Development of Antioxidant Packaging Material by Applying Corn-Zein to LLDPE Film in Combination with Phenolic Compounds

Hye-Yeon Park, Sung-Jin Kim, Ki Myong Kim, Young-Sun You, So Yeon Kim, and Jaejoon Han

Abstract: Functional active packaging materials were successfully developed by incorporating antioxidant agents into corn–zein–laminated linear low-density polyethylene (LLDPE) film. The minimum effective concentrations of the active compounds (for example, thymol, carvacrol, eugenol) were determined and these compounds were then laminated into LLDPE films to develop corn–zein–laminated films with antioxidant agents. The release rate of antioxidant agents in gas and liquid media were determined along with the mechanical and water barrier properties of the films containing these compounds. Tensile strength and percentage elongation at break were reduced in the corn–zein–laminated LLDPE films when compared to typical LLDPE film. Furthermore, the ability of the corn–zein–laminated films to repel moisture decreased by approximately 12.2%, but was improved by incorporating hydrophobic antioxidant compounds in the corn–zein layer. Examination of release kinetics in the gas and liquid phases verified that antioxidants were effectively released from the films and inhibited oxidation during testing. Finally, the films were used for fresh ground beef packaging, and effectively inhibited lipid oxidation and had a positive effect on the color stability of beef patties during storage. These results indicate that the developed antioxidant films are a novel active packaging material that can be effectively implemented by the food industry to improve the quality and safety of foods.

Keywords: antioxidant film, corn–zein, diffusion test, ground beef, mechanical property

Practical Application: Zein protein, a by-product of corn processing industry, was laminated into plastic films in combination with natural phenolic compounds to develop antioxidant packaging films. The films demonstrated their efficient release patterns of antioxidant compounds, which are suitable for packaging applications and food protection.

Introduction

The main purpose of food packaging is to maintain the quality and safety of food during distribution and storage. In recent years, there have been increasing concerns about food quality and safety, which have motivated the development of active packaging (Dallyn and Shorten 1988). Active packaging is an innovative type of packaging that prevents or slows deterioration in food quality (Buonocore and others 2008). Oxygen scavengers, carbon dioxide scavengers/emitters, moisture absorbers and ethanol generators are some examples of active packaging. Active packaging also involves the release of antioxidants and/or antimicrobial substances onto the food surface. Due to environmental and health concerns, active packaging has been made from eco-friendly and biodegradable food packaging materials combined with natural preservatives (Appendini and Hotchkiss 2002; Suppakul and others 2003). Polyethylene (PE), the most common and least expensive synthetic polymer, is a widely used plastic material in food packaging. PE is the family name for resins such as low-density polyethylene (LDPE), high-density polyethylene (HDPE), and linear low-density polyethylene (LLDPE) (Robertson 1993). However, despite the physical and economic advantages of PE films, they cause environmental problems due to their nondegradability and difficulty in recycling. Therefore, researchers have begun to use natural polymers as substitutions for synthetic polymers to develop biodegradable food packaging materials (Peppas 2004).

Biopolymers are biodegradable, nontoxic, recyclable polymers that are frequently applied as edible coatings or films (Cuq and others 1998). They are made from natural sources such as polysaccharides, proteins, and lipids. Zein is the major storage protein of corn (Zea mays L.) and comprises 45% to 50% of corn protein. It is a widely used biopolymer for producing biodegradable packaging materials. The advantage of corn–zein is its ability to form tough, relatively hydrophobic, and grease-proof films with excellent flexibility and compressibility (Lai and others 1997). However, corn–zein film has problems relating to its brittleness under dry conditions, which restricts its use as a free-standing film or a coating material (Pomes 1971; Shukla and Cheryan 2001). To overcome this problem, biopolymers are often coated on conventional synthetic plastic films to develop a multilayered film structure (Atik and others 2008).

