PRESERVATION OF COMMERCIAL FISH BALL QUALITY WITH EDIBLE ANTIOXIDANT-INCORPORATED ZEIN COATINGS

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ABSTRACT

*Fish ball, a surimi product rich in lipid and protein, is a popular food in Taiwan. Because lipid oxidation is one of the major deterioration reactions for fish ball, the feasibility of preservation of fish ball quality by the application of antioxidant-incorporated zein coating was investigated. Three antioxidants including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and n-propyl gallate (PG) were used to formulate the antioxidant zein coatings. Infrared spectroscopy was used to confirm the successful incorporation of antioxidant with zein protein; peroxide value (*POV*), thiobarbituric acid reactive substance (TBARS) and weight loss were used as the quality indicators of fish ball stored at 4C. While all three types of antioxidant-incorporated zein coatings significantly retarded the quality deterioration, PG-incorporated zein coating exerted better quality preservation effectiveness than BHA- and BHT-incorporated zein coatings.*

PRACTICAL APPLICATIONS

Edible coatings have been under research for several decades. However, most of the studies are conducted for the investigations of physiochemical or mechanical properties and usually using simulated food systems. The lack of applications on the commercial food products manufactured from food plants makes the edible coatings somewhat unrealistic. Not prepared in a laboratory for academic purpose only, the fish ball used in the present study was a real

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commercial product. The promising results of antioxidant-incorporated zein coatings on commercial products presented in this report will enhance the confidence of food manufacturers on the edible coatings.

INTRODUCTION

Active packaging can offer functionalities that are not provided by conventional packaging techniques (Dawson *et al.* 2003). Because of the biodegradability and compatibility with food ingredients, various biopolymeric materials originating from microorganisms, plants or animals, such as polysaccharides (gums, starches, cellulose, chitosan, etc.) or proteins (gelatin, soy protein, whey protein, zein, etc.), are used as the base materials for edible coatings or films in the development of active food packaging materials. Edible antioxidative or antimicrobial packaging materials are among the most commonly fabricated active packaging materials. Moreover, it is generally believed that oxidation and microbial growth begin in the food surfaces. Incorporation of additives in edible coatings concentrates the additive on the food surface and markedly reduces the amount of food additives when directly added to the entire food (Torres and Karel 1985; Chung *et al.* 2001; Kleen *et al.* 2002).

Zein, comprising 45–50% of total corn protein, is the major storage protein of corn. Zein has been used as edible coating or film in many applications because zein exerts tough, glossy, hydrophobic grease-proof characteristics (Shukla and Cheryan 2001; Hoa *et al.* 2002). With increasing demands for multifunctional properties for edible packaging materials, the functionality of zein coatings or films can be enhanced by incorporating various active ingredients such as antioxidants or antimicrobials (Torres and Karel 1985; Herald *et al.* 1996; Padgett *et al.* 2000; Carlin *et al.* 2001; Hoffman *et al.* 2001; Dawson *et al.* 2003; Mecitoglu *et al.* 2006; Güçbilmez *et al.* 2007).

Although research of antioxidant-incorporated zein have been found in the literature, results of its effectiveness are commonly demonstrated on the food-simulating model system (Kleen *et al.* 2002; Güçbilmez *et al.* 2007). Oleic acid at the level of 40–50% was incorporated with zein to plasticize the resulting biodegradable films (Kleen *et al.* 2002). The peroxide formation during accelerated UV storage was prevented by the addition of 4,000 ppm butylated hydroxyanisole (BHA). The results demonstrate that antioxidants in edible film could retard oxidative deterioration. Güçbilmez *et al.* (2007) prepared antimicrobial and antioxidant zein films by incorporating lysozyme, albumin protein and disodium ethylenediaminetetraacetic acid (EDTA) into zein films, and then the antioxidant activities of the zein films as measured by 2,2′azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) ($ABTS^{+}$) free radical

scavenging in phosphate buffer saline solution were demonstrated. Because the practical applications of zein films or coatings on commercial food products are scarcely reported, it is believed that the applications of edible coatings on food commodities actually manufactured by food plants will effectively promote the value of edible active packaging materials.

