Retardation of lipid oxidation using gelatin film incorporated with longan seed extract compared with BHT

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Abstract The aim of the present work was to apply the gelatin films with different levels of longan seed extract (LS) or butylated hydroxytoluene (BHT) on retardation of lipid oxidation in soybean oil. The films incorporated with various concentrations of aqueous LS (0, 50, 100, 300, and 500 ppm) or BHT (50, and 100 ppm) were developed. The films had transmittance percentages of 60–80 % at 570 nm and showed good light barrier properties when the concentration of LS or BHT increased. About 97 % protein solubility and 41 to 54 % water solubility were obtained for the developed films. Antioxidative activity of gelatin films incorporated with LS increased markedly with increasing storage time as indicated by the increase in DPPH radical scavenging activity (41–50 %) ($P < 0.05$). Films incorporated with LS or BHT showed the preventive effect on lipid oxidation of soybean oil during 30 days of storage. At the level of 500 ppm, LS provided the highest efficacy for lipid oxidation retardation as evidenced by lower conjugated diene (CD) values ($P > 0.05$). According to these findings, gelatin film incorporated with longan seed extract or BHT could be used as a tool to prolong the shelf-life of oily foods.

Keywords Active film · Antioxidant · Gelatin film · Lipid oxidation · Longan seed extract

Background

During storage, food quality is reduced quickly because of microbial spoilage and chemical reactions. The most important quality deterioration caused by chemical processes in food systems is lipid oxidation. In this process unsaturated fatty acids reacting with molecular oxygen via a free radical chain mechanism (Gray 1978). As a consequence, hydroperoxides are formed which are unstable and decompose relatively quickly into aldehydes, ketones, alcohols, acids, esters or hydrocarbons. These secondary oxidative products are responsible for food quality, nutrition, safety, color and consumers' acceptance (McClements and Decker 2000). The variables that affect the susceptibility of lipids to oxidation, including temperature, fatty acid composition, antioxidants, metals, enzyme-catalyzed reactions, and water (Schaich et al. 2013). Methods for controlling the lipid oxidation are extensively researched, especially by using antioxidative substances. The most commonly used are butyl hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary-butyl hydroquinone (TBHQ), and propylgalate by directly adding to food products (Schaich et al. 2013).

Nowadays, active packaging which antioxidants are incorporated into packaging materials is an alternative way for food spoilage preservation. It can avoid contacting between the substances and the food as well as decrease the risk of adverse reactions (Pereira et al. 2007). Active packaging extends the shelf life of food by preventing interacting of humidity or substances such as oxygen, ethylene, aroma or unusual flavors with the food (Salgado et al. 2011). Mixtures of fish gelatin with active compounds to be active packaging have been increasingly interested. For example, incorporation of synthetic antioxidants, such as BHT and BHA in polyethylene film, showed to protect the fresh beef color from oxidation (Moore et al. 2003). However, in recent years, there has been a growing interest in the use of natural antioxidants in food

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packaging applications such as green tea extract (Giménez et al. 2013a; Wu et al. 2013), brown algae extract (Haddar et al. 2012), essential oil (Tongnuanchan et al. 2012, 2013), tocopherols (Barbosa-Pereira et al. 2013), catechin-lysozyme (Rawdkuen et al. 2013). Longan seeds and leaves have been employed as a good source of phenolic compounds, showing good antioxidant activity (Soong and Barlow 2006; Rangkadilok et al. 2007; Sudjaroen et al. 2012). Therefore a wider application in foods or incorporated into food packaging materials are interesting. So, the aim of the present work was to apply the prepared gelatin films with different levels of longan seed extract or BHT on retardation of lipid oxidation in soybean oil.

Materials and methods

Chemicals

All chemicals were of analytical grade unless otherwise noted. Acetic acid and ethanol were obtained from Merck (Darmstadt, Germany). Absolute ethanol and trichloroacetic acid (TCA) were purchased from Merck (Darmstadt, Germany). Folin–Ciocalteu phenol reagent was obtained from Fluka (Steinheim, Germany). 2,2'-diphenyl-picrylhydrazyl (DPPH[•]), gallic acid, BHT, and 2-thiobarbituric acid (TBA) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Extraction of phenolic compounds

Longan seeds were collected and its pericarp tissues were removed and then washed with tap water and dried for 24 h using a hot air oven at 50 °C, and finally powdered with a hammer mill through a 20-mesh (0.84 mm) sieve. The sample powder was stored in a plastic bag and kept in a freezer at −20 °C until use for extraction.