For over 50 years, antioxidants have been added to the food-making process to delay auto-oxidation (Cuvelier and others 1994). In active antioxidant packaging, this consists of introducing antioxidants...
into food packaging material instead of adding them directly to the food (Tovar and others 2005). A wide range of compounds, most of them synthetic molecules, have been used as antioxidant agents in packaging (Padgett and others 1998). Currently, the most frequently and widely used synthetic antioxidants in active food packaging are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are highly stable and efficient, and are also cost-effective. Although these antioxidants can effectively inhibit the lipid oxidation of foods, the use of synthetic antioxidants is strictly regulated as they are known to cause health risks (Brenan 1975) and is becoming increasingly unacceptable to consumers because of their potential toxicity (Chan and others 2007; Yen and others 2008). Due to safety concerns in the active food packaging sector, natural compounds have been deemed more appropriate.

Essential oils, extracted from herbs or spices, are regarded as natural alternatives to synthetic preservatives and the use of these oils in food meets consumer demands for safe food products (Burt 2004). Many essential oils exhibit antioxidant activity, which is attributable to their high phenolic compound content. Phenolic compounds are free radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers (Proestos and others 2006). In particular, thymol, carvacrol, and eugenol are some of the most active phenolic antioxidants found in essential oils. They are the major phenolic compounds present in thyme, oregano (thymol and carvacrol), and clove (eugenol) essential oils. Roberto and Baratta (2000) reported that thymol, eugenol, and carvacrol exhibited the highest antioxidant activity in a study of 100 pure components of essential oils. In recent reports, eugenol, menthol, and thymol maintained the quality and safety of sweet cherries under modified atmospheric conditions (Serrano and others 2005).

Active packaging acts as an inert barrier between food products and the external environment, and the incorporation of antioxidant agents into polymeric packaging materials is an exciting development in food packaging (Nerin and others 2006). Antioxidants are released from packaging films by diffusion, and directly inhibit lipid rancidity on the food surface, especially in oxidation-sensitive foods. Antioxidant packaging also has the potential to extend the shelf life of foods (Anklam and others 1997; Morales-Arizpurua and Tenuta-Filho 2005).

The objective of the present study was to develop multilayer films based on corn-zein and LLDPE films incorporated with antioxidant agents (thymol, carvacrol, and eugenol). The mechanical properties and hydrophobic qualities of developed films and the release rate of antioxidant agents from the film matrix into model systems were measured. In addition, the effectiveness of the developed packaging films on color stability and lipid oxidation for the storage of fresh ground beef was evaluated.

Materials and Methods

Chemicals and reagents
Corn-zein, antioxidant agents (thymol, carvacrol, and eugenol), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, Mo., U.S.A.). Polyethylene glycol 400 (PEG 400), a plasticizer of corn-zein film, was obtained from Junsei Chemicals Co. Ltd. (Tokyo, Japan). LLDPE film was obtained from Hanjin P&K Co. Ltd. (Seoul, Korea), while propyl gallate (PG) and ethylenediaminetetraacetic acid (EDTA) were obtained from DAEJUNG (Siheung, Korea). Thiobarbituric acid (TBA) was purchased from Alfa Aesar (St. Ward Hill, Mass., U.S.A.).

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Film preparation
Corn-zein coating solutions were prepared according to the methods described by Mastromatteo and others (2009) with some modifications. Corn-zein was dissolved in 95% ethanol at 20 g/100 mL. As a plasticizer, PEG 400 was added to corn-zein solution at 4 g/100 mL and the solution was then heated at 70 °C for 15 min. After cooling, thymol, carvacrol, and eugenol were individually added to the corn-zein solutions at a designated concentration (1.5, 3, or 5%, v/v) and stirred for 15 min. Corn-zein solutions containing different concentrations of each antioxidant agent were coated onto corona-treated LLDPE films (surface tension 45 ± 5 dyne/min; thickness 40 ± 0.4 μm) using a Nr. 28 coating rod (RDS, Webster, N.Y., U.S.A.) and dried for 15 min at room temperature. The coated side of the double-layered film was laminated with another LLDPE film using a heat laminating machine (GMP Co., Paju, Korea). The final multilayered film (thickness 95 ± 2.2 μm) structure is shown in Figure 1.

