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Active packaging films based on chitosan and *Herba lophatheri* extract for the shelf life extension of fried bighead carp fillets

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Abstract

The effect of chitosan film incorporated with *Herba lophatheri* extract (HLE) on the quality of fried bighead carp (FBC) during refrigerated storage (4 ± 1°C) for 28 d was evaluated. The FBC wrapped in chitosan film with HLE showed fewer changes, when compared with the FBC wrapped in a pure chitosan film. At the end of the storage period, the moisture content and the water activity of the FBC wrapped in the chitosan film with HLE were 20.03% and 2.51% higher than those of the control, respectively. The peroxide value, thiobarbituric acid reactive substances, total viable count, and yeast and mould count of the chitosan film with HLE were 47.05, 61.82, 68.49, and 77.12% lower than those of the control, respectively. The best film was the chitosan film with 5% HLE, which extended the shelf life of the FBC by 14 d (50%) as compared to control.

Introduction

The use of petrochemical-based plastic raises food safety concerns as well as environmental and resource issues (Liang *et al.,* 2017), hence creating a wide research interest in biodegradable films based on polysaccharides, proteins, or lipids (Hedayatnia *et al.,* 2019). Biobased films are placed on the surface of food and can reduce food oxidation and spoilage during transportation, marketing, and storage (Zhou *et al.,* 2010).

Chitosan is a natural carbohydrate copolymer [β-(1-4)-2-acetamido-α-glucose and β-(1-4)-2-amino-α-glucose units] which is obtained from the deacetylation of chitin. Chitosan exhibits film-forming properties, antimicrobial activity, biocompatibility, and biodegradability (Wang *et al.,* 2013). There are many studies related to chitosan. Vacuum tumbling with a chitosan solution can deter the proliferation of aerobic bacteria in catfish fillets under refrigerated storage (Bonilla *et al.,* 2018). The chitosan film incorporated with thinned young apple polyphenols extends the shelf life of grass carp fillets during cold storage (Sun *et al.,* 2018). Chitosan-whey protein isolated coatings incorporated with tarragon (*Artemisia dracunculus*) essential oil could improve the deterioration of *Scomberoides commersonnianus* fillets under refrigerated conditions (Farsanipour *et al.,* 2020). Ice-glazing based on chitosan-gelatine incorporated with Persian lime peel essential oil effectively enhanced the quality and shelf life of rainbow trout fillets under superchilled conditions (Sarmast *et al.,* 2019). The chitosan-*Ferulago angulata* essential oil nano-emulsion extends the shelf life of rainbow trout fillets stored at 4°C (Shokri *et al.,* 2020).

Herba lophatheri extract (HLE) is obtained from *H. lophatheri*, which is also called "dan zhu ye" in Chinese (Ge *et al.,* 2013). *H. lophatheri* is a traditional medicine rich in active components (*e.g.*, polysaccharides, phenolic acids, flavonoids, specific amino acids, aromatic substances alkaloids, and mineral elements) (Ge *et al.,* 2013; Wang *et al.,* 2019a). Scientific research shows that HLE has antioxidant, antibacterial, antitumour, and antipyretic properties (Tang *et al.,* 2015; Kim *et al.,* 2016). Thus, HLE shows high potential for application in food preservation.

Bighead carp (*Aristichthys nobilis*) is rich in protein, fatty acids, vitamins, and trace elements, and widely consumed in China (Hong *et al.,* 2012; Zhuang *et al.,* 2019). Fried bighead carp (FBC) is a ready-to-eat meat product, and vulnerable to oxidation and rancidity. Therefore, appropriate packaging is crucial for the safety and quality of FBC, which can delay colour changes in FBC and lipid oxidation, prevent bacterial growth, and extend the shelf life of FBC. The sodium alginate coating enriched with horsemint essential oil extends the shelf life of bighead carp fillets during storage at 4°C (Heydari *et al.,* 2015), and the carboxymethyl cellulose coating with *Anethum graveolens* extract can improve the organoleptic properties and oxidation of FBC fillets (Sarmast *et al.,* 2019).

The physicochemical properties and

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biological activities of the chitosan film enriched with HLE were evaluated in our previous work (Wang *et al.,* 2019a). However, the effect of the practical application of this type of film has yet to be determined. Therefore, the present work aimed to evaluate the effect of the chitosan film incorporated with HLE (chitosan-HLE films) as an active packaging film on peroxide value (PV), thiobarbituric acid reactive substances (TBARS), microorganisms, moisture content, and water activity of FBC.