An amount of 5 g, was added to 100 ml of 53 % ethanol that had been pre-warmed to 58 °C and extracted by continuous stirring at 150 rpm in a warm-water bath at 58 °C for 139 min (the best condition from our previous work by using RSM technique). The aqueous extract was centrifuge at 8,000 rpm for 15 min and then supernatant was filtrated through filter paper. The resultant filtrate was freeze-dried and the dry matter was referred to as 'longan seed extract: LS'. The main phenolic fraction in longan seed extract are gallic acid (23.3 mg/100 seeds) and ellagic acid (156 mg/100 seeds) (Soong and Barlow 2006)

Preparation of gelatin films

Gelatin was extracted from farmed giant catfish skin according to the method of Rawdkuen et al. (2010). Gelatin powder

was mixed with distilled water to obtain the film-forming solution (FFS) with the protein concentration of 3 % (w/v). Glycerol was used as a plasticizer at the concentration of 25 % of protein. The solution was incubated at 60 °C for 30 min in a water bath with an occasional stirring. De-aerated film forming solution (4 ± 0.01 g) was cast onto a rimmed silicone resin plate (50×50 mm) and dried with a ventilated oven (Environmental chamber model H110K-30DM; Seiwa Riko Co., Tokyo, Japan) at 25 ± 0.5 °C and 50 ± 5 % relative humidity (RH) for 24 h. Dried films obtained were manually peeled off.

To study the effects of LS or BHT on film properties and antioxidative activity of incorporated film, LS or BHT were dissolved in ethanol and added to FFS to obtain a final concentration of 50–500 ppm for LS or 50–100 ppm for BHT. FFS was then stirred gradually for 10 min, cast and dried as previously described. Films incorporated without and with LS or BHT were subjected to the properties determinations and antioxidative activity during storage in a humidity control chamber (50 % RH) at 25 °C for 0, 2 and 4 weeks.

Determinations performed on the films

Light transmission

The light barrier properties of the films (film thickness ranged 35.60–37.60 µm, $P > 0.05$; Vichasilp et al. 2014) were measured according to Gómez-Guillén and Montero (2007). Films were exposed to light at wavelengths ranging from 570 to 270 nm, and absorbance was measured using a UV-1601 spectrophotometer. Results have been expressed as percentage transmittance.

Film and protein solubility

For water solubility, the film portions of 4 cm² were weighed and placed in tubes with 20 ml of distilled water and then gently shaken at a speed of 200 rpm at 25 °C for 24 h. The solution was then filtered through Whatman no. 1 filter paper to recover the remaining un-dissolved film, which was desiccated at 105 °C for 24 h. Film solubility was calculated by the equation:

$$FS(\%) = ((W_o - W_f) / W_o) \times 100$$

W_o was the initial weight of the film expressed as dry matter and W_f was the weight of the un-dissolved desiccated film residue. All tests were carried out in triplicate.

For protein solubility, the film samples (0.5 g) were dissolved in distilled water or 1 M NaOH (10 ml) and shaken at a speed of 200 rpm for 24 h at room temperature. The samples were centrifuged at $7,500 \times g$ for 20 min. The protein content

was measured in supernatant using the Biuret method (1940). Protein solubility was calculated by the equation:

$$PS(\%) = ((P_w - P_s)/P_w) \times 100$$

P_w was the protein content of the film dissolved in water and P_s was the protein content of the film dissolved in 1 M NaOH.

Total phenolic content

Total phenolic content was quantified spectrophotometrically at 750 nm using Folin–Ciocalteu reagent and gallic acid as standard and the results were expressed as gallic acid equivalents (GAE) mg /g of film, according to a standard curve of the same compound prepared in advance. Films were first dissolved in 0.5 M acetic acid at 40 °C. The resulting solution was shaken vigorously in a 95 % ethanol solution for 30 min to extract the phenols. The mixture was left to stand for 10 min, and the supernatant was employed as the extract for the total phenol determination and DPPH radical scavenging activity. All assays were performed at least in triplicate.

