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ORIGINAL PAPER

Hydroperoxide as a Prooxidant in the Oxidative Stability of Soybean Oil

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Abstract Fifteen milliliters of soybean oil having peroxide value (PV) of 0, 2, 4, 6, 8, or 10 meq/kg oil in a 35 mL serum bottle was sealed air-tight with a Teflon rubber septum and aluminum cap and was stored in a forced-air oven at 50 °C. The oxidative stability of soybean oil was evaluated daily for six days by measuring the headspace oxygen content and volatile compounds in the headspace of a sample bottle by gas chromatography. As the initial PV of the oil increased from 0 to 2, 4, 6, 8 and 10, the headspace oxygen decreased and the volatile compounds increased at $p < 0.05$. Hydroperoxide accelerated the oxidation of soybean oil. The correlation coefficient (R^2) between the headspace oxygen and the volatile compounds was 0.95. The increase of tertiary butyl hydroquinone (TBHQ) from 0 to 50 ppm for the oil of PV 4 or 8 had a significant effect on the oxidative stability at $p < 0.05$. The increase from 50 to 100 ppm for the oil of PV 4 or 8 did not significantly increase the stability at $p > 0.05$. The oxidative stability of PV 8 meq/kg and 50 ppm TBHQ was better than the control with PV 0 and 0 ppm TBHQ at $p < 0.05$. TBHQ was an effective antioxidant to improve the oxidative stability of soybean oil.

Keywords Headspace oxygen · Oxidative stability · Peroxide value · TBHQ · Volatile compounds

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Introduction

Lipid oxidation is one of the most common causes of flavor quality deterioration [1, 2]. It lowers the sensory perception, nutritional quality and safety of lipids. The oxidation not only makes the food less acceptable or unacceptable to consumers but also causes great economic losses to the food industry.

The initial product of lipid oxidation by autoxidation, enzymatic and photosensitized oxidations is hydroperoxide. The hydroperoxides are decomposed to produce volatile compounds [2, 3] and oxidized dimers, trimers or polymers [4]. The volatile compounds are aldehydes, ketones, alcohols, hydrocarbons, and furans, which are responsible for undesirable rancid flavor of oils. Some products from the decomposition of hydroperoxides are potentially toxic at relatively low concentration [2, 5].

Peroxide value determined by an iodometric method [6] measures only hydroperoxide [7, 8]. The hydroperoxide content is expressed as peroxide value in milliequivalent of hydroperoxides per kg of oil. This is based on the reduction of the hydroperoxide group (ROOH) by the iodide ion (I^-). Peroxide value measures only hydroperoxide, which is a transient product of oxidation. This method is empirical and any modifications may change the results.

The peroxide values of crude oil, degummed oil, refined oil, and bleached oil are 2.4, 10.5, 8.8, and 16.5 meq/kg oil, respectively [9]. Our preliminary analyses showed that the peroxide value of seven commercially available soybean oils in the supermarkets (Columbus, OH) ranged from 0.3 to 6.5 meq/kg oil. Hydroperoxide can be easily formed in the oil during processing, storage and marketing. Hydroperoxide can produce volatile compounds by decomposition. The oxidized compounds such as noncyclic hydroxyl dimers, dimers and trimers joined through carbon-to-oxygen

linkages are produced from hydroperoxide decomposition during oil processing [4]. Billek et al. [10] and Yoon et al. [11] reported that refined soybean oil contained 1.2% oxidized dimers, trimers or polymers of triglycerides. The oxidized dimers, trimers, or polymers of triglycerides accelerated oil oxidation [11].

Antioxidant is used to improve the flavor quality of oil. Tertiary butyl hydroquinone (TBHQ) is a synthetic antioxidant commonly used to minimize the rancid off-flavor produced by oil oxidation. Min and Wen [12] reported that TBHQ was a better antioxidant in vegetable oils than butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT).