Materials and methods

Materials

Chitosan and HLE were provided by Sinopharm Chemical Reagent and Xi'an Huilin Biotechnology Co. Ltd. (China), respectively. The HLE consisted of light-yellow powder, and the flavonoid content was 40.24%. Soybean oil and bighead carp (protein, 20.25%; fat, 1.87%; moisture, 72.52%; and ash, 1.38%) were purchased from a local market in Changchun (China). Acetic acid, sodium chloride, potassium nitrate, sodium thiosulphate, potassium iodide, and chloroform were supplied by Beijing Beihua Co. Ltd. (China).

Preparation of films and fried bighead carp fillets (FBC)

The films were prepared as described in our previous work (Wang *et al.,* 2019a). The chitosan solution (2 wt%) was mixed with the HLE solutions at a ratio of 1:1. The mixture was then ultrasonically treated for 20 min. After 12 h, the mixture was distributed into Petri dishes for drying at 60°C for 24 h. The peeled films were kept in a chamber at 75% RH for 48 h before use.

The meat of the bighead carp was cut into fillets (4 × 2 × 2 cm), marinated with salt (2%) and sugar (1%) for 20 min, and fried in soybean oil at 130°C for 6 min to obtain the FBC, which were then randomly assigned into six treatments.

The FBC was wrapped in the chitosan films with HLE at different concentrations (0, 0.1, 0.5, 1, 3, and 5%) and were assigned as the control, HLE0.1, HLE0.5, HLE1, HLE3, and HLE5, respectively. Each sample was individually packed in a low-density polyethylene bag under atmospheric conditions, and heat-sealed using an impulse sealer. All samples were stored at 4 ± 1°C for 28 d, and analysed on days 0, 7, 14, 21, and 28. All measurements were conducted on three separate samples.

Peroxide value

Hydroperoxide measurement was conducted as described in the study by Zhao *et al.* (2018) to determine the peroxide value (PV). The lipid sample (3 g) was treated with 30 mL of an organic solvent mixture (acetic acid:chloroform, 3:2). The mixture was shaken vigorously. Subsequently, 1 mL of saturated potassium iodide solution was added into the mixture, which was kept in darkness for 3 min. About 100 mL of distilled water was added to the mixture, which was again shaken; and 1 mL of starch solution (1% w/v) as an indicator was added into the mixture. PV was determined by titrating the iodine liberated from potassium iodide using a standardised 0.01 mol/L sodium thiosulfate solution. PV was reported in meq O_2/kg of lipid and calculated using Eq. 1:

$$PV = (V\text{-}V_0) \times C \times 1000 \div 2 \div M \quad \text{(Eq. 1)}$$

where, V and V_0 = volumes of the sodium thiosulfate solution consumed in the sample and blank test (mL), respectively; C = concentration of the sodium thiosulfate solution (mol/L); and M = weight of the lipid sample (g).

Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) were measured as described in the study by Cao *et al.* (2019) with slight modification. Briefly, the sample (5 g) and trichloroacetic acid solution (50 mL) were placed in an oscillator at 50°C for 30 min, and filtered through a filter paper. The filtrate (5 mL) was added to 20 mM thiobarbituric acid (5 mL) and then incubated in a water bath set to 95°C for 30 min. The mixture was allowed to cool, and the absorbance was measured at 532 nm by using a spectrophotometer (Ruili Analytical Instrument Company, Beijing, China). The TBARS values were expressed in mg of malondialdehyde (MDA) per kg of the FBC by using 1,1,3,3-tetraethoxypropane as a standard.

Microbiological analysis

Different microorganisms were measured using the spread plate method on different growth media (Sun *et al.,* 2019). Briefly, the FBC fillets (25 g) were homogenised with 225 mL of sterile 0.1% peptone buffer for 2 min by using a stomacher (Seward Laboratory, London, UK). Serial decimal dilutions were then performed. Each dilution factor was placed (1 mL) onto the surface of the plate count agar and coated evenly. In accordance with China National Standard (GB4789.15-2016), total viable count were determined using the plate count agar and incubated at 37°C for 48 h, and the yeast and mould count was determined using the potato dextrose agar and incubated at 25°C for 72 h. The total number of

microorganisms were then calculated. All counts were expressed as log CFU/g.