DPPH radical scavenging activity

DPPH radical scavenging activity was determined as described by Brand-Williams et al. (1995) with a slight modification. Samples (600 µl) were added to 600 µl of 0.20 mM DPPH in 95 % ethanol. The absorbance of the resulting solution was measured at 520 nm using a UV spectrophotometer. Results are expressed as DPPH radical scavenging activity (%) compared with the control.

Retardation of soybean oil oxidation by gelatin films

Soybean oil (2 ml) was filled into the active gelatin films bag (2×4 cm) (Fig. 1) and then sealed by desktop poly sealer. The samples were kept in dark in a humidity control chamber (50 % RH) at 28 °C. The samples were taken after 0, 1, 3, 5, 10, 15, 20, and 30 days for the analysis of conjugated diene (CD) according to the method of Frankel et al. (1996). The oil (0.1 ml) was dissolved in 5.0 ml of methanol and the absorbance was measured at 234 nm using a UV-1601 spectrophotometer. The conjugated diene was measured as the increase in absorbance at 234 nm. Soybean oil without active gelatin film covering was used as the control.

Statistical analysis

All chemical analyses were performed in triplicate. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test.

Analysis was performed by using a SPSS package (SPSS 10.0 for Windows, SPSS Inc, Chicago, IL, USA).

Results and discussion

Light transmission of the film

Transmission of UV and visible light at selected wavelength in the range of 270–570 nm of gelatin films (the thickness ranged 35.60–37.60 µm, $P>0.05$) incorporated with LS or BHT at different concentrations is shown in Fig. 2. Light transmission of all films tested was negligible <10 % at 270 to 300 nm. This result means that the developed gelatin films effectively blocked the UV light, while general plastic films have no this function. This result is similar to the investigation of Tongnuanchan et al. (2013) who reported that the gelatin films incorporated with ginger, turmeric and plai essential oils had the excellent barrier property to the light in UV at 200 nm. High transmittance (~10 %) in this region (<300 nm) was observed in the control and BHT incorporated gelatin films when compared with LS incorporated films. Increasing of LS concentration low transmittance was observed at each wavelength. Film incorporated with LS showed more light transmission in visible range (>350 nm) than the control, especially when the concentration of LS increased (300–500 ppm). No significant differences in transmittance (~50 %) of gelatin films incorporated with and without LS or BHT were observed after the wavelength was higher than 450 nm. With adding LS, the transmittance values became inversely proportional to the amount of LS added. These light barrier attributes are probably due to the formation of more compact structure between phenolic compounds in the extract and functional groups of gelatin molecule. Núñez-Flores et al. (2013) said that lose of transparency the gelatin film blending with lignin gained in light barrier properties. The background to support this finding may by the chromophoric nature of lignin which highly capable of protecting against UV radiation. High in light barrier properties could be of interest in certain food applications for preventing ultraviolet-induced lipid oxidation. Wu et al. (2013) also reported that gelatin film incorporated with green tea extract presented very low percent transmittance compared with gelatin film without green tea extract.

Water solubility and protein solubility of the gelatin films

The addition of LS or BHT to the gelatin films brought about a pronounced increase in film solubility, but there were no significant differences observed in terms of protein solubility (Table 1). The water solubility is a measure of the water soluble matter of a film, which is used as an indicator of the film's resistance to water (Giménez et al. 2013b). When compared with the control film, the gelatin film incorporated