The effects of light [13, 14], temperature [15], types of fatty acids of oil [16], types and amount of free fatty acids [17, 18], oxygen pressure [19], dissolved oxygen [20], types and amount of metals [19, 21], types and amount of chelating agents [22], the amount of monoglycerides and diglycerides [23], and types and amount of phospholipids [24] on the oxidative quality and stability of oils during storage have been extensively studied. The effects of hydroperoxide contents on the oxidative stability have not been reported, although the flavor quality and oxidative stability of oils have been commonly determined by measuring peroxide value. The objectives of this research are to study the effects of hydroperoxide contents on the oxidative stability of soybean oil and the quantitative effects of TBHQ on the oxidative stability of soybean oil with PV 2, 4, 6, 8, or 10 meq/kg oil.

Materials and Methods

Preparation of Purified Soybean Oil and Chemical Analyses

The fresh refined, bleached and deodorized soybean oil was from Karlshams Inc. (Columbus, OH). Three-hundred milliliters of fresh soybean oil was purified by passing it through a chromatographic column. The column (40 cm × 2.5 cm) was packed with a series of 20 g of activated silicic acid (100 mesh, Mallinkrodt Co., Paris, KY), 10 g of 2:1 mixture of activated silicic charcoal (J.T. Baker Chemical Co., Phillipsburg, NJ) and celite, 40 g of 2:1 mixture of powdered sugar and celite and 20 g of activated silicic acid. The silicic acid was activated by heating the silicic acid at 150 °C for 10 h. A vacuum of 635 mmHg was applied to a Buchner flask connected to the column. The column for the purification of oil was wrapped with aluminum foil to minimize the possible effect of light on the soybean oil during the purification process. The flow rate of oil through the column was 3 mL/h. The soybean oil which passed through the column was designated as the purified soybean oil. Phospholipids, chlorophylls and the free fatty acid of the purified soybean oil were determined by the AOCS methods [6]. Tocopherol in purified soybean oil was determined by normal-phase high-performance liquid chromatography (HPLC) using an ultraviolet (UV) detector by the AOCS method [6].

Preparation of PV 0, 2, 4, 6, 8 or 10 Soybean Oil

The soybean oil with PV 51 was prepared by purging the 200 mL purified soybean oil in a 250 mL beaker at a rate of 10 mL air per minute for 48 h at 50 °C. Two-hundred milliliters of soybean oil having PV 0, 2, 4, 6, 8, or 10 meq/kg oil was prepared by adding the proper amount of the soybean oil with PV 51 meq/kg oil to the purified soybean oil with PV 0 meq/kg oil. Fifteen milliliters of sample oil was transferred into a 35 mL serum bottle and sealed air-tight with a Teflon^{TM}-coated rubber septum (Supelco, Bellefonte, PA) and an aluminum cap. The samples were stored in a forced-air oven at 50 °C for six days.

Preparation of 50 and 100 ppm TBHQ Added Soybean Oil

TBHQ was added to the soybean oils having PV 4 or 8 meq/kg oil to achieve 50 or 100 ppm TBHQ. The 15 mL sample oil was pipetted into a 35 mL serum bottle and sealed air-tight with a Teflon^{TM}-coated rubber septum and an aluminum cap. The samples with 0, 50 or 100 ppm TBHQ were stored in a forced-air oven at 50 °C for six days.

Peroxide Value Determination

The peroxide value of oil was determined by AOCS method Cd 8-53 [6].