Fish ball is a favorable surimi product in Taiwan because of its relative low cost, simple manufacturing process, high nutrition value and versatile applications in culinary dishes as an important ingredient. Fish ball is highly perishable because it is made of fish meat, which is usually high in protein and fat contents. Moreover, unsaturated fatty acid content in fishery products is usually higher than in other types of animal products. Although they have better nutritional value, unsaturated fatty acids are more easily oxidized than saturated fatty acids. In order to retain the nutrition value as well as the sensory quality of fishery products, the oxidation reaction must be appropriately retarded. During the early stage of the oxidation process, unsaturated fatty acid will react with oxygen to form peroxides, such as hydroperoxide, which are usually defined as the primary oxidation products. Unstable peroxides will further degrade to secondary oxidation products including alcohols, ketones, aldehydes and acids. Therefore, peroxide value (*POV*) is often used as the quality indicator for lipid oxidation in food systems (Stenenson *et al.* 1984; Kleen *et al.* 2002). In contrast to *POV*, the content of thiobarbituric acid reactive substances (TBARS) is used as the indicator for the later stage of lipid oxidation. TBARS was used to demonstrate the antioxidative activity of green tea extract-incorporated zein films applied on the fried fish paste products (Lee *et al.* 2004). Thus, *POV* and TBARS are appropriate indicators for the quality of fish ball that contains high levels of lipid.

In this report, the oxidative deterioration of the commercially produced fish ball was retarded by coating with antioxidant-incorporated zein protein. Because each antioxidant possesses different physical and chemical characteristics, the antioxidative effectiveness will be different when a different antioxidant is used in zein film formulation. In order to select the most appropriate antioxidant to inhibit the oxidation reaction of fish ball, the antioxidative efficiency of three commonly used legal antioxidants, i.e., BHA, butylated hydroxytoluene (BHT) and n-propyl gallate (PG), was compared in the present study.

MATERIALS AND METHODS

Proximate Analysis of Fish Ball

Freshly manufactured fish balls (about 10 g for each individual fish ball) made of cod surimi were purchased from a local fishery product factory on the

day of experiment. Moisture, crude protein, ash, crude fat and crude fiber contents of the fish balls were determined according to AOAC 15.950.02, 15.976.05, 15.976.03, 15.920.39 and 15.962.09 (AOAC 1990), respectively.

Preparation of Antioxidative Zein Films

Yellow-colored zein of maize was purchased from Sigma-Aldrich (St. Louis, MO). To obtain the defatted zein, zein and n-hexane (Mallinckrodt Backer, Phillipsburg, NJ) (1:10, w/v) were mixed at room temperature for 2 h, and then were filtrated. The filtrate cake was transferred to glass Petri dishes (diameter of 15 cm) and was evenly spread to facilitate hexane removal. The Petri dishes were placed in a chemical ventilation hood for 2–3 h to allow the solvent to evaporate, and then the residue hexane was further removed by placing the Petri dishes in a vacuum oven (50C) overnight.

BHA, BHT and PG (Sigma Chemicals Co., Steinheim, Germany) were used as the antioxidants to prepare antioxidative zein films. The optimal ratio of defatted zein to propylene glycol (used as plasticizer) for zein film formation was determined in the preliminary tests. The antioxidant-incorporated zein film was prepared according to the method described by Padgett *et al.* (2000) with modifications. Defatted zein (5 g) was dispersed in 50 mL 95% ethanol (Taiwan Tobacco and Wine Co., Taipei, Taiwan). After adding propylene glycol (5 mL; Hayashi Pure Chemical, Osaka, Japan), 50 mg of antioxidant (BHA, BHT or PG) was mixed with the alcoholic zein solution. A portion of 2 mL zein solution was transferred into a polystyrene Petri dish (diameter of 9 cm). The Petri dishes were left in a chemical hood at room temperature to remove the solvent. After the solvent evaporated, the zein film was peeled off the plastic surface. Zein films without antioxidant were also prepared and used for comparison. The thickness of each zein film was randomly measured in at least five different points with a thickness micrometer (SM-114, Teclock Corporation, Nagano, Japan). Because the compositions were very similar for all types of film-forming mixtures and the preparation of zein films was carefully controlled, the thickness of zein films used in this study was relatively narrow with the thickness range of 0.075 ± 0.013 mm for all types of films.