Fig. 1 Pieces of the developed films and film sages from fish gelatin filled up with soybean oil. LS: Longan seed extract; BHT: butylated hydroxytoluene. Number indicated concentration (ppm) of LS or BHT in the gelatin films

with both LS and BHT showed higher water solubility. The highest level of both LS and BHT addition provided the highest level of water solubility (53.77 and 48.51 %, respectively). Increasing the concentration of the addition increased the film solubility ($p > 0.05$). The lowest film solubility (41.83 %) in distilled water was observed in the control. However, Wu et al. (2013) reported that water solubility of silver carp gelatin film was 43.75 %, which was obviously lower when green tea extract was added. They concluded that lower water solubility of the film incorporated with green tea extract might have been a result of the stronger structure of the film network, which had a higher extent of protein-polyphenol interactions. Bodini et al. (2013) also noted that the addition of ethanol-propolis extract did not provoke variations in water solubility probably because the extract did not interfere with the arrangement of the protein chains and did not change the hydrophilic characteristic of the films. Giménez et al. (2013a, b) also reported that the incorporation green tea extract both in

agar and agar-gelatin films produced approximately a 2-fold increase in water solubility, which was likely due to the hydrophilic nature of the tea extract. To reduce water solubility of a film, blending gelatin with chitosan has been reported to work (Fakhreddin Hosseini et al. 2013).

The protein solubility of the gelatin films with and without LS and BHT incorporation are presented in Table 1. The solubility of protein in the film was 97.59 to 98.26 % ($p > 0.05$). This high solubility may be due to the generation of low molecular weight peptides by hydrolysis. In addition, the high level of extractable protein in this experiment may confirm that the addition of LS and BHT did not alter the molecular organization and intermolecular interaction in the film matrix. Therefore, almost all of the proteins were extracted. Films with high solubility can potentially be used for developing edible packaging materials, which require easy solubility and release of the incorporated active compounds that exist in the film. However, potential applications often

Fig. 2 Changes in percentage of transmittance at wavelengths ranging from 270 to 570 nm for the developed gelatin films incorporated with LS or BHT. LS: Longan seed extract; BHT: butylated hydroxytoluene. Numbers represent the concentration in ppm

Table 1 Solubility of gelatin films incorporated with and without LS and BHT at different concentrations

Film samples	Film solubility (%)	Protein solubility (%)
Control	41.83±2.01 ^{a†}	97.79±0.26 ^{ab}
LS 50 ^{††}	52.47±6.82 ^{ab}	98.26±0.08 ^b
LS 100	52.81±2.05 ^{ab}	98.19±0.11 ^b
LS 300	53.57±6.30 ^b	97.66±0.21 ^a
LS 500	53.77±8.30 ^b	97.59±0.24 ^a
BHT 50	44.44±5.74 ^{ab}	97.61±0.22 ^a
BHT 100	48.51±6.06 ^{ab}	97.82±0.54 ^{ab}

[†] Different superscripts in the same column indicate the significant differences of mean ± SD ($n = 3$) ($p < 0.05$)

^{††} Number indicated concentration (ppm) of LS or BHT in the gelatin films

LS Longan seed extract, BHT butylated hydroxytoluene

also require water insolubility to enhance product integrity and water resistance.

Total phenolic content and antioxidative activity of the film

Total phenolic content and antioxidative activity of fish skin gelatin films incorporated with LS or BHT at the concentrations of 50–500 ppm during storage was monitored and presented in (Fig. 3). The increase of the LS in the gelatin film formulation gave a proportional increase in the amount of total phenolic compounds in the film. The gelatin film added with LS showed higher total phenolic content than the film added with BHT at the same concentration (Fig. 3a). The initial total phenolic content in the films incorporated with LS is in accordance with the concentration of LS addition. Sudjaroen et al. (2012) reported that the polyphenolic fraction of longan seed extract (80.90 g/kg dry weight) was dominated by ellagic acid which exhibiting strong antioxidant capacities. Soong and Barlow (2006) also reported that the ethanolic extract of longan seed contained 23.3 and 156 mg/100 seeds of gallic acid and ellagic acid, respectively. These major compounds contributed to potent antioxidant activity. Total phenolic content of gelatin film incorporated with LS or BHT was slightly increased when storage time increased (up to 4 weeks). However, not significantly increased at the week 4 of storage was observed in the films added with synthetic antioxidant, while the film added with LS showed markedly increased. Giménez et al. (2013a) said that the high recovery percentages of phenolic contents indicated the weak nature of the presumptive polyphenol-gelatin interactions in the film matrix, regardless the polyphenol concentration. Extracting the phenolic compounds from the gelatin film matrix during assay preparation may relate to the bonding formation between the phenolic compound in LS and the functional group of gelatin

molecules. This mechanism could be explained to the migration of active compound into food to play a role as the producer need from active film. Gómez-Estaca, et al. (2009) reported that by adding the oregano and rosemary extracts to the tuna-fish films raised the total phenol content in the film.