Measurement of film properties

Film thickness. Laminated film thickness was measured with a digital micrometer (ID-C112X, Mitutoyo, Kawasaki, Japan) at 5 random positions on the film. The mean of the 5 measurements was used to calculate tensile strength (TS) and water vapor permeability (WVP).

Mechanical properties. Mechanical properties of the films were determined by ASTM standard method D882-91 (1995) using an Instron universal testing machine (Model 5566, Instron Engineering Co., Canton, Mass., U.S.A.). Films were cut into strips with a test dimension of 25.4 mm × 100 mm. Before testing, all of the films were conditioned for 48 h in a thermostatic chamber adjusted to 30 °C and 90% RH. The equilibrated strip was placed between grip heads of the machine. The initial grip separation and cross head speed were set at 50 mm and 500 mm/min, respectively. TS was expressed in MPa and was calculated by dividing the maximum load (N) by the initial cross sectional area (m²) of the specimen. The percentage elongation at break (%E) was calculated as the ratio of the final length at the point of break to the initial length of a specimen (50 mm) and expressed as a percentage. TS and %E were replicated 5 times for each type of film.

Water vapor permeability. WVP was determined gravimetrically, based on ASTM standard method E96-95 (1997). Each film sample was mechanically sealed onto a polyethylene/methacrylate cup containing 30 g of anhydrous calcium chloride. The weights of the sealed cups were measured using an electronic scale with a precision of 0.001 g and cups were left in a controlled humidity chamber adjusted to 30 °C and 90% RH. After 24 h, they were reweighed and WVP was calculated according to the following:
formulas:

\[ \text{WVP} (\text{g} \cdot \text{mm/m}^2 \cdot \text{d} \cdot \text{kPa}) = W' \cdot x/A \cdot T (P_2 - P_1), \]

where \( W \) is the increase in weight of the cup (g) after 1 d, \( x \) is the average thickness of the film (mm), \( A \) is the exposed area of the film \((1.66 \times 10^{-3} \text{m}^2)\), and \((P_2 - P_1)\) is the differential vapor pressure across the films \((P_2 - P_1) = 1.70 \text{kPa at } 30 ^\circ \text{C})\). WVP measurements of each film sample were carried out in triplicate.

Release characteristics in different media

Release of antioxidant agent into the atmosphere. The release test was carried out according to the methods described in Gamege and others (2009), with some modifications. Corn-zein-laminated LLDPE films with antioxidant agent were put into a plastic tube \((500 \text{ mL volume})\) and carefully adhered to the inner wall to avoid gaps. The tube was tightly sealed with a cap and stored at room temperature. Air samples \((1 \text{ mL})\) were periodically withdrawn through a rubber septum on the tube using a syringe to determine the release of antioxidant agent from the films into the inner atmosphere. The amount of antioxidant in the gaseous medium was analyzed using the 6890A gas chromatography (GC) system (Agilent Technologies, Santa Clara, Calif., U.S.A.) equipped with an FID detector and HP5 MS capillary column \((30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \text{ mm})\). Gaseous samples \((1 \text{ mL})\) were injected manually, and the split ratio was 1:60. Helium was used as the carrier gas at a flow rate of 1 mL/min. The split/splitless injector temperature and detector temperature were set at 200 \(^\circ\text{C}\) and 350 \(^\circ\text{C}\), respectively. The initial column temperature was set at 60 \(^\circ\text{C}\) and increased to 200 \(^\circ\text{C}\) at a rate of 10 \(^\circ\text{C}/\text{min}\). The antioxidant compound was identified by comparing its mass spectra with those of authentic compounds in the computer library. The concentration of the identified compound in the atmosphere was quantified according to the peak area integrated by the data analysis program. Tests were conducted 4 times each.