Headspace Oxygen and Volatile Compounds Analyses

The oxidative stability of oil was evaluated daily for six days at 50 °C by a combination of headspace oxygen and headspace volatile compounds determination using gas chromatography. The depleted headspace oxygen was determined by injecting 100 μL headspace gas of a sample bottle into a HP 5890 gas chromatograph equipped with a thermal conductivity detector. A stainless-steel column (1.8 m × 0.32 cm) packed with a 60/80 molecular sieve 13× (Alltech Assoc. Inc., Deerfield, IL) was used. The flow rate of helium gas was 20 mL/min. The temperatures of the oven, injector, and thermal conductivity detector were 40, 120 and 150 °C, respectively. Electronic counts of the

100 μL headspace gas of a sample bottle were integrated using a Hewlett Packard 3390 electronic integrator. The percentage of headspace oxygen content of a sample bottle was determined by comparing the gas chromatographic peak area with that of air which was considered to have 20.9% oxygen [9].

The headspace volatile compounds in an air-tight sealed sample bottle were isolated with 65 μm polymethylsiloxane/divinylbenzene (PDMS/DVB) fiber (Supelco Co., Bellefonte, PA) in solid-phase microextraction (SPME). A sample bottle of soybean oil was put into a 55 °C water bath for 30 min while 65 μm PDMS/DVB of SPME solid phase was exposed to the headspace of a sample bottle. The isolated volatile compounds by SPME were separated in a gas chromatograph (HP 6890 series, Wilmington, DE) equipped with a flame ionization detector at 300 °C. The isolated volatile compounds in the solid phase of the SPME were desorbed at 250 °C from the GC injector for 2 min. Gas chromatographic analysis was carried out on a HP-5 column (30 m × 0.32 mm, 0.25 μm film thickness; Hewlett-Packard, Palo Alto, CA). Oven temperature was programmed from 60 to 120 °C at 4 °C/min and from 120 to 240 °C at 10 °C/min with initial and final hold times of 5 and 2 min, respectively. The rate of helium carrier gas was 2.2 mL/min.

Statistical Analyses

The analytical data of the effects of hydroperoxide contents and TBHQ on the headspace oxygen contents and volatile compounds of soybean oil were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range test at $\alpha = 0.05$. All statistical analyses were conducted with the Statistical Analysis System (SAS Inst., Cary, NC).

Results and Discussion

Characteristics of Purified Soybean Oil by Chromatography Column

The soybean oil eluted from the chromatography column was designated purified soybean oil. The purified soybean oil was colorless, tasteless, and odorless and the PV was 0 meq/kg oil. The analyses showed that free fatty acids, phospholipids, tocopherols, chlorophylls, and oxidized compounds were not present in the purified soybean oil (results not shown). The purified soybean oil was essentially made of triglycerides. Most impurity compounds from soybean oil were removed by using the column packed with silicic acid, charcoal, celite and sugar.

Reproducibility of the Analyses of PV, Headspace Oxygen Content and Volatile Compounds

Soybean oils in six replicates were analyzed to determine the reproducibility of the analyses of PV (meq/kg oil), headspace oxygen content (%) and volatile compounds (electronic counts). The coefficients of variation for the analyses of PV, headspace oxygen content and volatile compounds of soybean oil were 1.10, 0.39 and 1.91%, respectively, which were considered satisfactory.

Effects of Hydroperoxide Contents on the Oxidative Stability of Soybean Oil

The oxidative stability of soybean oil was determined by the combination of headspace oxygen content and volatile compounds. The means of headspace oxygen content and volatile compounds for soybean oils with PVs of 0–10 stored for up to six days at 50 °C are shown in Table 1. The means of headspace oxygen content determined daily for six days for PV 0 and PV 10 soybean oils were 15.7 and 11.2%, respectively (Table 1). The headspace volatile compounds for PV 0 and PV 10 soybean oils were 177 and 530 electronic counts, respectively (Table 1). Duncan's multiple range test showed that as the hydroperoxide contents of oil increased from PV 0 to PV 10 meq/kg oil, the headspace oxygen content decreased and the volatile compounds increased significantly at $p < 0.05$. The correlation coefficient between the depleted headspace oxygen content and the volatile compounds was 0.95 (Table 2). This good relationship showed that lipid oxidation could also be evaluated by measuring the formation of volatile compounds, as was expected.