DPPH radical scavenging activity of the gelatin films incorporated with LS or BHT presented in Fig. 3b. The result of antioxidant capacity of the films is in accordance with the presented of total phenolic content mentioned in Fig. 3a. Increasing the concentration of LS or BHT provided more antioxidant capacity indicated by DPPH radical scavenging activity. No significantly differences were found on changes of DPPH radical scavenging activity was found in the film added with LS or BHT. However, the increasing of this value was clearly observed at 4 weeks of storage. The results were in agreement with Jongjareonrak et al. (2008) who study in changes in antioxidative activity of fish skin gelatin films incorporated with BHT or α -tocopherol during storage. At the same level of LS addition with different storage periods showed that low amount of LS addition provided markedly increased of antioxidant activity when the storage time increased. This can explain by the changes of gelatin molecule into gelatin hydrolysates or conformation changes of the molecule and play a role of antioxidative properties. Giménez et al. (2013a, b) reported that fish gelatin hydrolysates have exhibited a high antioxidant activity in numerous reports. Jridi et al. (2013) also reported that release of some peptides exhibiting antioxidant activities from the gelatin partial hydrolysis caused the increase in antioxidant activity. In addition, exposure of hydroxyl group of phenolic compounds in LS or BHT or protein during storage could play a role in antioxidant capacity. Zheng et al. (2009) reported that eight polyphenols in longan seed extract exhibited scavenging activity towards DPPH radicals as well as superoxide radicals.

Retardation of soybean oil oxidation during storage

The formation of CD values of soybean oil kept in fish skin gelatin films bag (Fig. 1) with and without LS or BHT at a level of 50–500 ppm during 30 days dark storage at 28 °C are shown in Fig. 4. Changes in CD were expressed as an index of lipid hydroperoxides in the oil. This is a good indicator of oxidative changes in fats and oils. These changes were observed as the similar trend that already mentioned in peroxide value and thiobarbituric acid substances (Vichasilp et al. 2014). CD level increased continuously in all samples throughout the 30 days storage, especially in the sample packed with the film without LS or BHT. Stepwise decreased of CD in soybean oil was observed when the concentration of LS increased and showed the lowest when the highest concentration (500 ppm) of LS was incorporated into the gelatin film. Compared between LS and BHT at the same concentration addition, the later showed slightly more

Fig. 3 Total phenolic content (a) and DPPH radical scavenging activity (b) of gelatin films incorporated with LS or BHT during storage (50–500: Number indicated the concentration in ppm). Bars represent the standard deviation from triplicate determinations

effective for CD formation inhibition than the former. From the result, after 20 days storage no significantly increased of CD was observed in the soybean oil packed with the gelatin

films incorporated with LS >100 ppm or BHT 100 ppm. Chatha et al. (2006) said that lipids containing methylene-interrupted dienes or polyenes show a shift in their double

Fig. 4 Conjugated diene content of soybean oil packed in gelatin film incorporated with LS or BHT during 30 days storage. Number indicated the LS or BHT concentration in ppm. Bars represent the standard deviation ($n=3$)

bond position during oxidation which can be monitored by measuring the absorbance at 234 nm. The fresh olive oils contained significantly varied levels of carbonyl compounds (CD =5.0–18.6 $\mu\text{mol/g}$) which increased (to CD =42.7–77.6 $\mu\text{mol/g}$ after 16 h) with considerably different rates during the heating process (Farhoosh et al. 2012).

Conclusions

The present studies demonstrate that longan seed extract can be successfully incorporated into gelatin films and showed excellent antioxidant activities. Retardation of soybean oil was effectively obtained when filled with produced gelatin sag. It is interesting to note that, in addition to the potential antioxidant benefits afforded, films supplemented with added longan seed may also possess good light barrier properties, especially to ultraviolet light, a powerful lipid-oxidizing agent.

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Competing interests There are non-financial competing interests (political, personal, religious, ideological, academic, intellectual, commercial or any other) in relation to this manuscript to declare.

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