

Stabilization of Lipids in a Biodegradable Zein-Oleate Film by Incorporation of Antioxidants

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ABSTRACT

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Zein has been studied extensively for use as an edible, biodegradable food film. To function as such, as much as 40–50% oleic acid is added as a plasticizing agent. Over the course of time, these films became discolored and developed off-odors. It was speculated that this was due to oxidation of incorporated oleic acid. This study sought to reduce oxidative changes in film lipids by incorporation of antioxidants. Incorporation of 4,000 ppm of BHA into the films eliminated peroxide formation during accelerated UV storage conditions. Gas chromatography and headspace (GC-HS) chromatography revealed that total volatile components of UV-treated control films were 9× higher than

fresh control volatiles. Comparison of the profiles led to the demonstration of major differences in peaks of a specific region, suggesting the presence of oxidation products in that region. Differences in UV-treated BHA films and UV-treated control films appeared in the same specific region of the chromatogram. Solid-phase microextraction analysis of volatile components of the various film components and films also demonstrated a major increase in peaks associated with oxidation due to UV treatment and reduction of oxidative products in UV-treated BHA films. Antioxidant treatment was thus found to protect the films against oxidative deterioration.

Edible polymer films have been prepared from various proteins and polysaccharides, including methyl cellulose, high-amylose starch, collagen, wheat gluten, soy protein, whey protein, and zein (reviewed by Krochta and De Mulder-Johnston 1997). Novel research in this area is fueled by consumer demands for high-quality foods and for environmentally friendly packaging. Zein has been studied extensively as a protein source for biodegradable/edible food films (Lai and Padua 1997, 1998; Lai et al 1997).

Natural animal intestines used as sausage casings are one of the earliest edible packaging films (Hood 1987). Irregular quality, extensive labor needs, and higher production speed have brought about the widespread usage of manufactured casings. Collagen, a fibrous structural protein extracted from beef hides, has been the most successful material used in making edible casings (Pearson and Tauber 1984). In recent years, however, concern about the safety of the collagen supply, particularly imported collagen, has arisen due to the risk of bovine spongiform encephalopathy (BSE) or “Mad Cow Disease”.

Although no cases of BSE have been found in the United States, the reliance on imported collagen by casing manufacturers remains troublesome. This has motivated a search for alternative casing materials by sausage manufacturers. The potential for vegetable-protein-based sausage casings has been explored in past years (Turbak 1972). This has fueled some of our interest in the development of zein as a food packaging material.

Zein, a storage protein in corn, is useful to the food industry mainly because of its film and glaze-forming ability. It is an abundant by-product of the starch-gluten separation step in the corn wet-milling process. Roughly 60% of protein in gluten is in the form of zein (Reiners et al 1973). For every bushel of corn processed, 11.4 lb of gluten feed (20% protein) and 3 lb of gluten meal (60% protein) is produced (National Corn Grower’s Association 1995). Increased demands for starches, sweeteners, and ethanol produces an abundance of lower value gluten feed and meal. This has spurred the development of new, more profitable applications of corn zein.

The use of zein as a material for film formation allows the ability to produce high quality vegetable-based films for use with foods. Yet, the optimal formulation requires the use of a plasticizing agent; oleic acid was used at ≈40–50% of the weight of the film (Lai et al 1997;

Lai and Padua 1998). Zein film properties have been demonstrated to be dependent on the type and amount of fatty acids added as plasticizers (Padua et al 1997). Stearic and palmitic acids produced stiff and brittle materials of high tensile strength and high elastic modulus (Lai et al 1997). Samples containing oleic or linoleic acids were flexible and elastic, showing low modulus and high toughness (Lai and Padua 1997). Oleic acid was selected over linoleate because of these effective plasticizer properties, and because higher degrees of unsaturation in fatty acids leads to greater susceptibility to oxidation. It was our hypothesis that, on storage, zein-oleate films would undergo significant deterioration as a result of fatty acid oxidation and that this could be prevented by the incorporation of antioxidants. Thus, the objective of this study was to investigate the incorporation of antioxidants into zein-based films for stabilization of lipids against oxidation.

MATERIALS AND METHODS

Materials

Regular grade corn zein (F4000) was obtained from Freeman Industries, Inc. (Tuckahoe, NY). The antioxidants BHT, BHA, and α -tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO). Oleic acid and other chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI).

Preparation of Zein Film

Resin was prepared by dissolving 202.5 g of corn zein in 75% ethanol (technical grade). Oleic acid (202.5 g) was added and the mixture was blended (Omni-Mixer, Ivan Sorvall, Newton, CT) at 3,500 rpm for 10 min at 55–60°C. The resulting emulsion was cooled at room temperature to 40°C before precipitation in chilled water. The resulting doughlike resin was immersed in fresh chilled water (4°C) and refrigerated for 12–24 hr. The resin was kneaded using a farinograph (C.W. Brabender, Hackensack, NJ). The temperature of the bowl was maintained at 40°C for the first 20 min and at 20°C for the last 10 min. The resin was held at 4°C in resealable plastic bags until films were prepared. Films were produced by hand, stretching resins over the rims of plastic containers to form circular membranes or films. Those were left to air dry in a dark cupboard for 17 hr. Films measured 11 cm diameter and 0.1–0.2 mm thick.

