film could extend shelf-life of sea bass slice up to 12 days at refrigerated temperature, which was 8 days longer than the control. However, sea bass wrapped with FPI/FSG-ZnONP-BEO film showed a higher quality. Thus, FPI/FSG-ZnONP-BEO film could be used as an active packaging to maintain quality and extend shelf-life of sea bass slices at 4 °C.

**Acknowledgments** The authors would like to express their sincere thanks to Prince of Songkla University and the TRF Distinguished Research Professor Grant for the financial support.

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