Release of antioxidant agent into the liquid state. The release of antioxidant agent from the films into liquid medium was measured by 2,2-DPPH radical scavenging assay according to methods described in Shimada and others (1992), with some modifications. The films were tightly adhered to the inner wall of a 30 mL glass vial filled with 25 mL of 0.1 mM DPPH solution. The vial was wrapped with aluminum foil to avoid light, and then stored in a shaking incubator \((\text{Vision Scientific Co., Suwon, Korea})\) at 25 \(^\circ\text{C}\) and 70 rpm. The absorbance was measured with an Ultrospec 3100 pro UV spectrophotometer \((\text{Pharmacia Biotech Inc.})\). The results of DPPH radical scavenging activity were expressed as milligrams of malondialdehyde equivalents \((\text{MDA})\) per kilogram of meat sample. Tests were replicated 4 times.

Evaluation of lipid oxidation. Lipid oxidation of beef patties was determined by analysis of 2-thiobarbituric acid reactive substances (TBARS) according to the modified distillation method described in Rhie (1978). To prevent further oxidation, a mixed solution of PG and EDTA was added to each beef patty before distillation. After adding 4N HCl solution and distilled water, the mixture was distilled in a Kjeldahl flask and the distillate was collected in a 50 mL Falcon tube. Then, the distillate was reacted with 2-TBA in a water bath at 100 \(^\circ\text{C}\) for 35 min to induce color change. The optical density of the sample was read at 530 nm with an Ultrospec 3100 pro UV spectrophotometer \((\text{Pharmacia Biotech Inc.})\). The results of TBARS content were expressed as milligrams of malondialdehyde equivalents \((\text{MDA})\) per kilogram of meat sample. Tests were replicated 4 times.

Color changes in beef patty. Color changes were measured with a CR-400 colorimeter \((\text{Minolta Co., Japan})\). The patties were exposed to oxygen for 10 min before color evaluation. The colorimeter was calibrated using a standard white plate. Measurements were taken 5 times for each sample, and the mean values were used to determine the color coordinates \(L^*\) (lightness), \(a^*\) (redness), and \(b^*\) (yellowness). In order to monitor meat browning during storage, hue angle \((H = \arctan b^*/a^*); \text{higher values are more brown})\) was calculated. Tests were conducted 4 times.

Statistical analysis

Data analysis was performed using Statistical Analysis System (SAS) software, version 8.1 \((\text{SAS Inst., Cary, N.C., USA})\). The General Linear Models Procedure was used for analysis of variance, with main effect means separated by Student-Newman-Keuls test. Significance was defined as \(P \leq 0.05\).

Results and Discussion

Mechanical properties of film

The mechanical properties of the corn-zein laminated LLDPE films were measured by uniaxial tensile tests. The TS and %E of laminated films were determined from the attained stress-strain curves (Table 1).

The TS of simple LLDPE film was 31.33 MPa, which was the highest value among all tested films. When comparing to the simple LLDPE film, the TS of the corn-zein-laminated film \((\text{LLDPE}/\text{zein}/\text{LLDPE})\) decreased \((P \leq 0.05)\) by 28.6% \((22.38 \text{ MPa})\). The lower TS of the LLDPE/zein/LLDPE film was probably due to the physical weakness of the zein film layer. Rhim and others (1997) reported that the TS of simple zein film was 5.52 MPa, which is much lower than LLDPE film. In zein-laminated film groups, laminated films containing antioxidants had TS values ranging from 22.28 to 24.27 MPa. Thus, the addition of an antioxidant agent up to 5% \((\text{v/v})\) in the zein layer resulted in a slight but insignificant increase in TS \((P > 0.05)\). Generally, the TS of laminated films is dependent on the substrate or base film rather than the coating or laminated layer (Hong and others 2004). In this study, zein film was applied to LLDPE film as a coating material; LLDPE film was the substrate or base film in these laminated films. In this study, the TS of zein-laminated LLDPE film was
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22.38 MPa. Earlier studies reported that zein-coated polypropylene film had a TS of 45 MPa (Lee and others 2008), and the TS of zein-coated carrageenan films ranged from 26.38 to 37.73 MPa according to the concentration of zein-coating solutions (Rhim and others 1997). This difference may be attributed to the different substrate materials of the films.