Confirmation of Incorporation of Antioxidant with Zein

Zein films (with or without antioxidant) and the three antioxidants used in this study were characterized by the attenuated total reflectance/Fourier transform infrared (FTIR) spectroscopy. Film or solid antioxidant (in powder or granule form) was placed in a sample holder and the infrared (IR) spectrum (650–4,000 cm^{-1}, resolution 4 cm^{-1}, scanning 8) was obtained with an FTIR spectrometer (Spectrum 100 FTIR spectrometer, PerkinElmer, Beaconsfield, U.K.).

Preparation of Antioxidant-incorporated Zein-coated Fish Balls

The alcoholic zein/antioxidant solution was prepared as described earlier. The fish balls were submerged into the zein/antioxidant solution for 10–20 s with gentle rolling by chopsticks to ensure thorough covering of the zein solution. Then, the fish balls were individually stuck on a platform consisting of bamboo toothpicks and a thick foamed polystyrene board for about an hour to allow the solvent to evaporate. Each coated fish ball was weighed, placed individually in a plastic bag and stored at 4C for further analysis.

Determination of Fish Ball Quality

Before the fish balls were subjected to the following quality determinations, the weight of each fish ball was measured and used to calculate the weight loss during cold storage. The peroxide value (*POV*) of the fish balls was determined according to the methods described by Iwami *et al.* (1987) and Park *et al.* (2005) with modifications. Fish ball (5 g) and chloroform–methanol (25 mL; 2:1, v/v) were homogenized for 30 s and were filtrated through Whatman no. 1 filter paper (Whatman International Ltd. Maidstone, England). The filtrate (0.25 mL) was mixed with chloroform–methanol (4.55 mL; 2:1, v/v) for 2–4 s, and then 30% ammonium thiocyanate (0.1 mL) and 0.02 M iron (II) chloride tetrahydrate (0.1 mL) were added subsequently with brief mixing for 2–4 s between each addition. The resulting mixture was kept in room temperature for 5 min and then the absorbance at 500 nm was measured. The *POV* (meq/kg fish ball) was calculated against a calibration curve established with $FeCl_3$ standards.

The content of TBARS of the fish balls was determined according to the method described by Lemon (1975) with modifications. Fish ball (10 g) and extraction solution (20 mL, 7.5% trichloroacetic acid and 0.1% EDTA) were homogenized and filtrated (Whatman no. 1 filter paper). Filtrate (5 mL) and 0.02 M thiobarbituric acid solution (5 mL) were added into a screw-capped tube. The tubes were heated in a boiling water bath for 40 min and then cooled with running tap water to room temperature. The absorbance of the resulting mixture at 530 nm was measured and the TBARS was expressed as μmole malondialdehyde (MDA) per 100 g of fish ball after being calculated against a calibration curve established with 1,1,3,3-tetraethoxypropane (Sigma-Aldrich, St. Louis, MO) standards.

Statistical Analysis

Analysis of variance using the Statistical Analysis System software (SAS Institute Taiwan Ltd., Taipei, Taiwan) was conducted in a personal computer to determine if significant difference ($P < 0.05$) among treatments existed.

RESULTS AND DISCUSSION

Proximate Composition Analysis of Fish Ball, Zein and Defatted Zein

Inexpensive, simple manufacturing process and versatile cooking applications make fish ball a popular fishery product in Taiwan. In this study, fish ball was made of cod surimi, and the protein and fat contents of this type of fish ball were 40.67 and 13.84% (Table 1), respectively. The relatively high fat content (more than 10%) made fish ball subject to lipid oxidation, and in fact, lipid oxidation-related reactions (such as rancid flavor development) are among the major deteriorative causes of fish ball products. Thus, fish ball was chosen as the food model system to test the quality preservative capability of antioxidant-incorporated zein coatings.