Table 1 Means of headspace oxygen content and volatile compounds for PV 0, 2, 4, 6, 8, or 10 soybean oils of 1, 2, 3, 4, 5 and 6 days at 50 °C

Peroxide value (meq/kg)	Headspace oxygen content[a,c] (%)	Volatile compounds[b,c] (electronic counts)
0	15.7^{A}	177^{A}
2	14.3^{B}	263^{B}
4	13.2^{C}	354^{C}
6	12.8^{C}	413^{D}
8	12.3^{CD}	470^{CD}
10	11.2^{D}	530^{D}

[a] Overall mean of headspace oxygen contents for all days (1–6 days of storage)

[b] Overall mean of headspace volatile compounds of gas chromatographic peak (1–6 days of storage)

[c] Duncan's multiple range test were performed. Means with the same letter are not significantly different at $\alpha = 0.05$

Table 2 Correlation coefficients among peroxide value, headspace oxygen, headspace volatile compounds and storage

Relationship	Correlation coefficient (R^2)		
	Without TBHQ[a]	With TBHQ[b]	
		PV 4	PV 8
PV and HO	0.96	–	–
PV and HV	0.99	–	–
HO and HV	0.95	0.96	0.97
Day and HO	0.97	0.96	0.96
Day and HV	0.96	0.94	0.96

PV Peroxide value, *HO* depleted headspace oxygen content, *HV* headspace volatile compounds, *Day* storage days

[a] Oils of PV 0, 2, 4, 6, 8, or 10 without TBHQ

[b] Oils of PV 4 or 8 with TBHQ 50 or 100 ppm

The effects of hydroperoxide contents on the headspace oxygen and volatile compounds for soybean oils during six days of storage at 50 °C are shown in Figs. 1 and 2, respectively. The headspace oxygen content in soybean oil decreased as the PV increased from 0, 2, 4, 6, or 8 to 10 meq/kg oil during storage at 50 °C (Fig. 1). The headspace oxygen content in soybean oil at day 0 was 20.9% [9]. The headspace oxygen contents for PV 0 and PV 10 soybean oils after six days at 50 °C were 12.4 and 7.4%, respectively (Fig. 1). The higher the hydroperoxide content

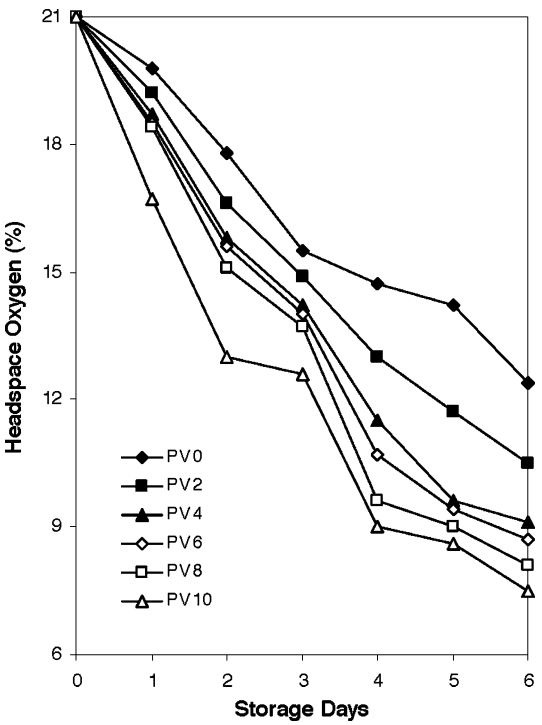

Fig. 1 Headspace oxygen content of soybean oil with PV 0, 2, 4, 6, 8, or 10 meq/kg oil during the storage of 6 days at 50 °C