The value of %E is associated with the elasticity and flexibility of the film. Values of %E for simple LLDPE and LLDPE/zein/LLDPE films were estimated to be 776.00% and 667.30%, respectively. Values of %E for LLDPE/zein/LLDPE film decreased by 14% in comparison to LLDPE film, but slightly increased when antioxidant agents were added. Gamage and others (2009) reported that the %E values of various essential oil-incorporated films were significantly higher compared to those of nonessential oil-incorporated films. These results indicate that antioxidant agents act as adhesives in the protein polymer matrix, similar to plasticizer. Therefore, the addition of essential oil improved the plasticity and %E of the films. Based on these results, antioxidants may be considered as potential replacements for plasticizers in protein biopolymer films. In addition, antioxidants can contribute positively to the mechanical properties of packaging materials composed of biopolymer.

Water barrier properties of films

WVP is determined by the chemical arrangement of film-forming polymers and the morphology of the film (Siriapatrawan and Harte 2010). WVP was calculated according to the aforementioned formula, and the results are depicted in Table 2. Simple LLDPE film had a lower WVP (1.778 g-mm/m²-d-kPa) than any other laminated film, indicating that LLDPE film provides a good barrier for moisture. In contrast, LLDPE/zein/LLDPE film had the highest WVP at 2.033 g-mm/m²-d-kPa, and thus performed the most poorly as a moisture barrier among the film samples tested in this study. Results showed that simple zein-laminated films had relatively high WVP compared to LLDPE film, which was probably due to the hydrophilic groups in zein molecules. These hydrophilic groups are formed from the amino acids that compose corn-zein protein, and that they weakened the water barrier properties of corn-zein-laminated film. The WVP of other laminated films containing phenolic antioxidant agents were estimated between 1.913 and 1.958 g-mm/m²-d-kPa. Regardless of the type of antioxidant agent used at 3% concentration (v/v), films containing antioxidants had improved water barrier properties when compared to simple zein-laminated film due to the strong hydrophobicity of the added antioxidant agents, which interrupts the penetration of water molecules. Thymol, carvacrol, and eugenol, which were used as antioxidant agents in this study, are hydrophobic and are major compounds of naturally occurring essential oils in thyme, oregano, and cloves. According to Sánchez-González and others (2011), the same behavior was observed when essential oils (bergamot, lemon, or tea tree oil) were incorporated into chitosan/hydropropylmethylcellulose composite films. They reported that WVP values of composite films showed a decrease in line with the increase in the concentration of hydrophobic antioxidant agent. The results of this study were in accordance with these observations.

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<tr>
<th>Table 1–Mechanical properties of zein-laminated LLDPE films with different antioxidant compounds and concentrations.</th>
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<tr>
<td>Film construction</td>
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<td>LLDPE</td>
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<td>LLDPE/zein/LLDPE</td>
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<td>LLDPE/zein with thymol 1.5%/LLDPE</td>
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<td>LLDPE/zein with carvacrol 1.5%/LLDPE</td>
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<td>LLDPE/zein with eugenol 1.5%/LLDPE</td>
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<td>LLDPE/zein with thymol 3%/LLDPE</td>
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<td>LLDPE/zein with carvacrol 3%/LLDPE</td>
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<td>LLDPE/zein with eugenol 3%/LLDPE</td>
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<tr>
<td>LLDPE/zein with thymol 5%/LLDPE</td>
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<tr>
<td>LLDPE/zein with carvacrol 5%/LLDPE</td>
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<tr>
<td>LLDPE/zein with eugenol 5%/LLDPE</td>
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<sup>ab</sup> Within a column, different superscripts indicate significant differences (P ≤ 0.05).