As for the proximate composition of zein and defatted zein (Table 1), it is not surprising that the protein contents were greater than 90% (wet basis) for both types of zein (Shukla and Cheryan 2001). The defatting process showed the most noted change in fat contents. While the crude fat content of zein was 2.33%, the fat content of defatted zein was reduced to 0.54%. The degree of yellowness of zein was also markedly reduced after the defatting treatment. Hydrophobic compounds such as carotene, zeaxanthin and lutein are responsible for the yellowness of zein (Sessa *et al.* 2003). Slight differences were observed for the other components (crude protein, ash, crude fiber and nitrogen-free extract) between zein and defatted zein (Table 1).

TABLE 1.
PROXIMATE COMPOSITIONS OF FISH BALL, ZEIN AND DEFATTED ZEIN

Component	Content (%)*		
	Fish ball	Zein	Defatted zein
Moisture	42.14 ± 0.11	3.36 ± 0.03	3.20 ± 0.03
Crude fat	13.84 ± 0.44	2.33 ± 0.04	0.54 ± 0.01
Crude protein	40.67 ± 0.16	92.3 ± 50.12	93.75 ± 0.14
Ash	1.84 ± 0.01	1.24 ± 0.03	1.27 ± 0.02
Crude fiber	Trace‡	0.11 ± 0.01	0.13 ± 0.01
NFE†	1.41	0.61	1.11

* Data are presented as mean ± SE ($n = 3$).
† NFE = 100 – (moisture + crude fat + crude protein + ash + crude fiber).
‡ Less than 0.1%.
NFE, nitrogen-free extract.

Confirmation of Incorporation of Antioxidant with Zein

Because each specific functional group in the molecule exhibits the absorption at specific frequency in the IR spectrum, IR spectrometry is commonly used as a useful tool to identify molecular structure. Although a slight shift of absorption peaks might occur because of the microenvironmental interactions (Pavia *et al.* 2001), the resulting IR spectrum will show all the component absorption peaks when different materials are physically incorporated together. Figures 1–3 are the IR spectra that demonstrate the incorporation of BHA, BHT or PG into the zein film, respectively. Using Fig. 1 as an example, all major absorption peaks for BHA (top) and zein film (middle) appeared in the spectrum of BHA-incorporated zein film (bottom). Similar results for BHT and PG incorporation were observed in Figs. 2 and 3, respectively. The amide I band (around 1,644 cm^{-1}) of zein protein (Forato *et al.* 2004) and aromatic C=C stretch (between 1,409 and 1,537 cm^{-1}) of phenyl ring in antioxidants clearly appear in all of the spectra of antioxidant-incorporated zein films. However, the overlap of the absorption peaks might also cause some peaks to disappear in the combined spectrum, e.g., the independent O-H stretch peaks (3,452 and 3,324 cm^{-1} in Fig. 1) of antioxidants are not observed in the combined spectrum, but a broader and stronger peak (3,292 cm^{-1}) appears in the spectrum of antioxidant-incorporated zein film.

