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# Edible oxygen barrier bilayer film pouches from corn zein and soy protein isolate for olive oil packaging

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#### ABSTRACT

An edible oxygen barrier film pouch was fabricated from a heat sealable corn zein (CZ) layer laminated on soy protein isolate (SPI) film and used to package olive oil condiments for use with instant noodles. The mechanical, barrier, and physical properties of the CZ/SPI bilayer films were then investigated and the oxidative stability of olive oil in the pouches was measured during storage under dry and intermediate relative humidity conditions. When compared to the SPI film, lamination with an additional layer of CZ film led to increased tensile strength and water barrier properties, while it had a lower elongation at break and decreased oxygen barrier properties. Nevertheless, the oxygen permeability of the CZ/SPI film  $(0.81 \times 10^{-18} \, \text{m}^3 \, \text{m/m}^2 \, \text{s Pa})$  was lower than that of nylon-metalocene catalyzed linear low-density polyethylene (NY/mLLDPE) film  $(3.51 \times 10^{-18} \, \text{m}^3 \, \text{m/m}^2 \, \text{s Pa})$  which is the material usually used for such condiments. The CZ/SPI bilayer films generated here were heat sealable at 120–130 °C and produced a seal strength greater than 300 N/m. The higher oxygen barrier property of the CZ/SPI bilayer films resulted in reduced oxidative rancidity of olive oil packaged in the CZ/SPI film when compared to olive oil packaged in NY/mLLDPE films.

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#### 1. Introduction

Convenience foods enable the consumer to save time and energy associated with food-related activities such as shopping, meal preparation, cooking, and consumption (Buckley, Cowan, & McCarthy, 2007). Among the convenience foods, instant noodles have become very popular due to their reasonable price and ease of preparation. As the standard of living has increased, instant noodle products with improved organoleptic and nutritional qualities have gained popularity. Often, edible vegetable oils such as olive oil are added to instant noodles to improve the flavor. Such added oils have generally been provided in a separately packaged plastic film pouches designed for single usage.

However, packaged oil condiments are susceptible to lipid oxidation since plastic films offer only limited protection against oxygen transmission. Lipid oxidation not only causes an off-flavor in oil condiments that leads to decreased organoleptic quality, but they also produce several by-products those are potentially toxic to human such as malonaldehyde, glyoxal, and methylglyoxal (Fujioka

& Shibamoto, 2004; Kanavouras, Hernandez-Munoz, & Coutelieris, 2006). Additionally, direct contact of the oil and films for sufficient lengths of time has been found to result in the transfer of additives originally incorporated in the plastic films into the oil condiments; therefore, there is the potential for additives to threaten the health of the consumer. In addition, there are a number of drawbacks associated with the using plastic film pouches for packaging oil condiments including; (1) the generation of waste associated with the materials used to package convenience foods; (2) tearing the pouches during the application of oil condiments to the food may cause reduced convenience; (3) leftover oil may remain in the pouch after application to food.

Edible packaging materials produced from natural products may provide an alternative to the problem of disposing of plastic materials. Among biopolymers, soy protein produces films that are more flexible, smooth, water soluble, and clear than those produced by other plant protein sources (Brandenburg, Weller, & Testin, 1993). Soy protein-based edible films have received considerable attention due to their excellent film forming abilities, low cost and barrier properties against oxygen permeation under dry RH conditions (Rhim, Mohanty, Singh, & Ng, 2006). However, there are some limitations to the application of soy protein-based films for packaging due to their poor mechanical properties and heat sealability compared to synthetic polymer films such as LDPE

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and polyester film. In general, the tensile strength (TS) of soy protein films range between 3 and 9 MPa which varies with the plasticizers used and environmental RH, whereas TS of LDPE films and polyester films had 9–17 MPa and 175 MPa, respectively (Krochta, 2002). In addition, heat sealability of soy protein film has not been reported yet (Su et al., 2010).

The properties of protein-based films can be improved by laminating additional film layers with desired film properties such as corn zein films (Ghanbarzadeh & Oromiehi, 2009; Pol, Dawson, Acton, & Ogale, 2002). Corn zein produces a film with high tensile strength and low water vapor permeabilities when compared to other protein-based films, and the resulting films have desirable heat seal characteristics (Cho, Park, & Rhee, 2002; Park, Testin, Vergano, Park, & Weller, 1996). It is also expected that the lamination of corn zein layers onto soy protein isolate films will produce oxygen barrier bilayer films that will enable pouches to be readily formed by heat sealing of the corn zein layer.