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<th>Table 2–Water vapor permeability of zein-laminated LLDPE films with different antioxidant compounds.</th>
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<td>Film construction</td>
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<td>LLDPE</td>
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<tr>
<td>LLDPE/zein/LLDPE</td>
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<td>LLDPE/zein with thymol 3%/LLDPE</td>
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<tr>
<td>LLDPE/zein with eugenol 3%/LLDPE</td>
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</table>

<sup>ab</sup> Within a column, different superscripts indicate significant differences (P ≤ 0.05).

Release into the atmosphere

GC analysis was used to measure changes in the concentration of antioxidants released from the developed films into the atmosphere (Figure 2). Overall, the maximum concentration of antioxidants in the headspace was correlated with the concentrations of antioxidants added to corn-zein film solution, and the released concentrations of antioxidants reached a maximum within 2 d in all experimental groups. Films containing higher concentrations of antioxidants also had faster diffusion rates of antioxidants into the air yet still maintaining higher concentrations of antioxidants than films with lower concentrations.

GC analyses of headspace gas composition revealed that the concentrations of diffused antioxidants in the container reached saturation after approximately 1 to 2 d, after which the amount of antioxidants was reduced or maintained. Groups containing thymol and carvacrol displayed a similar quantitative trend. There were no significant changes in the concentration of antioxidants in these groups after day 2, indicating that all antioxidants escaped from these films within 2 d.

The diffused antioxidants of zein-5% eugenol-laminated film displayed the highest concentration and the longest holding time in the gas state. In this film, concentration of eugenol remained at a high level for approximately 3 d and decreased over the following 2 d. From day 5 to day 7, eugenol levels inside the headspace remained constant. The maximum value of this group was observed up to day 3, and then it gradually reduced over last time period. This might suggest that eugenol leaked out of the container through the lid or septum of the container, even though the container was sealed carefully. However, LLDPE/zein/LLDPE films containing 1.5% and 3% eugenol showed a similar pattern, even though they contained different initial amounts of antioxidants.
Release into the liquid state

DPPH radical assays were performed with some modifications, to evaluate the effectiveness of the antioxidant agents released from the films into the liquid medium. DPPH radical scavenging activities of the samples consistently increased with storage time in all of the experimental groups, suggesting that there was a continuous release of antioxidant agents (Figure 3).

There were slight changes in the radical scavenging effects of LLDPE film and zein-laminated film even though they did not contain any antioxidant agent. These changes might have been caused by the instability of DPPH radicals resulting in a gradual extinction of radicals over time. By contrast, zein-laminated films containing antioxidant (thymol, carvacrol, or eugenol) displayed considerable changes in the activity of released antioxidants. Antioxidant film with eugenol had the maximum DPPH radical scavenging activity of all the tested films. In addition, the reaction mechanism of DPPH radicals and eugenol molecules released from the film was terminated much sooner than thymol and carvacrol, at a concentration one-tenth that of the others. After 21 h, absorbance at 517 nm of eugenol-containing film dropped below 0.1, which is a value that was too small to be measured correctly by a UV spectrophotometer with the optimum measurable absorbance range of 0.1 to 2.0. Therefore absorbance of this film was not evaluated after 21 h. The DPPH radical scavenging activities of thymol and carvacrol were similar, although thymol activity was not significantly higher than that of carvacrol, perhaps because they are isomers with the same molecular formula and a similar chemical nature.