Antioxidant was added to the film in one of two ways: spraying (SP) or direct incorporation (DI). Direct incorporation involved dissolving the antioxidant in 95% ethanol and stirring into the resin after cooling and before precipitation. For spraying, the antioxidant was dissolved in 100% ethanol that would deliver the desired concentration in ≈1 mL of sprayed solution. A 125-mL reagent sprayer

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(Kontes, Vineland, NJ) was used to spray the solution. The exact volume delivered was calculated by measuring volume difference of solution before and after spraying.

Storage of Films and Oleate

Films were stored in resealable plastic bags and kept in a dark drawer at ambient temperatures before light treatment and analysis. To prevent scalping of odors from the plastic bags before headspace analysis, aluminum foil was used to encase the films during storage. Oleate was stored at 4°C under nitrogen before storage and analysis.

Accelerated oxidation of samples was induced by exposing film and oleate to UV light for 10 hr. For oleate oxidation trials, an amount of oleate equal to the amount in three films was spread in an 11-cm petri dish. A box with a 30W germicidal UV lamp (General Electric, Fairfield, CT) was used. The distance between the sample and the lamp was 19 cm. Sample location within the box was completely random. Temperature inside the box ranged from 18–26°C during 10 hr of exposure. Analysis began immediately after the light treatment. Other trials were conducted using a combination of heat and oxygen as a more realistic condition of oxidation. Films were stored in 2L plastic containers at 37°C. A continuous flow of oxygen was passed through the system. Films were checked periodically for oxidation odors.

Lipid Extraction

Lipids were extracted from films using Soxhlet extraction. Three films of each sample replicate were cut into small (0.5 mm²) uniform pieces with a razor blade. Samples of ≈0.8 g were extracted with 500 mL of HPLC-grade hexane for 48 hr. Extracts were dried almost to completion on a rotary evaporator (Rotavapor R111, Büchi, Zurich, Switzerland) and then were quantitatively transferred to a screw-top test tube. Drying was completed under nitrogen.

Free Fatty Acid Analysis

Measurement of free fatty acids (FFA) was conducted on films according to Official Method Ca 5a-40 (AOCS 1998). This protocol was slightly modified to accommodate smaller oil samples. Neutralized isopropyl alcohol (50 mL) was used as the solvent and 0.01N NaOH was used as the titrant.

Peroxide Value Analysis

Fresh and stored oleate and lipid extracted from fresh and stored films were iodometrically titrated to determine peroxide value (PV). Measurements were made following Official Method 965.33 (AOAC 1995).

Gas Chromatography-Headspace (GC-HS) Analysis

All measurements were made on a purge and trap concentrator (Tekmar 3000, Cincinnati, OH) and an HP5890 series II gas chromatograph (Hewlett Packard, Palo Alto, CA). Each treatment was analyzed in triplicate. A sample of 20 mg was placed into a 32-mL sample tube. The sample was heated to 90°C.

Tubes were purged with helium gas for 11 min (40 mL/min) at 40°C and cryofocused onto a Tekmar trap containing Carboxpak B and Carboxieve SIII at -185°C. The cold trap was desorbed (10

mL/min, 15 min) at 240°C, and volatile compounds were transferred (line temperature 180°C) onto the GC column.

GC oven temperature was initially kept at 40°C for 5 min and was programmed to rise 5°C/min until a temperature of 250°C was reached. The maximum temperature was maintained for 6.5 min. Separation was performed on a DB-WAXetr column (J&W Scientific, Folsom, CA), 50 m long × 0.32 mm i.d. × 1.0 μm film, split with two 0.3 m × 0.25 mm deactivated fused silica lines to FID and FPD (sulfur mode) detectors.

Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry (SPME-GC-MS)

A sample of either 100 mg of film or 5 mg of zein was placed into a 22-mL vial and fitted with a septum lid. The sample was pretreated in a 60°C water bath for 10 min and then a SPME fiber (Carboxen/polydimethylsiloxane SPME fiber, 57344-U; Supelco, Bellefonte, PA) was exposed to the vial headspace for 20 min. Volatile compounds were desorbed by injecting the fiber into a splitless GC injector (injector temperature 240°C, splitless time 4 min, vent flow 50 mL/min) and analyzed by GC-MS.

An HP6890 GC/5973 mass selective detector (MSD; Agilent Technologies) was used. A fused-silica capillary column DB-Wax (50 m × 0.32 mm i.d. × 1 μm film thickness) was operated with helium at a constant flow of 1.2 mL/min. GC oven temperature was programmed from 40 to 225°C at a rate of 4°C/min with initial and final hold times of 5 and 30 min, respectively. MSD conditions were 250°C capillary direct interface; 70 eV ionization energy; 35–300 a.m.u. mass range; EM voltage (Stune + 200 V); 5.27 scan/sec scan rate. Compound identifications were based on comparison of GC retention indices and mass spectra of unknowns with those of authentic reference compounds analyzed under identical experimental conditions.

Color

Samples were cut in pieces 0.5 mm² and placed as a uniform layer in an 11-cm diameter petri dish. A colorimeter (HunterLabScan XE, Reston, VA) was calibrated with white and black standard tiles. Color was recorded where *L** indicates lightness, *a** indicates hue on a green (-) to red (+) axis, and *b** indicates hue on a blue (-) to yellow (+) axis. Readings were taken at randomized locations over the surface of samples. The average of three readings was recorded per sample.