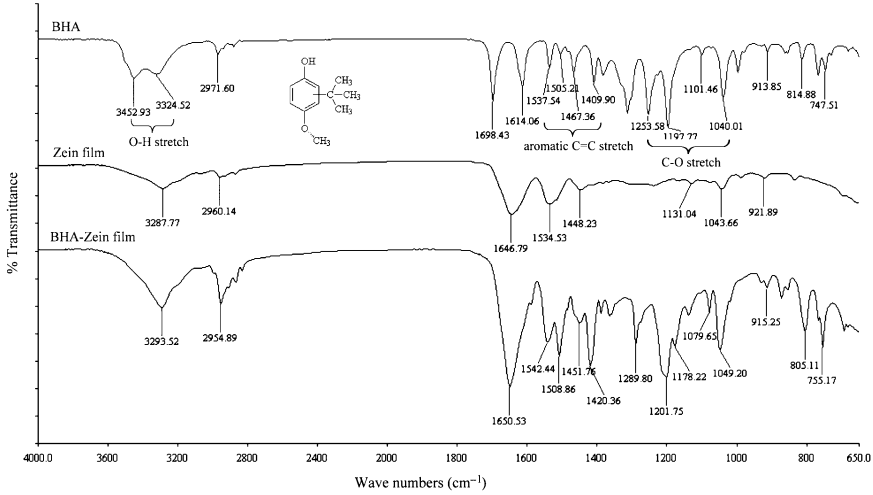

FIG. 1. INFRARED SPECTRA OF BUTYLATED HYDROXYANISOLE (BHA), ZEIN FILM AND BHA-INCORPORATED ZEIN FILM

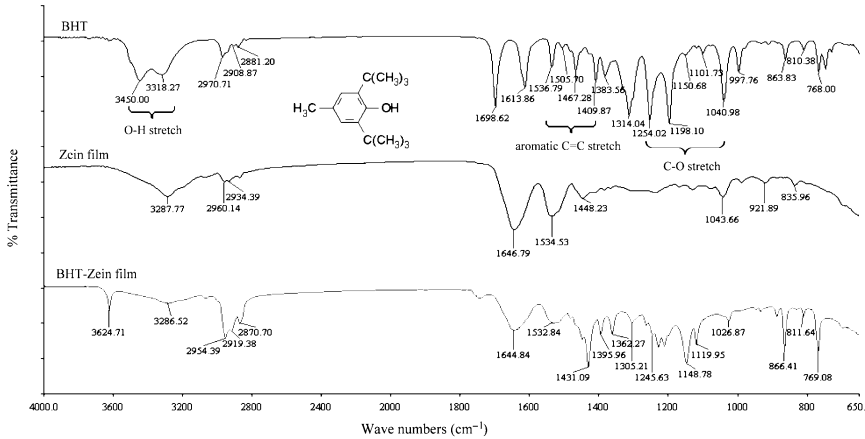

FIG. 2. INFRARED SPECTRA OF BUTYLATED HYDROXYTOLUENE (BHT), PLAIN ZEIN FILM AND BHT-INCORPORATED ZEIN FILM

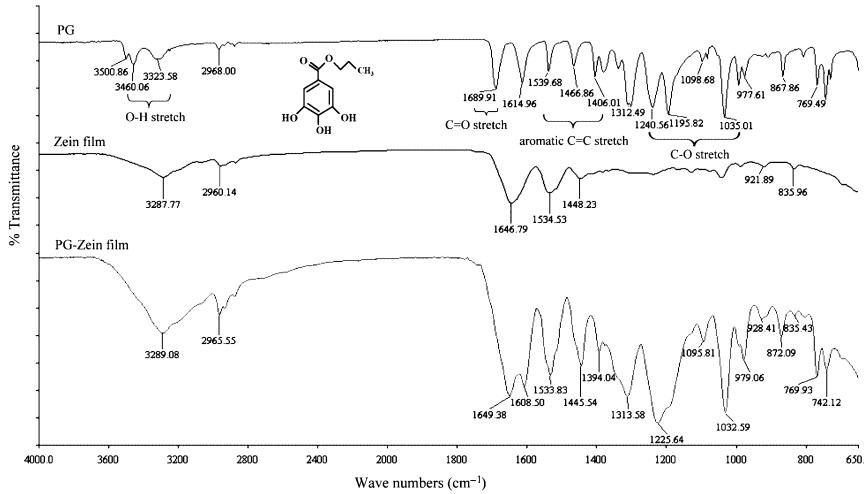

FIG. 3. INFRARED SPECTRA OF n-PROPYL GALLATE (PG), ZEIN FILM AND PG-INCORPORATED ZEIN FILM

Preservation of Fish Ball Quality

The *POV* for fish ball coated with the antioxidant-incorporated zein coating are shown in Table 2. While the *POV* for all groups of fish ball kept under refrigeration (4C) increased as the storage time increased, no significant