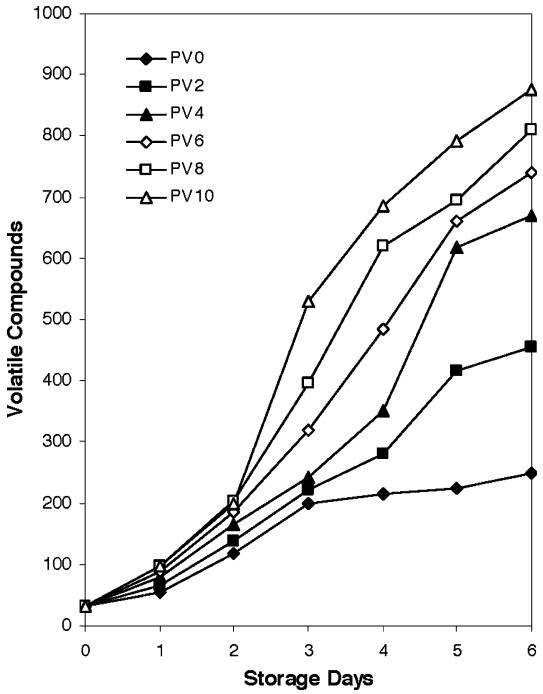

Fig. 2 Headspace volatile compounds of soybean oil with PV 0, 2, 4, 6. 8, or 10 meq/kg oil during the storage of 6 days at 50 °C

of soybean oil was, the faster the reaction between the oxygen and the oil in the bottle to decrease the headspace oxygen was.

The headspace volatile compounds in soybean oil increased as the PV increased from 0, 2, 4, 6, or 8 to 10 meq/kg oil during six days of storage at 50 °C (Fig. 2). The amount of volatile compounds in the purified soybean oil at 0 day was 32 in electronic counts. The headspace volatile compounds for PV 0 and PV 10 soybean oils after six days at 50 °C were 250 and 950 in electronic counts, respectively (Fig. 2). The higher the initial hydroperoxide contents in the oil were, the greater the volatile compounds formed by lipid oxidation. The combined results of headspace oxygen and volatile compounds of oil during storage showed that hydroperoxides acted as prooxidants to accelerate the reaction between oxygen and oil to produce the volatile compounds (Table 1; Figs. 1, 2).

The detailed mechanisms and relationships of the headspace oxygen depletion, the formation and decomposition of hydroperoxides, and the formation of volatile compounds by lipid oxidation are shown in Fig. 3. The headspace oxygen in a gas-tight sample bottle continuously disappears to form volatile compounds as the lipid oxidation progresses during storage. The formation of nonvolatile oxidative products by hydroperoxide decomposition is shown in Fig. 4. The RO· or ROO· formed by the decomposition of ROOH in the presence of a transitional metal or energy are prooxidants for free-radical lipid