In the present study, the corn zein layer laminated soy protein isolate (CZ/SPI) films were produced, and the mechanical, barrier, and physical properties such as solubility in hot water and heat sealability of CZ/SPI film was investigated and compared with those of the synthetic films. The film solubility in hot water is a very important characteristic of the protein-based films because the films needed to be disintegrated during cooking and release the oil from the pouches for improved convenience. A nylon layer laminated metalocene catalyzed linear low-density polyethylene (NY/mLLDPE) film was used as the control synthetic film, that is currently used for the package of oil condiments used in instant noodles. And also the potential application of CZ/SPI films to produce an oxygen barrier pouch was evaluated based on the oxidative stabilities of olive oil in the pouches during storage under dry and intermediate relative humidity (RH) conditions.

#### 2. Materials and methods

#### 2.1. Materials

A commercial soy protein isolate (SPI, 86.2% protein content, Donghae Chem. Co., Korea) and corn zein (CZ, 85.0% protein content, Sigma Chemical Co., USA) were used to make films. NY/mLLDPE (nylon/metalocene catalyzed linear low-density polyethylene) films were obtained from Youl Chon Chemical, Ltd. (Korea). Olive oil was purchased from Showa Chemical Company (Japan). Other reagents including glycerol, sorbitol and polyethylene glycol (PEG) 400 were of analytical grade.

#### 2.2. Preparation of bilayer films

The bilayer films were prepared by fabricating an additional CZ layer onto a dried SPI film. The SPI film layers were formed by casting a SPI film solution, which was prepared by dissolving 5.0 g of SPI, 1.25 g of glycerol and 1.25 g of sorbitol in 100 mL of distilled water. The film solutions were then homogenized (T-25, IKA Labotechnik, Germany) at 10,000 rpm for 2 min, after which the pH of each solution was adjusted to 10 using ammonium hydroxide. The solutions were then heated on a hot plate while stirring for 10 min at 80 °C, after which they were poured onto a 25 × 25 cm Teflon<sup>TM</sup> film coated glass plate and allowed to dry overnight at room temperature (  $\approx$  25 °C).

The CZ solution for lamination was prepared by dissolving 2 g of CZ and 0.6 g of polyethylene glycol (MW, 400) in 30 mL of aqueous ethanol (95%, Duksan Chemical Co., Republic of Korea). The solution was then homogenized (T-25, IKA Labotechnik, Germany) at 6000 rpm for 30 s, after which it was heated to  $\approx\!85\,^{\circ}\text{C}$ . Next, the solution was allowed to cool to 40–50 °C and then poured onto

a dried SPI film. The plates were left at room temperature (  $\approx$  25 °C) for 10 min and then dried at 80–85 °C for 30 min, after which they were allowed to rest overnight at room temperature.

#### 2.3. Film characterization

#### 2.3.1. Mechanical properties

Ten or more specimens (80 mm  $\times$  25 mm) were conditioned at 25 °C and 50% relative humidity (RH) for 48 h in an environmental chamber (Sang Woo Co., Korea). A texture analyzer (TA-XT2, Stable Micro Systems, England) was used to measure tensile strength [TS (MPa)] and the percentage elongation at break [E(%)] according to ASTM Standard Method D 882-01 (ASTM, 2002c). The initial grip separation was set at 50 mm and the crosshead speed was set at 500 mm/min.

#### 2.3.2. Water vapor permeability

The water vapor transmission rate (WVTR) was determined gravimetrically using a modification of ASTM Standard Method E 96-00 (ASTM, 2002d), which is also known as the cup method, as described in Cho, Park, Batt, and Thomas (2007). The water vapor permeability (WVP) was calculated using Eq. (1), which was as follows:

$$WVP = WVTR(L/Dp) \tag{1}$$

where WVTR ( $ng/m^2$  s) is the water vapor transmission rate of films measured at 25 °C and 50% RH gradient, L(m) is the mean thickness of the film specimens, and  $\Delta p$  (Pa) is the actual difference in partial water vapor pressure between the two sides of the film specimens.