Generally, the release of an active compound from the swelled polymeric network occurs in 2 steps. First, water molecules penetrate into the polymeric matrix, causing the matrix to swell. Then, active compounds diffuse through the widened mesh of the polymeric network (Del Nobile and others 2008). Therefore, release of an active compound is affected by the structural properties of the polymer or polymeric network and the diffusion rate of the active compound. However, in this study, the structural properties of the films were likely not factors because the antioxidant active films had the same structure as the LLDPE/zein/LLDPE film. Moreover, the 3 antioxidant compounds added to the developed films have similar molecular weights and water solubility, and therefore differences in diffusion rates were also negligible. Consequently, the factor that influenced DPPH radical scavenging activities the most was the antioxidant ability of the antioxidant agent. Yanishlieva and others (1999) reported that thymol is a better antioxidant than carvacrol due to the greater steric hindrance of the phenolic group in thymol. According to Mastelić and others (2008), eugenol exhibited outstanding DPPH radical scavenging activity, followed by thymol and carvacrol. This study reported similar results.

TBA values

In the diffusion test of antioxidants in a liquid state, zein-laminated film with 0.3% eugenol showed the greatest antioxidant
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...properties. Therefore, this film formulation was used for raw beef patty packaging in this study. TBA value was measured in order to examine the preventive effects of antioxidant-containing films on lipid oxidation of meat during storage (Figure 4). In all experimental groups, TBARS contents increased consistently throughout storage. The control group, over-wrapped with the oxygen-permeable PVC film, had a higher TBA value (P ≤ 0.05) than all of the vacuum-packaged groups. In contrast, vacuum packaging effectively protected beef patties from the beginning of storage, with a TBA value lower than 2 mg MDA/kg over the course of 14 d. There were few differences in TBA values among the vacuum-packaged groups, and vacuum-packaged patties with zein-laminated film with 3.0% eugenol had the lowest TBA values. Even though vacuum packaging alone inhibited lipid oxidation effectively, the developed antioxidant films demonstrated a synergistic effect in combination with vacuum treatment against lipid oxidation, and completely suppressed lipid oxidation during the 14 d. Specifically, TBA value of “vacuum only packaging” increased 86.1% after 14 d, but TBA values of “vacuum packaging with 0.3% eugenol-containing film” and “vacuum packaging with 3% eugenol-containing film” increased 40.9% and 27.7%, respectively, after 14 d. This result implied that the antioxidant films improved the efficacy of vacuum packaging and stabilized raw beef patties against oxidation. The antioxidant property of prepared film containing 3% eugenol was similar to that of the film containing 0.3% eugenol, which suggests that eugenol is a strong antioxidant and 0.3% concentration was sufficient to completely retard lipid oxidation in beef patties.

The inhibitory effect of eugenol on lipid oxidation is induced by the phenolic group, which contains an electron-repelling group in the ortho-position. This phenolic group is capable of reducing both lipid radicals and ferric iron (Dorman and others 2000). Ogata and others (1997) suggested that eugenol prevents lipid oxidation by trapping active oxygen species, such as O$_2^-$ or hydroxyl radicals.

Lipid oxidation has been associated with off-flavors and off-taste in meat and meat products. According to Insauti and others (2001), humans detect off-flavors or off-taste in meat when TBA values are greater than or equal to 5 mg MDA/kg. In this study, TBA values above 5 mg MDA/kg were only observed in the PVC-wrapped control group. In the other vacuum-packaged groups, TBA values remained below 2 mg MDA/kg at all times during storage.

Color changes

Color values including L* (lightness), a* (redness), and b* (yellowness) of the oxygen-permeable control group and the vacuum-packaged groups using antioxidant active films were measured and used to calculate the hue index. The color of meat is one of the most important factors in customer selection because color is an indicator of freshness (Mancini and Hunt 2005). Color changes during storage at 4 °C are presented in Table 3.