Tensile Properties

Films were laminated to achieve a uniform sample for mechanical testing. A three-ply laminate was prepared using a laboratory press (model C, Fred. S. Carver, Menomonee Falls, WI). Films were stacked so that obvious striations of individual films would run in the same direction. Parameters for pressing films were 210°C under a load of 5 psi for 5 min for the BHT/control sample set, and 160°C under a load of 4 psi for 1 min for the BHA/control set. Parameters were optimized to avoid melt-out, which occurred if the temperature was too high.

Tensile properties were determined using Standard Method D638-91 (ASTM 1994). Film was cut into barbell shape specimens of

TABLE I
Effects of Resin Processing and Film Lamination^a on Free Fatty Acids in ≈50% Oleic Acid Zein Films

Sample	Treatment	Film Wt. (g)	% Oil of Film	% FFA ^b	Replicates
Control	Fresh	0.6764	52.4	100 ± 3.4	3
	Laminated	0.6862	52.1	101 ± 4.8	2
	UV	0.8394	52.6	94.4 ± 0.57	3
BHT, 100 ppm	Fresh	0.7373	51.6	98.8 ± 0.72	3
	Laminated	0.7058	52.9	99.1 ± 0.69	2
BHA, 4,000 ppm	UV	0.8470	56.2	91.3 ± 2.6	3

^a Laminating parameters: three layers of film, 5 psi, 5 min, 210–220°C.

^b % Free fatty acids shown as mean values ± standard deviation.

Type I dimensions, taking one specimen per film. Mean thickness was calculated from five random measurements taken with a dial-gauge micrometer (B.C. Ames, Waltham, MA). Samples were pre-conditioned at 23°C and 50% rh for 48 hr before testing. A testing system (model 1011, Instron Engineering, Canton, MA) measured tensile strength. Samples were placed, machine-direction, in pneumatic grips on the testing machine. Initial grip separation was 10 cm and the extension rate was 5 mm/min. Eight to ten replicates were run for each sample.

Data Analysis

Statistical software (v. 7.00, SAS Institute Inc., Cary, NC) was used for statistical analysis. Data were treated by analysis of variance (ANOVA) with differences between means compared by least significant differences.

Sensory Analysis

Two treatments, control and a test film with 4,000 ppm of BHA (DI), were evaluated for difference by the paired comparison method with the hypothesis that a detectable difference in aroma exists between the two films after 10 hr of exposure to UV light. A total of 23 panelists (15 females and 8 males) were used with no replicates.

Each circle of film was cut into small pieces (0.5 mm²) immediately after UV storage was completed, and a portion 0.2–0.3 g was put into a 20-mL glass vial and covered with a plastic-foil lined screw-top lid until testing (≈2 hr). All samples were coded with random three-digit numbers and presented at room temperature. Four possible orders were counterbalanced.

Test subjects were recruited from faculty, staff, and students from the University of Illinois. No prescreening was used. Panelists were seated in isolated booths under ambient temperature and humidity. A fluorescent red light was used to eliminate any biases due to color differences. Testing was conducted in three separate afternoon sessions. The subjects were instructed to choose the sample that seemed “most rancid” or “painty” smelling. The number of correct responses was analyzed with the table for critical number of correct responses in a paired comparison given by Meilgaard et al (1999).

Functionality Tests

An ersatz sausage casing was made from the zein-oleate films to test under typical storage (refrigeration and freezing) and cooking (boiling and microwaving) conditions that might be encountered. Three replicates of both control and films with 4,000 ppm of BHA (DI) were tested. Each circle of film was folded in half and two edges were sealed (Dazey Micro-Seal, Industrial Airport, KS). Freshly

made casings were dropped into boiling water and removed after 5 min. Casings were also microwaved on high power for 4.5 min in a microwave oven (model R-3A88; Sharp Electronics, Mahwah, NJ). To test the effects of cold storage, casings were put in a resealable plastic bag and kept at –20 and 4°C. Casings were checked for changes in appearance at various intervals.

RESULTS AND DISCUSSION

Free Fatty Acids (FFA)

The recovery of lipid from the films matched their approximate 50% content of oleic acid (Table I). All freshly prepared films and laminated films had >98% FFA. This indicates that little or no polymerization or breakdown of oleic acid occurred during resin preparation or resulted from film lamination. This lack of oxidation of resin was anticipated. Light and heat are catalysts of oxidation (Coe 1937; Rhee and Stubbs 1978), and films were stored in the dark at 4°C to prevent this. The extent of lipid recovery and the high % FFA suggests that oleic acid did not bind to zein protein during processing.

Use of high levels of free fatty acids as plasticizers in films may adversely affect the food and packaging system. Free fatty acids from foods are known to migrate into food packaging (Arora and Halek 1994). Problems with adhesion of laminated cartons have been blamed on such migrations (Pieper and Petersen 1995). Therefore, the effects of lipid migration from zein films may be important in choosing secondary packaging materials if they are necessary.