Moisture content

The moisture content of the FBC fillets was determined using a moisture meter (HB43-S, Mettler Toledo, Switzerland).

Water activity

The water activity of the FBC fillets was determined using a HygroLab 2 four-channel desktop water activity meter (Rotronic, Switzerland) in accordance with the method applied by Wang *et al.* (2019b).

Statistical analysis

Differences between factors and levels were determined using ANOVA. All data were expressed as mean ± standard deviation.

Results and discussion

Peroxide value

Changes in the PV of the FBC are presented in Figure 1. The initial PV of the FBC was 3.61 meq O_2/kg of lipid which was higher than that of Beluga sturgeon fillets studied by Gharibzahedi and Mohammadnabi (2017), because the bighead carp was fried in the present work. The change in PV was not apparent in the first 7 d because the interval between sample processing and PV analysis was too short to show the possible effects of antioxidants on oxidative stability (Serrano-León *et al.,* 2018). The PV of the samples in all treatments significantly increased ($p < 0.05$) after storage for 7 d. The PV of the control consistently exhibited the highest production of hydroperoxides, reaching the maximum at the end of storage. Moreover, the FBC wrapped in chitosan-HLE films with 0.1 - 5% concentrations of HLE showed PVs that were 15.04 - 47.05% lower than that of the control on day 28. The higher the concentration of HLE, the lower the PV. The lowest PV was observed in the FBC wrapped in chitosan-HLE films at 5% concentration. Under this treatment, the PV concentrations were consistently the lowest among the treatments at any observation time. HLE delayed the formation of primary oxidation products in the FBC during refrigerated storage. The main active compound in HLE was flavonoid, which plays an important antioxidant role (Wang *et al.,* 2019a). Heydari *et al.* (2015) also reported a lower PV content in the bighead carp fillets treated with a sodium alginate coating enriched with horsemint. Similarly, Shahosseini *et al.* (2019) found a lower PV content in fried bighead carp treated using a carboxymethyl cellulose coating with *Anethum graveolens* extract. The incorporation of thinned young apple polyphenols into chitosan could also delay the increase in PV in grass carp fillets during storage at 4°C for 8 d (Sun *et al.,* 2018). Alsaggaf *et al.* (2017) reported that fungal chitosan incorporated with pomegranate peel extract as an edible coating could inhibit the increase in PV in Nile tilapia fillets.

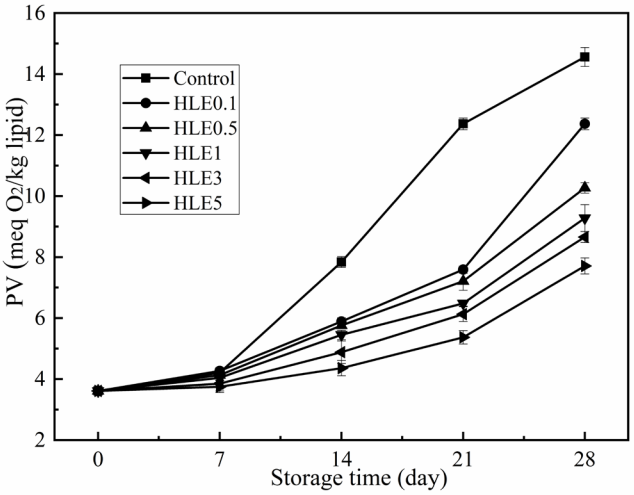

Figure 1. Peroxide values (PV) of fried bighead carp wrapped with chitosan-HLE films during 28-day storage at 4 ± 1°C.

Thiobarbituric acid reactive substances

Changes in TBARS in the FBC during refrigerated storage are presented in Figure 2. The initial TBARS of the FBC was 0.155 mg MDA/kg, which is similar to that of the catfish fillet in the study by Bonilla *et al.* (2018). The change trend in TBARS was generally similar to the change trend in the PV of FBC. The samples under all treatments showed significant increases ($p < 0.05$) in TBARS over the entire storage period. In addition, the TBARS levels of the FBC wrapped in chitosan-HLE films were 40.93 - 61.82% lower than that of the control at the end of storage. The lowest TBARS level was detected in the FBC packed in chitosan-HLE films at 5% concentration. Under this treatment, the TBARS concentrations were consistently the lowest at any observation time. The result showed that the incorporation of HLE into the chitosan film could delay the formation of secondary oxidation products in the FBC during refrigerated storage, indicating that chitosan-HLE films could be used as antioxidant active packaging to protect fish against lipid oxidation. The inhibitory effect of HLE on lipid oxidation was related to its flavonoid, constituents that mainly contributed to antioxidant activity (Wang *et al.,* 2019a). The incorporation of horsemint into sodium alginate coating could also