#### 2.3.3. Measurement of oxygen permeability

Oxygen permeability (OP) was determined using an Ox-Tran system (Mocon Inc., Minneapolis, MN, USA) at 25 °C and 50  $\pm$  1% RH according to ASTM Standard Method D3985-95 (ASTM, 2002a). Film specimens were placed in precut aluminum masks that allowed an exposure area of 5 cm². Three replicates of each film were evaluated.

#### 2.3.4. Color measurement

The color values of the films were measured using a Chroma meter (CR 300 Chroma Meter, Minolta Camera Co., Osaka, Japan). Film specimens were placed on the surface of a white standard plate (calibration plate CR-A43, L = 95.91, a = 0.00, and b = 2.27) and the Hunter L, a, and b color values were measured.

#### 2.3.5. Scanning electron microscopy (SEM)

Cross-sectional microscopic images of CZ/SPI bilayer films and heat-sealed bilayer films were obtained using a scanning electron microscope (SEM, S-450, Hitachi, Japan). The heat-sealed film specimens were cut into a strip (1 mm  $\times$  20 mm) with a razor. The specimens were mounted on aluminum stub using a double-sided tape and then coated with thin gold layers about 20 nm thick. The samples were examined using an accelerating voltage of 5 kV.

#### 2.3.6. Film solubility in hot water

The film solubility of the CZ/SPI bilayer films during solubilization in a hot water was calculated as the ratio of the solubilized material after solubilization in water to the initial dry matter in the sample (Rhim, Wu, Weller, & Schnepf, 1999). The film samples (2.5 cm  $\times$  2.5 cm) were dehydrated in a desiccator over phosphorus pentoxide (P<sub>2</sub>O<sub>5</sub>) at 25 °C for 3 days and then accurately weighed to obtain the initial film dry weight. Next, a piece of film was placed in a beaker with 30 mL of hot distilled water that had previously been heated to 80, 90 or 100 °C and then gently shaken at 40 rpm. After

heating the film specimens in the water for a predetermined time  $(30-150\,\mathrm{s})$  and temperature  $(80,90,100\,^\circ\mathrm{C})$ , dry materials that had not solubilized were removed from the beakers and dried in an oven at  $105\,^\circ\mathrm{C}$  for  $24\,\mathrm{h}$ . Next, the weights of the dried insoluble materials were determined. The weight of water-soluble material was then calculated by subtracting the weight of unsolubilized dry matter from that of the initial dry matter. Film samples were weighed to the nearest 0.0001 g before and after drying. The solubility of the films in hot water were determined in triplicate for each test condition.

#### 2.3.7. Heat sealability

The heat sealabilities of the CZ/SPI bilayer films were determined according to ASTM Standard method F88-00 (ASTM, 2002b) using a heat gradient tester (HG-100, Toyo Seiki Co., Ltd, Japan). Laminated films were mounted on the hot plate of the gradient tester with the corn zein layers facing together. The film specimens were then lap sealed ( $10~\text{mm} \times 25~\text{mm}$ ) under a pressure of 3 atm for 3 s at varying temperatures of 85-155~°C. Ten specimens with a size of  $80~\text{mm} \times 25~\text{mm}$  that included 10~mm of lap seal length in the middle were then conditioned at 25~°C and 50%~RH for 48~h in an environmental chamber (Sang Woo Co., Korea). A texture analyzer (TA-XT2, Stable Micro Systems, England) was used to measure the maximum force required to split the film specimens with a crosshead speed of 500~mm/min. The maximum force required for a film of unit width to cause seal failure was reported as the seal strength (N/m) (Kim & Ustunol, 2001).