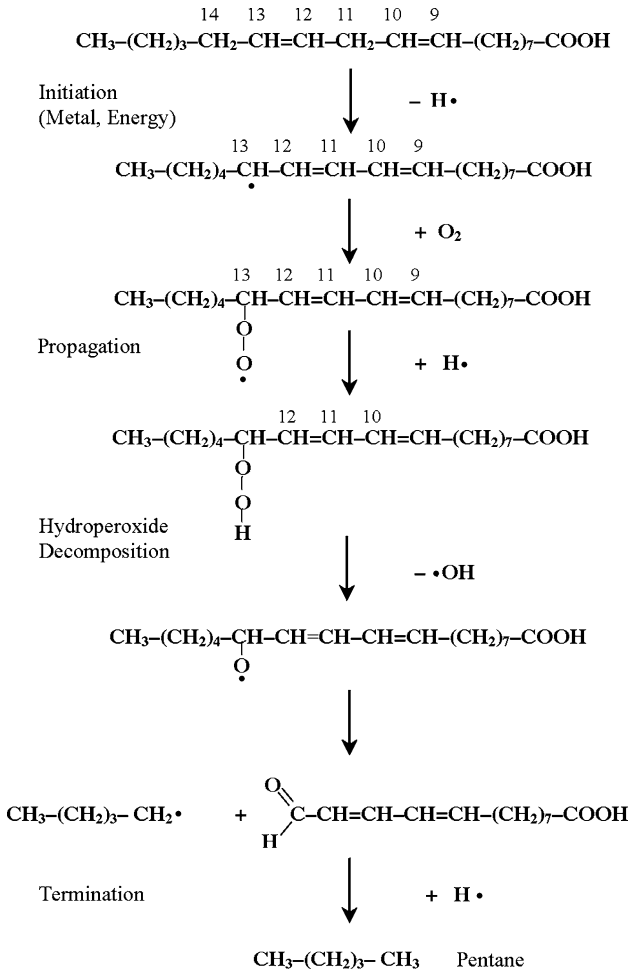

Fig. 3 Mechanisms of pentane and aldehyde formation and relationships among headspace oxygen, hydroperoxide, and volatile compounds during linoleic acid oxidation

oxidation. The ROOR and $^1O_2$ (singlet oxygen) which are formed from ROO and ROO·· are also prooxidants [16, 25]. Therefore, hydroperoxides acted as prooxidants by the formation of ROOR and $^1O_2$. The oxidized compounds including noncyclic hydroxyl dimers and dimers and trimers joined through carbon-to-oxygen linkage are formed from the decomposition of hydroperoxide [4]. Yoon et al. [11] reported that oxidized dimers, trimers, and polymers of triglycerides were prooxidants.

Paulose and Chang [26] also reported that the oxidized dimers, trimers, and polymers formed from trilinolein during deep fat frying contain hydroxyl, carbonyl and epoxide groups. Min and Jung [27] reported that monoglycerides, diglycerides and lipid compounds containing hydroxyl, carbonyl or epoxide groups in the bulk oil system are present in the surface of the oil. The oxidized compounds with hydrophilic groups formed during lipid oxidation lower surface tension in the bulk oil, increase the introduction of oxygen into the oil, and accelerate the oil oxidation to act as prooxidant.

Peroxide value determined by an iodometric titration method measures only the hydroperoxides (ROOH) and does not measure the peroxides between fatty acids in dimers (ROOR), trimers, polymers and endoperoxides of fatty acids. Hydroperoxide content is used as an index of the oxidative state of oil. If the rate of hydroperoxide decomposition to produce oxidized polymer is greater than the rate of hydroperoxide formation from oil, oils with high amounts of oxidized polymers could have very low hydroperoxide content. The oil at the end of oxidation period could have a low peroxide value although the oil is highly oxidized and has a rancid flavor. Oil with a large amount of oxidized polymers could have a very low peroxide value. Oil with a low peroxide value and a large amount of oxidized polymers could have a very low flavor quality and stability during storage. Peroxide value could not be a reliable indication for the oxidative stability of oil at the later stage of lipid oxidation.

Hydroperoxides in oil are also formed during the process of degumming, refining, bleaching and deodorizing.

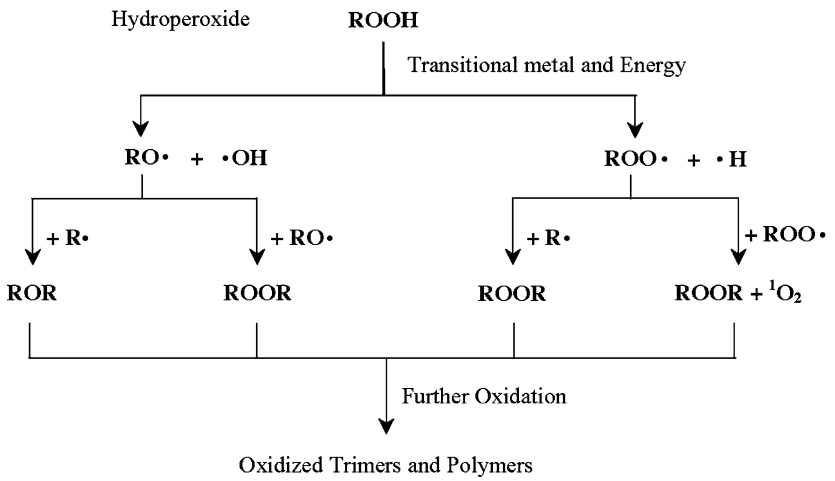

Fig. 4 Formation mechanism of oxidized dimers, trimers, and polymers from hydroperoxide