Evaluation of BHT Levels and Application Methods in UV-Exposed Films

Peroxide values (PV) of films with 0–20,000 ppm of BHT were determined (Fig. 1). Adding 100 ppm directly to the resin-inhibited oxidation, but to a limited extent. This minimal inhibition of oxidation would not be sufficient to prevent oxidative deterioration. Although films with 1,000 ppm of BHT had higher PV, they were not statistically different than films with 100 ppm of BHT. The lack of effectiveness of BHT at these concentrations was theorized to be due to its volatilization. This is consistent with the finding of Hannah (1962) that BHT-treated cereal liners lost up to 87% of antioxidant after about four months storage, with only 0.5% lost to the cereal through migration. In fact, up to 82% was lost between the time of application and the start of the study (10 days), with only 0.33% lost to the cereal. Therefore, the alternative method of spraying was investigated. Spraying at 500 ppm was equally as effective as 100 and 1,000 ppm DI. Although not statistically different than 100 ppm DI, PV of 4,000 ppm SP and 20,000 ppm SP showed a downward trend.

Comparisons of Antioxidant Types

The fact that BHT was not as effective as anticipated led to the investigation of BHA and tocopherol, two commonly used phenolic antioxidants. Tocopherol at 100 ppm (DI) did not effectively control oxidation under UV storage (Table II). The mean PV was not statistically different from that of the control film exposed to UV and was significantly higher than 100 ppm BHT (DI). Bose and Chatterjee (1994) found that tocopherol inhibited hydroperoxide

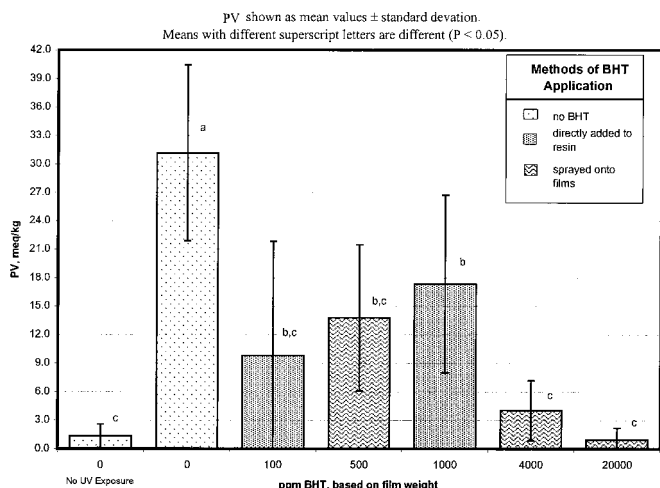


Fig. 1. Effect of BHT levels on peroxide values (PV) in UV-exposed (10 hr) films.

TABLE II
Influence of BHT and Tocopherol at 100 ppm on Peroxide Values (PV) of UV-Exposed Films

Treatment	PV (meq/kg) ^{a,b}	Replicates
Control, no UV	1.35 ± 1.2b	4
Control, 10 hr UV	31.2 ± 9.3a	9
BHT, 10 hr UV	9.78 ± 1.2b	8
Tocopherol, 10 hr UV	25.0 ± 4.8a	4

^a PV are mean values ± standard deviation.

^b Values followed by the same letter are not significantly different ($P < 0.05$).

formation in dried egg lecithin films exposed to UVA light to a greater extent than did BHT. Crystallization of BHT during drying and formation of the films was blamed for poor inhibitory results. A more recent study on marine oils comparing the performance of some common antioxidants found that 200 ppm of BHT was more effective at lowering peroxide values than 500 ppm of tocopherol in both seal blubber and menhaden oils (Wanasundara and Shahidi 1998). After six days of storage at 65°C Schaal oven conditions, PV was reduced by 9 and 19% with tocopherol and by 48 and 60% by BHT in seal blubber oil and menhaden oils, respectively.

BHA added at 4,000 ppm was as effective as the same concentration of BHT (Table III). Incorporating BHA directly into the resin eliminated peroxide formation during UV storage. The stronger inhibitory effect of BHA over BHT is thought to be due to its lower volatilization. Hannah (1962) found that when antioxidant-treated waxed papers were exposed to the atmosphere, 50% of BHA was retained while BHT was completely lost after 24 hr. The less volatile nature of BHA, and its effectiveness at suppressing oxidation as suggested by low PV, prompted its further use in our studies. Because DI was less laborious and resulted in 0 PV at 4,000 ppm, it was the method used in these studies.

Protective Effects of Zein

The PV of oleic acid alone when stored under conditions conducive to oxidation rose dramatically from 0 to 182 meq/kg (10 hr of UV storage) and 552 meq/kg (two weeks and two days of heat/O₂ storage) (Table IV). PV of films without antioxidant stored under UV light (10 hr) were significantly lower. Additionally, films stored with heat and oxygen (two weeks and two days) had PV <math>< 1</math>. This data reveals that the zein interactions with oleate in films is protective against oxidation under these storage conditions. The known antioxidative properties of zein explains these findings. The antioxidative effect is influenced by water activity (Wang et al 1991). Tocopherols in zein (usually 5.8 ppm) were thought to be largely responsible for antioxidative activity at ambient humidity.