TABLE 2.
PEROXIDE VALUE (*POV*)* (meq/kg FISH BALL) OF FISH BALL COATED WITH BHA-, BHT- OR PG-INCORPORATED ZEIN STORED AT 4C

Treatment†	Storage time (days)				
	3	6	9	12	15
Noncoated	$29.05^{a} \pm 1.61$	$34.12^{a} \pm 1.90$	$40.35^{a} \pm 0.65$	$44.58^{a} \pm 1.42$	$51.17^{a} \pm 0.72$
Zein only	$28.81^{a} \pm 1.82$	$32.33^{a} \pm 1.39$	$38.65^{ab} \pm 0.47$	$42.55^{ab} \pm 1.27$	$47.63^{b} \pm 0.71$
BHA–zein	$27.06^{a} \pm 0.66$	$29.74^{a} \pm 1.52$	$34.34^{c} \pm 1.25$	$37.28^{c} \pm 0.28$	$41.56^{c} \pm 1.67$
BHT–zein	$28.16^{a} \pm 1.78$	$30.74^{a} \pm 2.72$	$35.80^{bc} \pm 1.68$	$40.75^{b} \pm 1.38$	$44.48^{bc} \pm 0.41$
PG–zein	$26.35^{a} \pm 2.05$	$29.38^{a} \pm 0.34$	$33.42^{c} \pm 0.77$	$35.25^{c} \pm 0.73$	$36.62^{d} \pm 1.62$

* The initial *POV* of fish ball used in this study was 24.03 ± 2.04 meq/kg. Means ($\pm$SE) with different superscript letters within each column are significantly different ($P < 0.05$); $n = 4$.

† Noncoated: fish ball used without coating. Zein only: fish ball coated with zein without antioxidant. BHA–zein: fish ball coated with BHA-incorporated zein. BHT–zein: fish ball coated with BHT-incorporated zein. PG–zein: fish ball coated with PG-incorporated zein.

BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; PG, n-propyl gallate.

difference was detected among all treatments up to 6 days. At days 9 and 12, the fish ball coated with BHA-incorporated zein and with PG-incorporated zein showed significantly lower *POV* content than the fish ball without coating. At the same refrigerated storage period, the fish ball coated with zein and BHT-incorporated zein did not show significantly different *POV* content from that without coating control. At the end of storage (15 days), the *POV* of the fish ball (36.62 ± 1.62 meq/kg fish ball) coated with PG-incorporated zein was significantly ($P < 0.05$) lower than the *POV* of all the other groups. The fish ball sample coated with zein without antioxidant had significantly low *POV* than the fish ball without coating; the barrier property provided by zein coating alone might explain the phenomenon. In general, lower peroxide contents were observed for all three types of antioxidant-incorporated zein-coated fish ball when compared with fish ball either without coating or coated with zein alone.

In contrast to *POV*, the content of TBARS is used as the indicator for the later stage of lipid oxidation. MDA is formed through β-γ unsaturated peroxide radicals originated from polyunsaturated fatty acids. MDA reacts with 2-thiobarbituric acid and forms pink complexes which can be detected at 530 nm. TBARS was used to demonstrate the antioxidative activity of green tea extract-incorporated zein films applied on fried fish paste products (Lee *et al.* 2004). The TBARS contents of fish ball during cold storage are shown in Table 3. The TBARS contents of fish ball coated with antioxidant-incorporated zein were significantly lower than those of uncoated and coated with zein without antioxidant starting from day 6. Although the TBARS value (2.32 ± 0.05 μmole MDA/100 g fish ball) for fish ball coated with

TABLE 3.
THIOBARBITURIC ACID REACTIVE SUBSTANCE (TBARS) VALUES* (μmole MDA/100 g fish ball) OF FISH BALL COATED WITH BHA-, BHT- OR PG-INCORPORATED ZEIN STORED AT 4C