The hydroperoxide contents of crude oil, refined oil, degummed oil, and bleached oil were 2.4, 8.8, 10.5, and 16.5 meq/kg of oil, respectively [9]. The high hydroperoxide contents in degummed oil could be due to oxidation caused by moisture and high temperatures (70–80 °C) [28]. Bleaching (80–100 °C) also increased hydroperoxide content in oil [28]. Hydroperoxide formation in oil during degumming and bleaching processes should be prevented to improve the oxidative stability as much as possible by blanking the oil processing with nitrogen gas if economically and technically possible. The hydroperoxides in the crude, bleached, or refined oils should be destroyed during deodorization, which is the last step in soybean oil processing.

Effects of TBHQ on the Oxidative Stability of Soybean Oil with Hydroperoxides

The addition of 0, 50, or 100 ppm TBHQ on the headspace oxygen contents for PV 0, PV 4 and PV 8 soybean oils during six days of storage at 50 °C are shown in Fig. 5. The addition of 50 or 100 ppm TBHQ to PV 4 and PV 8 soybean oils increased the headspace oxygen content. The headspace oxygen content for PV 0, PV 4 and PV 8 soybean oils was 12.4, 9.1 and 8.1%, respectively. As the PV increased, the headspace oxygen decreased. The addition of 0, 50, or 100 ppm TBHQ to PV 4 oil increased the headspace oxygen from 9.1 to 17.4 or 17.7%, respectively (Fig. 5). The addition of 0, 50, or 100 ppm TBHQ to PV 8 oil increased the headspace oxygen from 8.1 to 15.7 or 17.0% (Fig. 5). The increase of TBHQ from 0 to 50 ppm for the oils with PV 4 or PV 8 had significant effect to minimize the reaction between oxygen and oil during storage at $p < 0.05$. However, the increase from 50 to 100 ppm TBHQ for the oils with PV 4 or PV 8 did not have a significant effect at $p > 0.05$.

The effect of the addition of 0, 50, or 100 ppm TBHQ on the headspace volatile compounds for PV 0, PV 4 and PV 8 soybean oils during six days of storage at 50 °C is shown in Fig. 6. The headspace volatile compounds for PV 4 or PV 8 soybean oil decreased as the TBHQ content increased from 0 to 50 ppm. The headspace oxygen content for PV 0, PV 4 and PV 8 soybean oils was 250, 670, and 810 in electronic counts, respectively. As the PV increased, the volatile compounds increased. The addition of 0, 50, or 100 ppm TBHQ to the PV 4 oil decreased the volatile compounds from 670 to 140 or 120 in electronic counts, respectively (Fig. 6). The addition of 0, 50, or 100 ppm TBHQ to PV 8 oil decreased the volatile compounds from 810 to 198 or 165 in electronic counts, respectively (Fig. 6). The increase of TBHQ from 0 to 50 ppm for the oils with PV 4 or PV 8 had a significant effect, reducing the formation of headspace volatile compounds at $p < 0.05$. However, the increase of TBHQ from 50 to 100 ppm for