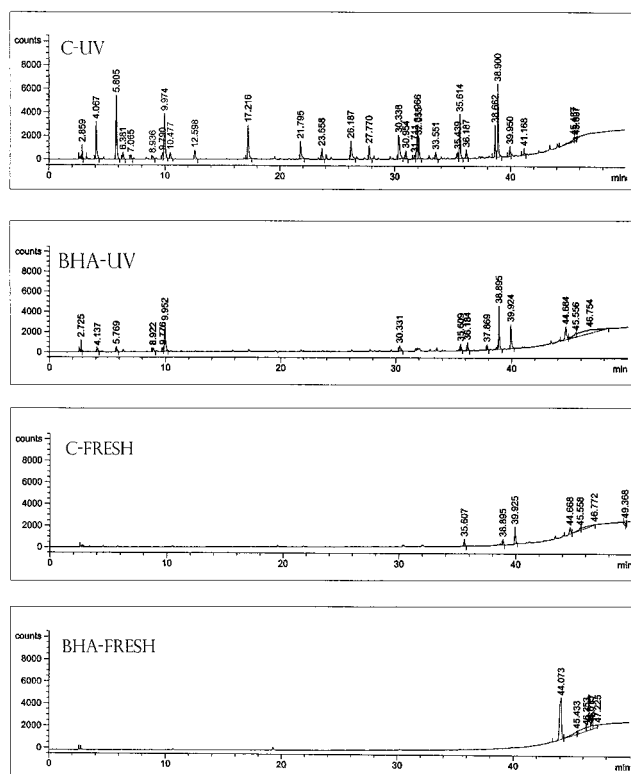


Fig. 2. Gas chromatography and headspace (GC-HS) analysis of stored and fresh zein films. C = control; UV = ultraviolet light treated (10 hr).

However, at a higher A_w (0.9) hydrophobic residues of zein were thought to delay oxidation by either shielding oil from oxygen or binding it to protein.

GC-HS Analysis

GC-HS chromatograms (Fig. 2) revealed that the areas of total volatile components of UV-treated control (C-UV) films were 9× higher than those of fresh control (C-Fresh) volatiles. Additionally, the profiles of C-Fresh and C-UV films demonstrate major differences at 10–30 min, suggesting that oxidation products appear in that region, with the exception of a peak at 10.7 min. Differences in UV-treated BHA (BHA-UV) films and C-UV also lie in that specific region of the chromatogram.

Chromatograms of individual components of zein-oleate films exposed to UV (oleate-UV, zein-UV, and BHA only-UV) (Fig. 3) helped identify peaks associated with oleate oxidation and breakdown products of zein and BHA. The absence of peaks at 10–30 min of zein-UV and BHA only-UV confirms that peaks in that time period are most likely lipid oxidation products. The peak at 10.7 min is apparently contributed by zein.

SPME-GC-MS

Solid-phase microextraction (SPME) analysis of volatile components of the various film components and films is presented in Table V. Five volatile compounds were identified in fresh zein powder. The largest peak at 8.68 min was identified as isopropanol, the solvent used in the processing of commercial zein. None of the compounds identified in zein showed up in any of the zein-oleate films. The conditions of processing the film resin are probably responsible for their absence. Volatiles may be driven off by thermal treatment during blending of the zein and oleic acid mixture. Residual amounts are most likely dissolved in the water during precipitation and overnight refrigeration.

A total of 24 volatile compounds were identified in zein-oleate films using SPME-GC-MS. A constant sample size allows for the relative comparison of their amounts. Chromatograms of fresh films, UV-stored films, and fresh zein powder are presented in Figs. 4–6, respectively. UV light increased total volatiles of control films by ≈481%. BHA decreased total volatiles of UV films by 60%. Subtracting BHA itself, which eluted at 40 min, from total volatile increases the reduction to 62%.

TABLE III
Influence of BHT and BHA at 4,000 ppm on Peroxide Values (PV) of UV-Exposed Films

Antioxidant Type	Application Method	UV (hr)	PV (meq/kg) ^{a,b}	Replicates
Control	None	0	1.35 ± 1.2b	4
	None	10	31.2 ± 9.3a	9
BHA	Direct	0	0.00 ± 0.0b	5
	Spray	10	2.12 ± 2.9b	7
BHT	Direct	10	0.00 ± 0.0b	3
	Spray	10	4.02 ± 3.1b	6

^a PV are mean values ± standard deviation.

^b Values followed by the same letter are not significantly different ($P < 0.05$).

TABLE IV
Comparison of Peroxide Values (PV) of Zein Films and Oleic Acid Under Various Storage Conditions

	Treatment	Replicates	PV (meq/kg) ^a
Oleic acid	Control, fresh	6	0.00 ± 0.0
	UV, 10 hr	3	182.2 ± 20.8
	O ₂ /heat, 2 weeks, 2 days	3	551.7 ± 10.7
Zein films	Control, fresh	4	1.35 ± 1.2
	UV, 10 hr	9	31.2 ± 9.3
	O ₂ /heat, 2 weeks, 2 days	2	0.70 ± 0.01

^a PV are mean values ± standard deviation.