#### 2.4. CZ/SPI film pouches for olive oil packaging

CZ/SPI bilayer films were cut into 40 mm × 50 mm pieces and two pieces of film were then superimposed with the corn zein layers facing together. The three sides of the film cuts were then heat sealed using an impulse sealer (Lee Tech Systems Co., Korea) to form a CZ/SPI film pouch. Next, 2.0 g of olive oil were placed in the pouch, which was subsequently heat sealed to form a four sided sealed pouch. Commercially available NY/mLLDPE (Youl Chon Chemical, Ltd., Korea) films were used as a control pouch film for the olive oil packaging. The olive oil samples in the CZ/SPI film pouches (CSP oil) and in the NY/mLLDPE film pouches (NMP oil) were stored in the desiccators at a controlled RH (32%, 43%, and 52%) at one of three storage temperatures (30, 40, and 50 °C) for 4 months. The RH of the inside of the desiccators was controlled during the storage by placing the pouch samples over various saturated salt solutions (MgCl<sub>2</sub>, 32%; K<sub>2</sub>CO<sub>3</sub>, 43%; and Mg(NO<sub>3</sub>)<sub>2</sub>, 52%) in the desiccator.

During the 4 months of the storage, the changes in the rancidity of the olive oil in the pouches were determined every 30 days by measuring the peroxide values (POV). The POV of the olive oil was determined according to the IUPAC standard method 2.501 (IUPAC, 1987).

#### 2.5. Statistical analysis

Statistics on a completely randomized design were conducted using the analysis of variance (ANOVA) procedure available in the SAS software. Duncan's Multiple Range Test (p < 0.05) was used to detect differences among film properties.

#### 3. Results and discussion

#### 3.1. Film appearance and cross-sectional view

Film color, which is an important factor that determines the films appearance, is presented in Table 1. The SPI-based film layer

**Table 1** Hunter values (L, a, and b) of soy protein film (SPI), corn zein/soy protein isolate (CZ/SPI) bilayer film and nylon-metalocene catalyzed linear low-density polyethylene (NY/mLLDPE) film.<sup>a</sup>

	Film	Thickness (μm)	$L^{\mathbf{b}}$	a <sup>c</sup>	$b^{d}$
Ī	SPI	$115.9 \pm 3.7$	$85.59 \pm 1.09b$	$-1.46\pm0.07b$	$16.04 \pm 0.28 b$
	CZ/SPI	$114.6 \pm 2.7$	$83.96 \pm 0.39c$	$-2.58 \pm 0.13 \text{c}$	$18.50 \pm 0.46 a$
	NY/mLLDPE	$\textbf{76.5} \pm \textbf{2.0}$	$93.95 \pm 0.27 a$	$-0.08\pm0.01a$	$2.54 \pm 0.08 \text{c}$

Means in the same column with the same letter do not differ significantly (p > 0.05).

- <sup>a</sup> Films were placed on top of a standard white plate (L = 95.91, a = 0.00, b = 2.27).
- <sup>b</sup> Lightness.
- c Redness.
- d Yellowness.

had a yellow color and Hunter L, a, and b values of 93.59, -1.46, and 16.04, respectively. Lamination with additional CZ film layers resulted in a greenish yellow colored film with Hunter L, a, and b values of 91.96, -2.58 and 18.50, respectively. The increased yellowness of the CZ/SPI laminated film was due to the yellow character of the corn zein films (Weller, Gennadios, & Saraiva, 1998).

The lamination performance of the two layers was evaluated by scanning electron microscopy (SEM) of a cross section of the CZ/SPI film (Fig. 1). The 153-µm thick CZ/SPI laminated film in Fig. 1 was produced by laminating a 40-µm thick CZ film layer onto a 114-µm thick SPI film layer. The individual layers of SPI and CZ could be seen clearly in the micrograph. The CZ/SPI bilayer film was produced by pouring alcohol solubilized corn zein solution on the SPI film that was prepared from SPI dissolved in water. The alcohol solution of corn zein did not dissolve the SPI film layer. The different solvent systems used for the SPI film and CZ film produced an intact interface at the bilayer film. If the same solvent were used for making the film components of the bilayer films, the interaction between film components would have produced a non-uniform cross section of bilayer films such as occurs in gelatin/chitosan bilayer films (Rivero, Garcia, & Pinotti, 2009).