delay the increase in TBARS in bighead carp fillets during storage at 4°C for 16 d (Heydari *et al.,* 2015). Sarmast *et al.* (2019) demonstrated that glazing based on chitosan-gelatine incorporated with Persian lime peel essential oil postponed the increase in TBARS in rainbow trout fillets stored at superchilled condition. The chitosan-*Ferulago angulata* essential oil nano-emulsion postponed the increase in TBARS in Rainbow trout fillets stored at 4°C (Shokri *et al.,* 2020). According to Farsanipour *et al.* (2020), chitosan-whey protein isolated coatings incorporated with tarragon essential oil could inhibit the increase in TBARS in *Scomberoides commersonnianus* fillets under refrigerated conditions.

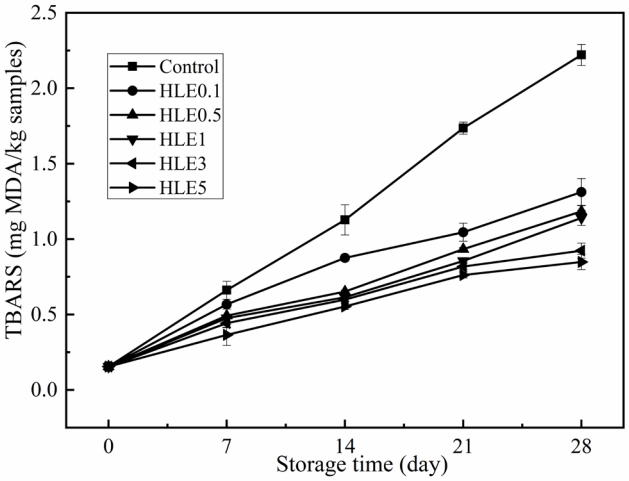

Figure 2. TBARS values of fried bighead carp wrapped with chitosan-HLE films during 28-day storage at 4 ± 1°C.

Microbiological analysis

Total viable count (TVC), and yeast and mould count of the FBC are shown in Table 1. These microorganisms are considered as spoilage microflora, and their presence could affect the organoleptic properties of food (Siripatrawan and Noipha, 2012). The initial TVC in the FBC was log 1.12 CFU/g, which was less than that in bighead carp fillets (Heydari *et al.,* 2015), indicating low bacterial loads in the FBC in the present work. The TVC significantly increased with time, particularly in the control group. Moreover, the TVC of the FBC wrapped in chitosan-HLE films were 8.79 - 68.49% lower than that of the control on day 28. The higher the concentration of HLE, the lower the TVC. The lowest TVC was found in the FBC packed in chitosan-HLE films

Table 1. Total viable count, and yeast and mould count of samples wrapped with chitosan-HLE films during 28-day storage at 4 ± 1°C.