Acetic acid was the only peak identified in the fresh films. UV treatment resulted in a substantial increase in number of peaks. Many compounds identified in C-UV films were either completely eliminated (pentanal, 2-octenal, 2-nonenal, 2-decenal, 1-pentanol, 1-hexanol, pentanoic acid, hexanoic acid, 5-hexenoic acid, 2-ethyl-hexanoic acid, 2-pentyl-furan, and toluene) or substantially reduced (2-methyl butanal, hexanal, heptanal, benzaldehyde, heptanol, 1-octanol, and acetic acid) in BHA-UV films.

Pentanal and hexanal are useful indicators for monitoring lipid oxidation in soybean oil (Warner et al 1978). In the current study, reduction of hexanal by 47% and elimination of pentanal by incorporation of BHA and the considerable reduction in peak numbers and area counts indicate suppression of lipid oxidation.

A few of the identified peaks are not associated with lipid oxidation. Toluene was identified by Huang et al (1987) as a carotenoid oxidation product in zein. Its presence in C-UV films but not in BHA-UV films explains the difference in color results. The aldehydes 2-methyl butanal and 3-methyl butanal, previously found in corn products (Buttery et al 1994; Buttery and Ling 1998) were also produced. These are aromatic products of the Strecker degradation pathway in the Maillard reaction. As this involves the reaction of reducing sugars with free amino groups, it is assumed that zein contains carbohydrate impurities. Promotion of the Maillard reaction by heat (Baltes, 1982) accounts for presence of Strecker aldehydes in films made from heat-processed resins and their absence in fresh zein powder.

Sensory Analysis

Eleven of 23 panelists (48%) correctly identified the control sample as "most rancid" indicating that with an $\alpha = 0.05$ of Type I error, there is no detectable difference in aromas of zein-oleate films made with and without BHA. This is in direct contrast to the demonstrated lowering of peroxide values. The difficulty in detecting rancidity may have arisen from the complex aroma profile of the films. White

and Miller (1988) noted that flavors not related to oxidation might have contributed to flavor intensity scores in soybean oils. Likewise, the panelists in this study may have confused intensity of odor with rancidity even though contributing aromas in films may or may have not been associated with lipid oxidation. Additionally, the variety of descriptors for oxidized odor and flavor makes discrimination difficult for untrained panelists who may have incompatible ideas about rancidity.

Color

All of the colorimeter values were influenced by UV exposure (all significantly different at $P < 0.05$). The values presented in Table VI indicate that control films are lighter in color when exposed to UV light. The higher L^* value of control indicates lighter color. The lower a^* and b^* values indicate loss of redness and yellowness. Rhim et al (1999) reported similar findings in UV-treated zein films.

The pigments responsible for yellow, orange, and red colors in plant tissues are mainly carotenoids. This lipid soluble class of pigments occurs naturally in corn in the form of zeaxanthin, lutein, and β -carotene (Konings and Roomans 1997). Their oxidation is facilitated by a substantial system of conjugated double bonds. The oxidation of carotenoids by light and heat has been demonstrated in the past (Chen et al 1996). Woodall et al (1997) found

TABLE VI
Effects of 4,000 ppm of BHA and 10 hr of UV Light on Hunter Colorimetry Values of Zein Films^{a,b}

Sample	L	a	b
BHA-UV	60.67 ± 1.27b	3.28 ± 0.80a	30.90 ± 1.48a
Control-UV	70.08 ± 0.78a	-1.60 ± 0.48b	19.52 ± 0.21b

^a Three replicates for each treatment. Values means ± standard deviation.

^b Values followed by the same letter are not significantly different ($P < 0.05$).

TABLE V
Area Counts^a of Volatile Compounds Identified by Solid-Phase Microextraction-Gas Chromatography-Mass Spectrometry (SPME-GC-MS)

Peak Identification	Retention Time (min)	Zein Powder	Control	Control-UV	BHA	BHA-UV
Dimethyl sulfide	4.84	—	—	7.06×10^6	—	2.92×10^7
3-Methyl butanal	8.13	—	—	1.48×10^7	—	4.09×10^7
2-Methyl butanal	8.25	—	—	1.10×10^9	—	5.64×10^7
Isopropanol	8.68	9.99×10^8	—	—	—	—
Pentanal	9.94	—	—	2.23×10^7	—	—
Toluene	11.64	—	—	1.87×10^7	—	—
Hexanal	12.87	—	—	3.99×10^7	—	2.10×10^7
Heptanal	15.70	—	—	2.61×10^7	—	1.11×10^7
2-Pentyl-furan	16.85	—	—	5.89×10^7	—	—
1-Pentanol	17.35	—	—	5.46×10^7	—	—
Octanal	18.30	—	—	1.79×10^7	—	2.08×10^7
1-Hexanol	19.75	—	—	2.90×10^7	—	—
4-Hydroxy-4-methyl-2-pentanone	20.08	1.40×10^7	—	—	—	—
Nonanal	20.71	—	—	2.57×10^7	—	8.04×10^7
2-Octenal [E]	21.52	—	—	1.04×10^7	—	—
Acetic acid	21.79	—	6.88×10^7	1.16×10^9	7.57×10^7	4.93×10^7
Heptanol	21.98	—	—	7.92×10^9	—	9.45×10^6
1-(2-Methoxy-1-methoxy)-2-propanol	22.66	6.93×10^7	—	—	—	—
1-(2-Methoxypropoxy)-2-propanol	23.50	1.41×10^8	—	—	—	—
Benzaldehyde	23.56	—	—	5.16×10^7	—	9.76×10^6
2-Nonenal [E]	23.74	—	—	1.16×10^7	—	—
1-Octanol	24.07	—	—	8.52×10^7	—	2.53×10^7
2-(2-Ethoxyethoxy)-ethanol	25.42	2.36×10^8	—	—	—	—
2-Decenal [E]	25.85	—	—	1.15×10^7	—	—
Pentanoic acid	27.45	—	—	2.81×10^7	—	—
Hexanoic acid	29.34	—	—	1.06×10^9	—	—
5-Hexenoic acid	30.39	—	—	9.47×10^7	—	—
2-Ethyl-hexanoic acid	30.84	—	—	1.65×10^7	—	—
BHA	40.02	—	—	—	2.35×10^8	6.67×10^7
Total unknowns		1.05×10^8	3.90×10^7	1.07×10^9	3.21×10^7	1.40×10^8
Total volatiles ^b		1.18×10^9	7.85×10^8	4.56×10^9	1.11×10^9	1.81×10^9