#### 3.2. Mechanical and barrier properties of the bilayer films

The mechanical properties (TS and *E* values) and barrier properties (WVP and OP values) of the bilayer films are presented in Table 2. The application of a relatively thin layer of corn zein to form a CZ/SPI bilayer film resulted in a more than two-fold increase in the TS value (5.9 MPa). In addition, the *E* value of SPI decreased drastically to 7.3% after production of the CZ/SPI bilayer film. The low *E* value of the CZ/SPI bilayer film was attributed to the limited stretchability of the PEG plasticized corn zein film at 50% RH (Lawton, 2004). In general, the mechanical properties of films are affected by the type and amount of plasticizer incorporated into the

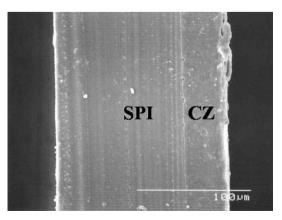


Fig. 1. Scanning electron micrograph of the cross-section of the CZ/SPI bilayer film.

**Table 2**Tensile strength (TS), percent elongation at break (*E*), water vapor permeability (WVP), and oxygen permeability (OP) of soy protein film (SPI), corn zein/soy protein isolate (CZ/SPI) bilayer film and nylon-metalocene catalyzed linear low-density polyethylene (NY/mLLDPE) film.

Film	TS (MPa)	E (%)	WVP ( $\times 10^{-12}$ kg m/m <sup>2</sup> s Pa)	OP ( $\times 10^{-18}$ m <sup>3</sup> m/m <sup>2</sup> s Pa)
SPI	$2.5 \pm 0.5 \text{c}$	178.6 ± 19.7a	$0.94\pm0.06a$	$0.60 \pm 0.03 b$
CZ/SPI	$\textbf{5.9} \pm \textbf{0.4b}$	$\textbf{7.3} \pm \textbf{2.0b}$	$0.61 \pm 0.05 b$	$0.81 \pm 0.05b$
NY/mLLDPE	$31.6 \pm 6.5a$	$179.2 \pm 25.9a$	$< 0.001^{a}$	$3.51 \pm 0.08a$

Means in the same column with the same letter do not differ significantly (p > 0.05). <sup>a</sup> WVP value was less than  $0.001 \times 10^{-12}$  kg m/m<sup>2</sup> s Pa.

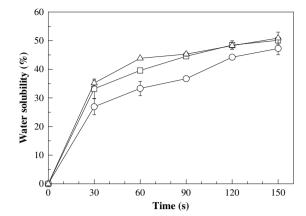
film, with the *E* value increasing and the TS values and barrier properties decreasing as the amount of plasticizer increases. The CZ/SPI film with 7.3% elongation was not brittle and had a film integrity that was acceptable for pouch fabrication, although the TS and *E* values of the CZ/SPI film were much lower than those of the NY/mLLDPE control films.

The WVP and OP are measures of barrier properties of CZ/SPI bilayer films against moisture and oxygen movement across the membrane, respectively. The WVP and OP of the NY/mLLDPE film were used as a reference for comparison of the moisture and oxygen barrier property. Although the moisture barrier properties of the CZ/SPI bilayer film were still lower than those of the NY/ mLLDPE film, the WVP of the SPI film layer was significantly (p < 0.05) reduced when an additional CZ film layer was introduced. In general, the WVP of laminated film is determined by the thickness and WVP values of each film layer comprising the laminated film (Cho et al., 2002). Films produced using CZ have an extraordinarily high hydrophobicity when compared to other plant protein films due to the high content of hydrophobic amino acids in CZ (Fu, Weller, & Wehling, 1999). The decrease in WVP value in response to lamination of the CZ film layer was attributed to the low WVP value of the CZ film layer. CZ film layers have also been used to reduce the WVP of biodegradable hydrophilic films including starch (Ryu, Rhim, Roh, & Kim, 2002), and whey protein isolates (Ghanbarzadeh & Oromiehi, 2009).