Films	Storage time (day)				
	0	7	14	21	28
Total viable count (log CFU/g)					
Control	1.12 ± 0.09^{eA}	3.46 ± 0.29^{dA}	5.02 ± 0.29^{cA}	6.36 ± 0.14^{bA}	9.33 ± 0.18^{aA}
HLE0.1	1.12 ± 0.09^{eA}	2.93 ± 0.16^{dA}	4.84 ± 0.20^{cA}	5.46 ± 0.20^{bB}	8.51 ± 0.26^{aB}
HLE0.5	1.12 ± 0.09^{eA}	1.85 ± 0.16^{dB}	2.98 ± 0.13^{cB}	3.77 ± 0.15^{bC}	6.07 ± 0.09^{aC}
HLE1	1.12 ± 0.09^{dA}	1.23 ± 0.13^{dC}	1.90 ± 0.15^{cC}	2.52 ± 0.10^{bD}	4.54 ± 0.18^{aD}
HLE3	1.12 ± 0.09^{dA}	1.21 ± 0.05^{cdC}	1.36 ± 0.08^{cD}	2.22 ± 0.20^{bD}	4.26 ± 0.14^{aD}
HLE5	1.12 ± 0.09^{cA}	1.14 ± 0.14^{cC}	1.28 ± 0.08^{cD}	2.14 ± 0.19^{bD}	2.94 ± 0.21^{aF}
Yeast and mould count (log CFU/g)					
Control	ND	1.29 ± 0.03^{dA}	4.58 ± 0.14^{cA}	6.99 ± 0.31^{bA}	11.67 ± 0.26^{aA}
HLE0.1	ND	1.08 ± 0.06^{dB}	2.76 ± 0.11^{cB}	6.14 ± 0.43^{bA}	9.62 ± 0.52^{aB}
HLE0.5	ND	0.98 ± 0.02^{dC}	2.51 ± 0.09^{cC}	4.45 ± 0.14^{bB}	9.25 ± 0.36^{aB}
HLE1	ND	0.85 ± 0.01^{dD}	2.33 ± 0.12^{cC}	3.85 ± 0.09^{bC}	6.16 ± 0.19^{aC}
HLE3	ND	0.67 ± 0.04^{dE}	2.08 ± 0.16^{cC}	2.43 ± 0.15^{bD}	4.27 ± 0.71^{aD}
HLE5	ND	0.48 ± 0.01^{dF}	1.39 ± 0.21^{cD}	1.96 ± 0.19^{bE}	2.67 ± 0.28^{aE}

Values are means ± standard deviations. Means with different lowercase superscripts within a row, and means with different uppercase superscripts within a column indicate significant differences ($p < 0.05$).

at 5% concentration. Under this treatment, the TVC concentrations were consistently the lowest at any observation time. It could be inferred that HLE was a superior antibacterial agent and could inhibit the growth of microorganisms in the FBC during refrigerated storage (Tang *et al.,* 2015; Wang *et al.,* 2019a). TVC below log 4 CFU/g was acceptable in accordance with China National Standard (GB2726-2016), and this limit was exceeded between days 7 and 14 in the control group and the HLE0.1 group, as well as between days 21 and 28 in the HLE0.5, HLE1, and HLE3 groups. However, HLE5 maintained TVC values below the limit during the entire experiment, which proved that the chitosan-HLE films could effectively control the TVC of the FBC stored at 4°C.

The increased population of yeasts and moulds may cause the formation of slime (Siripatrawan and Noipha, 2012), indicating the need to inhibit their growth. Initially, neither yeasts nor moulds were detected in the FBC. The reason was that the products were cooked at high temperatures before storage. Subsequently, the growth of yeasts and moulds in the FBC significantly ($p < 0.05$) increased with time. On days 7, 14, 21, and 28, the growth of yeasts and moulds in the samples wrapped in chitosan-HLE films was lower than that in the control because HLE could control microbial growth (Tang *et al.,* 2015; Kim *et al.,* 2016). The higher the concentration of HLE, the higher the inhibition of yeasts and moulds. The yeasts and moulds in the FBC wrapped in chitosan-HLE films groups were 17.57 - 77.12% lower than those in the control group at the end of storage.

The result suggested that the incorporation of chitosan film with HLE could inhibit microbial growth in the FBC. HLE was known to act as an antimicrobial agent with luteolin and its glycoside as its effective constituent (Tang *et al.,* 2015; Wang *et al.,* 2019a). However, the mechanism underlying the antimicrobial action of HLE has yet to be elucidated.

Moisture content

As illustrated in Figure 3, the initial moisture content of the FBC was lower than the result observed by Duan *et al.* (2010) in fresh and frozen lingcod fillets, given that the bighead carp in the present work partly losed water while being cooked. The moisture content of the samples significantly decreased ($p < 0.05$) regardless of the treatment as storage time was extended. This downward trend was similar to that observed in the double-filleted Indian oil sardine studied by Mohan *et al.* (2012). The moisture contents of the FBC wrapped in chitosan-HLE films were consistently higher than that of the control, suggesting that HLE could reduce water loss in the FBC. At the end of storage, the moisture content of the FBC wrapped in chitosan-HLE films was 5.12 - 20.03% higher than that of the control. The higher the concentration of HLE, the lower the water loss. Our previous work reported that the degree of swelling of the pure chitosan film was markedly higher than those of chitosan-HLE films, indicating that the ability of pure chitosan film to absorb water was higher than that of chitosan-HLE films. The reason is that HLE promoted the interactions with the polar groups in the film, resulting in fewer polar groups that were accessible to water molecules (Wang *et al.,* 2019a). Thus, the pure chitosan film might have absorbed more water from the FBC than the chitosan-HLE films, resulting in a decrease in the moisture content of the FBC.