Treatment and storage time had no significant effects on L* and b* values of beef patties. In the control group, beef patty samples suffered considerable decreases in a* values, in contrast to the other groups which exhibited relatively high a* values. Vacuum packaging and the addition of eugenol made it possible to maintain initial a* levels over time. Allen and Conforth (2010) also observed that after 14 d, the a* value of beef patties mixed with 0.05% eugenol was significantly more reddish, and therefore more desirable to consumers, than the control group. Control samples had significantly higher (P ≤ 0.05) hue angle values (more reddish and less yellow) in comparison with the hue angle values of vacuum-packaged samples, regardless of eugenol existence within films. A significant increase in the hue angle value of a control sample...

Figure 4—Effects of packaging material on the TBARS values of beef patties after 14 d.

Table 3—Color value of beef patties packaged with the developed film.

<table>
<thead>
<tr>
<th>Color value</th>
<th>Type of packaging</th>
<th>Time of storage (d)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>L*</td>
<td>PVC wrap</td>
<td>36.44 ± 2.57$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>vacuum packaging only</td>
<td>38.09 ± 3.10$^{a,b}$</td>
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<tr>
<td></td>
<td>vacuum packaging with antioxidant film (0.3% eugenol)</td>
<td>36.77 ± 3.71$^{a,b}$</td>
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<tr>
<td></td>
<td>vacuum packaging with antioxidant film (3% eugenol)</td>
<td>34.96 ± 1.87$^{ab}$</td>
</tr>
<tr>
<td>a*</td>
<td>PVC wrap</td>
<td>13.57 ± 2.07$^{ab}$</td>
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<tr>
<td></td>
<td>vacuum packaging only</td>
<td>12.19 ± 0.97$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>vacuum packaging with antioxidant film (0.3% eugenol)</td>
<td>12.01 ± 1.01$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>vacuum packaging with antioxidant film (3% eugenol)</td>
<td>12.65 ± 1.19$^{ab}$</td>
</tr>
<tr>
<td>b*</td>
<td>PVC wrap</td>
<td>4.49 ± 1.17$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>vacuum packaging only</td>
<td>3.61 ± 1.50$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>vacuum packaging with antioxidant film (0.3% eugenol)</td>
<td>2.80 ± 0.81$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>vacuum packaging with antioxidant film (3% eugenol)</td>
<td>2.98 ± 0.66$^{ab}$</td>
</tr>
<tr>
<td>Hue angle</td>
<td>PVC wrap</td>
<td>18.09 ± 2.05$^{b}$</td>
</tr>
<tr>
<td></td>
<td>vacuum packaging only</td>
<td>14.54 ± 2.66$^{a,b}$</td>
</tr>
<tr>
<td></td>
<td>vacuum packaging with antioxidant film (0.3% eugenol)</td>
<td>13.06 ± 1.66$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>vacuum packaging with antioxidant film (3% eugenol)</td>
<td>13.24 ± 2.14$^{ab}$</td>
</tr>
</tbody>
</table>

Superscripted letters indicate significant differences (P ≤ 0.05).
Antioxidant packaging film...

indicated discoloration. The control group became brownish (decreased redness and increased yellowness) with an increase in storage time. Color change in the control group might be associated with lipid oxidation and the formation of methyglycol (Djene and others 2002; Nerín and others 2006). Beef patties vacuum-packed with antioxidant films showed lower changes in hue angle values. In these groups, the films acted as an oxygen gas barrier preventing the direct contact of meat with oxygen. Moreover, the strong antioxidant property of eugenol released from the films contributed to protection of the beef patties from lipid oxidation. These data suggest that diffusion of eugenol from the packaging delays oxidation of beef color pigments.

Conclusion

We developed zein-laminated LLDPE films with natural antioxidants and measured their physical and functional properties. In addition, diffusion modes of antioxidants in the gas and liquid states were estimated. Zein-laminated films with antioxidants had reduced TS and %E values compared to LLDPE films. These films also had higher WVVP than LLDPE film. The antioxidant film was tested in fresh beef patty packaging. Antioxidant films effectively retarded lipid oxidation and inhibited color change in beef during storage. These results suggest that zein-laminated films containing antioxidants are suitable for packaging applications and food protection.

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References