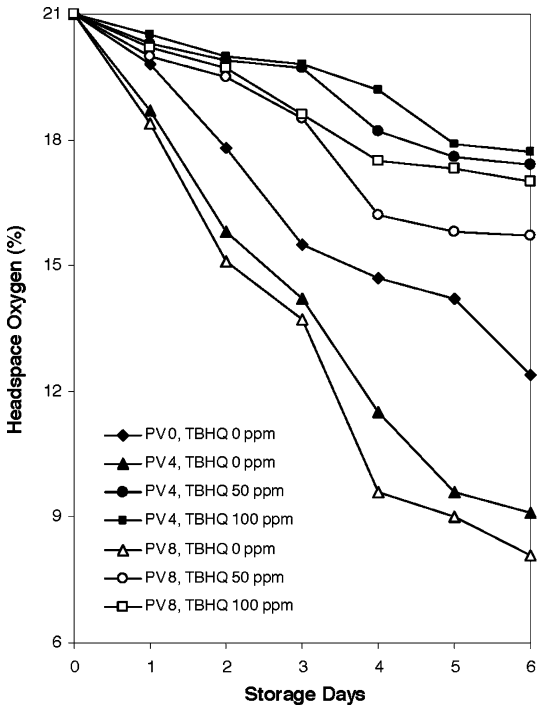

Fig. 5 Effect of 0, 50, or 100 ppm TBHQ on the headspace oxygen content of soybean oil with PV 4 or PV 8 meq/kg oil during the storage of 6 days at 50 °C

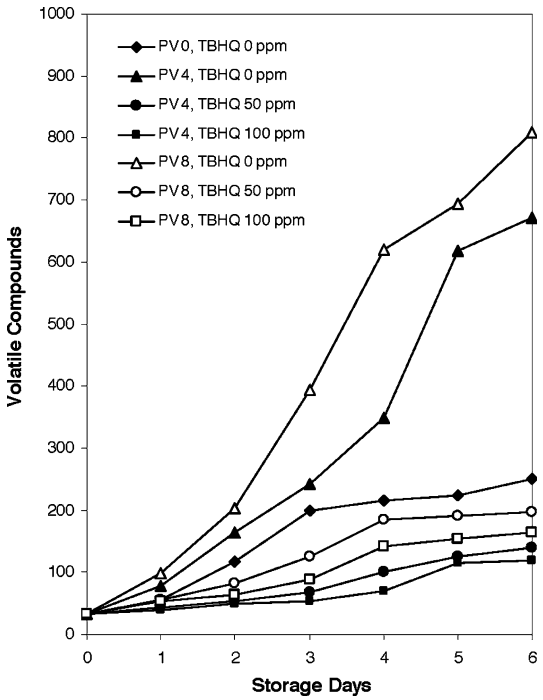

Fig. 6 Effect of 0, 50, or 100 ppm TBHQ on the headspace volatile compounds of soybean oil with PV 4 or PV 8 meq/kg oil during the storage of 6 days at 50 °C

the oils with PV 4 or PV 8 did not have a significant effect at $p > 0.05$.

The addition of 50 ppm TBHQ had a significantly different effect on the combination of headspace oxygen and volatile compounds at $p < 0.05$. However, the increase from 50 to 100 ppm TBHQ did not have a significant effect at $p > 0.05$. Oil with PV 4 and 50 ppm TBHQ had better oxidative stability than oil with PV 8 and 100 ppm TBHQ according to the results of headspace oxygen and volatile compounds (Figs. 5, 6). The addition of TBHQ improved the oxidative stability of the oil. Since the result of oxidative stability for PV 4 and 50 ppm TBHQ oil is better than the oil with PV 8 and 100 ppm, it seems to be more important to have a low hydroperoxide content than to add extra amounts of TBHQ for the minimization of the lipid oxidation.

The relationship between the headspace oxygen contents and volatile compounds for PV 4 and PV 8 soybean oils with TBHQ are shown in Table 2. The correlation coefficients (R^2) for PV 4 and PV 8 oils were 0.96 and 0.97, respectively. The good relationship suggested that lipid oxidation in oil could be evaluated by the combination of headspace oxygen and volatile compounds analyses.

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