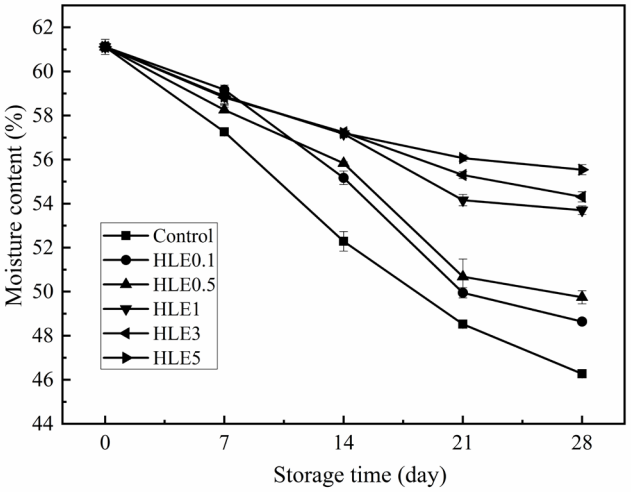

Figure 3. Moisture content of fried bighead carp wrapped with chitosan-HLE films during 28-day storage at 4 ± 1°C.

Water activity

Water activity plays an important role in the safety and stability of products with respect to physical properties, microbial growth, and chemical/biochemical reaction rates (Polatoğlu *et al.,* 2011). Changes in the water activity of the FBC are shown in Table 2. The initial water activity of the FBC was 0.95 a_w, which was similar to that of *Cololabis saira* in the study by Bian *et al.* (2017). As storage time increased, the water activity of the FBC slightly decreased regardless of the treatment. On days 0, 7, 14, and 21, no significant differences in water activity were observed between the samples. However, the water activity of the FBC wrapped in chitosan-HLE films was significantly ($p < 0.05$) higher (1.85 - 2.51%) than that of the FBC wrapped in chitosan film only on day 28, suggesting that HLE could prevent the decrease in the water activity of the FBC.

Table 2. Water activity of samples wrapped with chitosan-HLE films during 28-day storage at 4 ± 1°C.

Films	Storage time (days)				
	0	7	14	21	28
Control	0.950 ± 0.01^{aA}	0.946 ± 0.01^{aA}	0.946 ± 0.00^{aA}	0.934 ± 0.01^{abAB}	0.918 ± 0.02^{bB}
HLE0.1	0.950 ± 0.01^{aA}	0.949 ± 0.01^{aA}	0.945 ± 0.00^{aA}	0.936 ± 0.01^{aA}	0.935 ± 0.01^{aA}
HLE0.5	0.950 ± 0.01^{aA}	0.950 ± 0.00^{aA}	0.937 ± 0.00^{aA}	0.937 ± 0.01^{aA}	0.936 ± 0.01^{aA}
HLE1	0.950 ± 0.01^{aA}	0.952 ± 0.01^{aA}	0.943 ± 0.00^{aA}	0.938 ± 0.01^{aA}	0.941 ± 0.00^{aA}
HLE3	0.950 ± 0.01^{aA}	0.949 ± 0.00^{aA}	0.942 ± 0.01^{aA}	0.942 ± 0.01^{aA}	0.936 ± 0.01^{aA}
HLE5	0.950 ± 0.01^{aA}	0.948 ± 0.01^{aA}	0.943 ± 0.01^{aA}	0.941 ± 0.01^{aA}	0.941 ± 0.01^{aA}

Values are means ± standard deviations. Means with different lowercase superscripts within a row, and means with different uppercase superscripts within a column indicate significant differences ($p < 0.05$).

Conclusions

The present work demonstrated that chitosan film incorporated with *Herba lophatheri* extract (HLE) could maintain the physical and organoleptic qualities of the fried bighead carp (FBC) by delaying the lipid oxidation and microbial growth during refrigerated storage at 4 ± 1°C. The best film was the chitosan film with 5% HLE, which extended the shelf life of the FBC by 14 d as compared to control. Thus, the active packaging film consisting of chitosan incorporated with HLE can be potentially used as a film to protect the quality and safety of fish products.

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