The OP of the CZ/SPI film is very important because it determines the amount of lipid oxidation of packaged olive oil that occurs, which is the primary determinant of quality. The OP of the SPI film and CZ/SPI bilayer film measured at 50% RH were  $0.60 \times 10^{-18} \,\mathrm{m}^3 \,\mathrm{m/m}^2 \,\mathrm{s} \,\mathrm{Pa}$  and  $0.81 \times 10^{-18} \,\mathrm{m}^3 \,\mathrm{m/m}^2 \,\mathrm{s} \,\mathrm{Pa}$ , respectively (Table 2). The OP of the SPI film used in the present study was comparable to the previously reported OP values of SPI film measured at 50% RH (Cho et al., 2007; Rhim et al., 2006). It is well known that SPI films show extremely low OP values under dry RH conditions and that the OP of SPI films at 0% RH were about 70% lower than those of corn zein films formed under similar conditions (Brandenburg et al., 1993; Gennadios, Weller, & Testin, 1993). The OP of hydrophilic protein films are susceptible to environmental RH conditions, and increase exponentially as the RH increases beyond intermediate RH conditions (Rhim et al., 2006). The application of an additional layer of lower oxygen barrier CZ film and an elevated RH was found to have an adverse effect on the OP of the CZ/SPI bilayer film. Despite these drawbacks, the CZ/SPI bilayer film still had higher oxygen barrier properties than the NY/mLLDPE film  $(3.51 \times 10^{-18} \text{ m}^3 \text{ m/m}^2 \text{ s Pa})$  and other plastic films including the LDPE  $(216.44 \times 10^{-18} \text{ m}^3 \text{ m/m}^2 \text{ s Pa})$  and HDPE  $(49.42 \times 10^{-18} \text{ m}^3 \text{ m/m}^2 \text{ s Pa})$  $10^{-18} \,\mathrm{m}^3 \,\mathrm{m/m}^2 \,\mathrm{s} \,\mathrm{Pa}$ ) films (Rhim et al., 2006).

#### 3.3. Solubility in hot water

The film solubility in hot water was a very important characteristic of the CZ/SPI films because they must disintegrate during

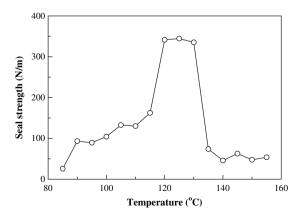


**Fig. 2.** Water solubility of the CZ/SPI bilayer film at various water temperatures (- $\bigcirc$ - 80 °C, - $\square$ - 90 °C, - $\triangle$ - 100 °C).

cooking while releasing the oil from the pouches. The disintegration of pouch increases the convenience of releasing oil condiment into food system. As shown in Fig. 2, approximately 50% of the CZ/SPI film dissolved when placed in hot water (90 °C) for 150 s. The partially dissolved bilayer film easily disintegrated, thereby releasing the olive oil. Additionally, the dissolution speed of the bilayer film accelerated as the water temperature increased for each film sample. Su, Huang, Yang, and Yuan (2008) reported that an SPI film completely dissolved within 180 s when placed in 100 °C hot water. The relatively lower water solubility of the bilayer film in the present study could be attributed the less hydrophilic nature of the corn zein film.

#### 3.4. Heat sealability

The optimum heat sealing temperature was determined by measuring the lap seal strength of film specimens that were heat sealed at various temperatures (Fig. 3). The seal strengths of the CZ/SPI bilayer films were measured for film specimens that were heat sealed at temperatures ranging from 85 to 155 °C, and the resulting seal strengths ranged from 25 to 345 N/m. The seal strengths of the films were primarily affected by the heat sealing temperature. Specifically, 120–130 °C was found to be the optimal temperature range for sealing the corn zein layer. Indeed, seals formed in the optimal temperature range had a strength of 330 N/m, while those formed at other temperatures had a strength of less than 165 N/m. The heat sealing temperature of a protein film is closely related to



**Fig. 3.** Seal strength (N/m) of heat-sealed CZ/SPI films at various sealing temperatures. Films were heat-sealed on the corn zein side.

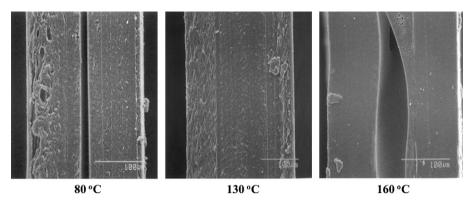


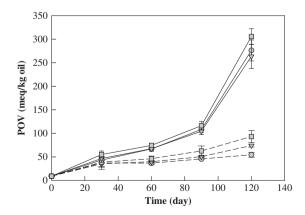
Fig. 4. Cross-section of CZ/SPI bilayer films heat sealed at various temperatures.

its thermal transition temperature. This is because the sealing temperature of a polymer is typically conducted near the onset temperature of thermal transition, which is a function of the amount of moisture and plasticizer in the film (Hernandez-Izquierdo, Reid, McHugh, Berrios, & Krochta, 2008; Kim & Ustunol, 2001). The decreased strength of seals formed above 130 °C was due to excessive heat treatment, which resulted in a distorted or nonfunctional seal (Kim & Ustunol, 2001). The seals were also visualized by SEM analysis (Fig. 4). A cross-sectional view of the heat-sealed CZ/SPI films showed that the films heat sealed at 80 °C separated due to insufficient heat treatment, while the zein film layer of pouches sealed at 160 °C were deformed due to excessive heat.