Treatment†	Storage time (days)				
	3	6	9	12	15
Noncoated	$2.14^{a} \pm 0.07$	$2.28^{a} \pm 0.09$	$2.76^{a} \pm 0.05$	$2.88^{a} \pm 0.05$	$2.94^{a} \pm 0.06$
Zein	$2.07^{a} \pm 0.03$	$2.15^{ab} \pm 0.09$	$2.59^{b} \pm 0.04$	$2.64^{b} \pm 0.05$	$2.73^{b} \pm 0.05$
BHA–zein	$1.92^{a} \pm 0.08$	$2.00^{b} \pm 0.07$	$2.20^{c} \pm 0.02$	$2.20^{d} \pm 0.02$	$2.40^{cd} \pm 0.05$
BHT–zein	$2.03^{a} \pm 0.04$	$2.04^{b} \pm 0.04$	$2.34^{d} \pm 0.04$	$2.38^{c} \pm 0.07$	$2.54^{c} \pm 0.04$
PG–zein	$1.95^{a} \pm 0.06$	$1.97^{b} \pm 0.05$	$2.25^{cd} \pm 0.03$	$2.23^{d} \pm 0.05$	$2.32^{d} \pm 0.06$

* The initial TBARS value of fish ball used in this study was 1.96 ± 0.06 μmole MDA/100 g. Means ($\pm$SE) with different superscript letters within each column are significantly different ($P < 0.05$); $n = 4$.
† Treatment as described in Table 2.
MDA, malondialdehyde; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; PG, n-propyl gallate.

TABLE 4.
WEIGHT LOSS* (%) OF FISH BALL COATED WITH BHA-, BHT- OR PG-INCORPORATED ZEIN STORED AT 4C

Treatment†	Storage time (days)				
	3	6	9	12	15
Noncoated	$0.71^{a} \pm 0.03$	$0.82^{a} \pm 0.05$	$1.63^{a} \pm 0.06$	$2.60^{a} \pm 0.04$	$3.38^{a} \pm 0.06$
Zein	$0.32^{b} \pm 0.03$	$0.31^{b} \pm 0.03$	$0.97^{b} \pm 0.04$	$1.64^{b} \pm 0.03$	$2.42^{b} \pm 0.08$
BHA–zein	$0.36^{b} \pm 0.04$	$0.35^{b} \pm 0.06$	$0.78^{bc} \pm 0.04$	$1.57^{b} \pm 0.12$	$2.01^{c} \pm 0.05$
BHT–zein	$0.28^{b} \pm 0.03$	$0.34^{b} \pm 0.01$	$0.78^{bc} \pm 0.08$	$1.62^{b} \pm 0.09$	$2.30^{b} \pm 0.05$
PG–zein	0.25 ± 0.04	$0.25^{b} \pm 0.04$	$0.63^{c} \pm 0.06$	$1.21^{c} \pm 0.06$	$1.31^{d} \pm 0.03$

* Means ($\pm$SE) with different superscript letters within each column are significantly different ($P < 0.05$); $n = 4$.
† Treatment as described in Table 2.
BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; PG, n-propyl gallate.

PG-incorporated zein was the lowest in day 15 among all treatments, no significant difference was detected among three antioxidant-incorporated groups. The results imply that the antioxidant effectiveness of zein incorporated with the three tested antioxidants is similar in the case of TBARS formation for fish ball.

The weight loss of fish ball during cold storage is shown in Table 4. Compared to noncoated fish ball, all types of zein coatings could significantly reduce the weight loss of fish ball during cold storage. Because zein coatings

or films are relatively hydrophobic and can serve as relatively good water vapor barriers (Pol *et al.* 2002), zein coatings were reported to reduce the weight loss of apples (Bai *et al.* 2003) or deep-fat frying products (Mallikarjunan *et al.* 1997).

In conclusion, edible antioxidant-incorporated zein films had been successfully prepared as confirmed by IR spectroscopy. The quality of commercially manufactured fish ball could be preserved by this type of active packaging material. More interestingly, the three types of antioxidants showed different antioxidative potency, which might be the result of physical and chemical differences existing among different antioxidants. Therefore, it is suggested that different active compounds in the same catalog should be tested during the screening test when active packaging materials are under investigation.

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