^a Area counts presented as mean values of duplicates.

^b Total volatiles include unidentified peaks and silane compounds produced by SPME

that oxidative degradation of zeaxanthin and lutein, major carotenoids in corn, may proceed by peroxy radicals when in solution. Degradation and isomerization of carotenoid pigments has a bleaching effect in powder (Tang and Chen 2000) and juice systems (Pesek and Warthen 1987). In the present study, results indicate that 4,000 ppm of BHA protects zein carotenoids from oxidation.

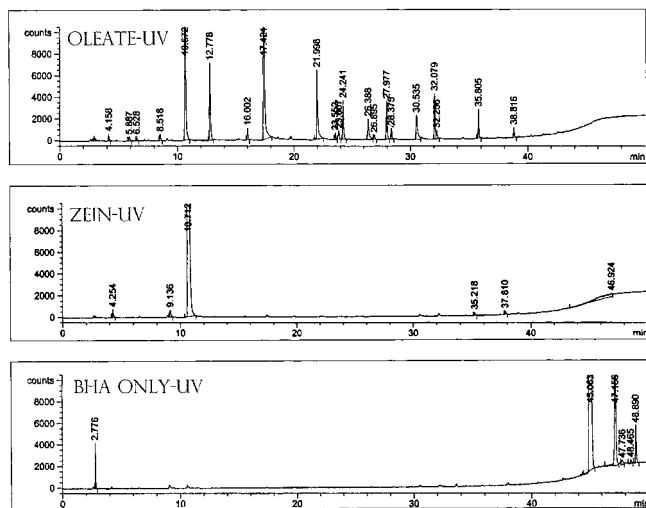
Tensile Strength (TS) Testing

Tensile strength is defined as force per unit area, or the maximum pulling stress at which failure occurs. TS of control films and 100 ppm BHT films as a function of UV exposure were compared. BHT had no effect on either fresh or stored films (4.68 ± 0.76

MPa and 4.46 ± 0.42 MPa, respectively). UV storage lowered TS by 17% in BHT films and 14% in control films.

Results of tests comparing 4,000 ppm BHA films and control films showed that TS of fresh films were not significantly different. This is in agreement with findings by Herald et al (1996) that 100 ppm BHA did not affect TS of fresh zein films. In the present study, TS of fresh BHA (2.17 ± 0.35 MPa), C-Fresh (1.92 ± 0.14 MPa), and C-UV (1.95 ± 0.24 MPa) films were not significantly different. However, TS of BHA-UV films (2.45 ± 0.42 MPa) was 25% higher than TS of C-UV films.

The effects of plasticizing agents on tensile properties of zein films have been studied extensively (Lai et al 1997; Parris and Coffin 1997; Santosa and Padua 1999). In general, TS decreases with increasing plasticizer content; the TS reported in this study are considerably lower than those reported by others in similar films. Oleic acid at a 50% (w/w) level provided TS of 8–9.5 MPa (Lai and Padua 1997, 1998; Santosa and Padua 1999) in single layer



Amounts of individual components were equal to the amount analyzed in a film sample. UV exposure was equal to that of films (10 h).

Fig. 3. Gas chromatography and headspace (GC-HS) analysis of individual components of zein films exposed to UV light.