## 3.5. Oxidative stability of olive oil packaged in the bilayer film pouch

Olive oil packaged in NY/mLLDPE film pouches (NMP oil) and in CZ/SPI bilayer film pouches (CSP oil) was stored at 32%–52% RH, which was similar to the internal RH of instant noodle packages. The oxidative stabilities of the oil samples determined by measuring the peroxide values (POV) during storage are shown in Fig. 5.

The POV of NMP oil stored at 50 °C increased as the storage time increased. Specifically, during 90 days of storage at 50 °C, the POV of NMP oil increased from 9.05 meq/kg to 104.5—116.3 meq/kg, and an additional 30 days of storage led to a drastic increase in POV to 250—300 meq/kg. The oxidative stabilities of vegetable oils are affected by the environmental RH and oxygen level (Maté, Saltveit,



**Fig. 5.** Peroxide values (POV) of olive oil stored in corn zein/soy protein isolate (CZ/SPI) bilayer film pouches (dotted line) and in NY/mLLDPE film pouches (solid line) at 50 °C:  $(-\bigcirc -30\%, -\Box -40\%, -\triangle -50\%)$ .

& Krochta, 1996). The rates of oxidation in vegetable oil are accelerated at high RH because more water molecules can act as reaction media, while the rates of oxidation are higher at RH values below the monolayer moisture content because the oil is directly exposed to external oxygen (Partanen et al., 2008). However, in the present study, the environmental RH did not have a significant effect (p < 0.05) on the rancidity of NPP oil samples stored at 32–52% RH. This was probably due to the uniform oxygen permeability of the plastic films under a wide range of RH conditions and to the constant RH of the inside headspace in the package that was produced by the high barrier properties of the NY/mLLDPE film against the movement of moisture across the membrane (Table 2).

The CSP oil showed a reduced increase in POV when compared to NMP oil during 120 days of storage. The POV of CPS oil after 120 days of storage at 50 °C and varying environmental RH ranged from 54 to 93 meg/kg. During the early stages of storage, lipid oxidation is propagated by consuming oxygen in the headspace of the pouch. The loss of oxygen in the pouch causes movement of oxygen across the film toward the inside of the packaging, which provides substrates for oxidation. Protein films are generally good barriers against oxygen at low and intermediate RH (Javanmard, 2008). The higher oxygen barrier property of CZ/SPI bilayer films resulted in a lower oxygen concentration in the pouch headspace than in the NY/mLLDPE film pouches, which led to reduced lipid rancidity in CSP oil. Unlike NMP oil, the POV of CSP oil was affected by the environmental RH, and this RH dependence of POV was attributed to increased oxygen permeability of hydrophilic biopolymer films under elevated RH conditions. Moisture has a plasticizing and swelling effect on protein-based films that may result in elevated oxygen permeability. Additionally, an exponential effect of RH on the OP of hydrophilic films has been reported for protein-based films subjected to high RH conditions (Hong & Krochta, 2006; Tomasula, Yee, & Parris, 2003). In this study, the packaging of olive oil in the CZ/SPI bilayer film pouch was found to control lipid rancidity under practical storage conditions with dry and intermediate RH ranges.

#### 4. Conclusions

The heat sealability of soy protein isolate film was improved by laminating the film with an additional layer of corn zein film. The packaging of olive oil in edible and water disintegrable CZ/SPI bilayer film pouches would increase the convenience of packaged oil consumption since the film pouch disintegrated during cooking. In addition, the high moisture barrier properties of the CZ/SPI bilayer film under dry and intermediate RH conditions may extend the shelf life of olive oil by preventing oxidative rancidity during storage.

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