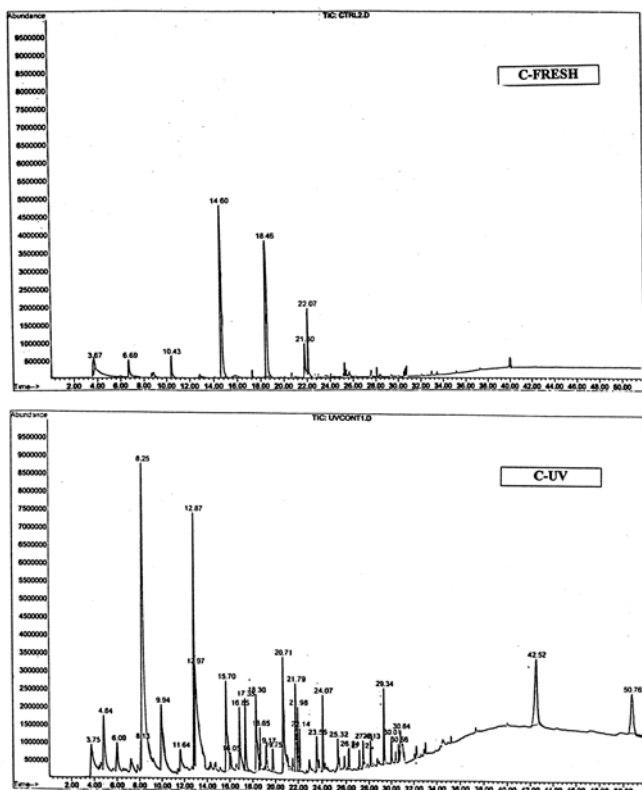


Fig. 4. Solid-phase microextraction and gas chromatography (SPME-GC) of fresh and UV-exposed control (C) films.

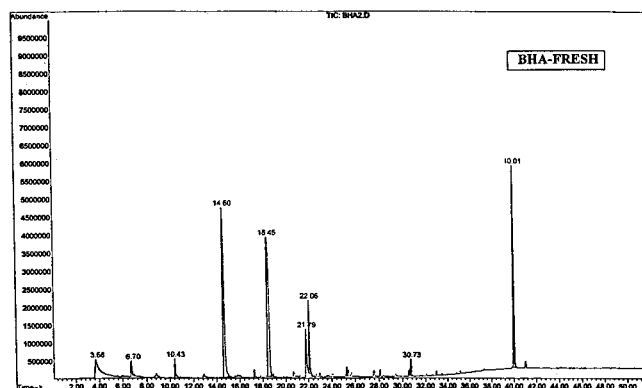


Fig. 5. Solid-phase microextraction and gas chromatography (SPME-GC) of fresh and UV-exposed films with 4,000 ppm of BHA.

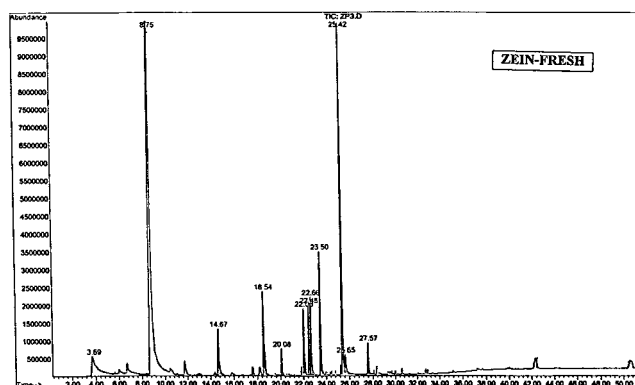


Fig. 6. Solid-phase microextraction and gas chromatography (SPME-GC) of fresh zein powder.

films. Rakotonirainy (2000) found that lamination of three or more layers of zein films increased TS from 2.1 to 3.0 MPa. These values are more in order with TS values found in this study.

Irradiation by UV light increases TS in zein and other protein films. Rhim et al (1999) suggested cross-linking within the film structure of cast zein films was responsible for a 20% increase in TS with 24 hr of UV irradiation. Cast films are, however, more homogeneous in nature than resin films. Resin films are made of many layers and often have imperfections. The conflicting data on the effect of UV light on zein films reported in this study suggest that lamination itself or lamination conditions may have influenced irradiation effects.

Storage Performance

No visible changes occurred in any films after 24 hr of refrigerated storage. White patches were observed on control and BHA films after 48 hr of storage; these were believed to be due to precipitation of the oleate. Precipitation was extensive at 20 days. Oleate solidifies at 4°C to a crystalline mass (Windholz et al 1983). Therefore, precipitation of oleate from the films was not unanticipated; this suggests that oleate and zein protein were not tightly bound.

Frozen storage is typically longer than refrigerated storage for foods. For that reason, films were frozen for a longer time period (two weeks). No visible changes occurred in frozen films after 3 hr of freezing. Freezing for one week brought about precipitation of oleate. No dramatic alterations were observed in the films between one and two weeks. With the exception of one of the three BHA films, there were no large white patches in frozen films. Crystallized oleate was more uniformly distributed throughout in frozen films.

Cooking Performance

All films became misshapen after 5 min of boiling. Most films had some loss of shape during the boiling period. However, total loss of shape occurred in all films on removal from the water. The sticky nature of the boiled films caused them to clump together when handled. Microwaved films did not lose their shape and remained flexible. There was little difference in appearance between microwaved and nonmicrowaved films after 4.5 min. Microwaving seemed to melt the oleate only very slightly.

CONCLUSIONS

Zein-oleate films stored for periods up to one year at ambient conditions have been affected deleteriously. Films became lighter in color, were less flexible, and off-odors were apparent. It is believed that lipid oxidation is responsible for these changes. Time limits have restricted investigation of these long-term effects. This research was designed to mimic oxidation conditions under accelerated conditions. In this system, we have effectively demonstrated the protection of lipids in zein films against oxidation by incorporation of antioxidant. Anticipation of development of other edible films using lipids as plasticizing agents lends importance to these findings. While previous studies have investigated antioxidant use in films and coatings to protect foods, this is the first study to investigate antioxidants to suppress oxidation of the edible film itself.

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