Effects of selected herb extracts on iron-catalyzed lipid oxidation in soybean oil-in-water emulsion

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Abstract Effect of herb extracts on the oil oxidation of an oil-in-water emulsion containing iron was studied. The emulsion comprised tocopherol-stripped soybean oil (40 g), citrate buffer (60 g, pH 4.0), xanthan gum (35 mg), and FeSO₄ (0.5 mg) with 80% ethanol extracts of rosemary (*Rosmarinus officinalis*), basil (*Ocimum basilicum*), peppermint (*Mentha piperita*), thyme (*Thymus vulgaris*), or oregano (*Origanum vulgare*). Hydroperoxide contents and *p*-anisidine values were significantly ($p<0.05$) lower in the emulsions with the added herb extracts compared with the control emulsions. The antioxidant activity of the basil and peppermint extracts containing higher proportions of rosmarinic acid was significantly higher ($p<0.05$) than that of the other herb extracts, but the polyphenol degradation rate was not significantly different. The antioxidant activity of the basil and peppermint extracts showed concentration-dependence within 400 mg/kg. This study suggests that the oxidative stability of oil-in-water emulsion containing iron can be improved by addition of basil or peppermint extract.

Keywords: culinary herb, oil oxidation, acidic oil-in-water emulsion, polyphenols, concentration dependence

Introduction

Herbs refer to plants that have been used, in part or whole, for medical treatment and food seasoning or coloring and have nutritive value (1). Basil, oregano, mint, rosemary, and thyme are examples of typical culinary herbs that impart characteristic flavors to salads and their dressings. The most common and important functional compounds in herbs are polyphenols, which have been reported to possess antioxidant, anticancer, antiatherosclerosis, anti-inflammatory, and antimicrobial activities (2-7). The antioxidant activity of polyphenols such as rosmarinic acid and catechin in herbs depends on their structure (8-10), which suggests that herbs with different polyphenol composition show different antioxidant activity. Among herbs, rosemary and sage have proven to be effective for controlling lipid oxidation in bulk oil and/or oil-in-water (O/W) emulsions (8,11) and thyme has proven to be an effective antioxidant in an O/W emulsion and rapeseed oil (12-14).

Reports on herbs and their polyphenols have been confined to the evaluation of the antioxidant activity of herbs and their individual polyphenols in terms of radical scavenging or absorption capacity (15-19), with a low number of studies on bulk oil or O/W emulsions having relatively high oil content. It is much more difficult to find a study on the antioxidant activity of herbs related to their polyphenol composition. This study was conducted to compare the antioxidant

activity of culinary herbs, rosemary (*Rosmarinus officinalis*), basil (*Ocimum basilicum*), peppermint (*Mentha piperita*), thyme (*Thymus vulgaris*), and oregano (*Origanum vulgare*), on acidic O/W emulsions with iron and a high oil content as a model system of salad dressing and to determine the concentration dependence of their antioxidant activity. Degradation of polyphenols during the oxidation of the emulsion was also evaluated.

Materials and Methods

Materials and reagents Fresh rosemary (*R. officinalis*), peppermint (*M. piperita*), and thyme (*T. vulgaris*) were purchased from Young Grower Farm (Hwasun, Korea), and sweet basil (*O. basilicum*) and oregano (*O. vulgare*) were purchased from Greenfarm (Seoul, Korea); these herbs were then freeze-dried (TFD5505 freeze-dryer; Ilshinbiobase, Dongducheon, Korea). Refined, bleached, and deodorized soybean oil, which is commonly used for the manufacturing of salad dressing, was obtained from Samyang Corp. (Seoul, Korea). Minor compounds such as tocopherols, phospholipids, pigments, and metals that may be present in oil were removed by passing the oil through a glass column (30×2.5 cm) packed with activated silicic acid (10 g; Sigma-Aldrich Co., St. Louis, MO, USA), alumina (70 g; Sigma-Aldrich Co.), and celite (10 g; Junsei Co., Tokyo, Japan) from

the bottom (20). Rosmarinic acid, caffeic acid, gallic acid, *p*-coumaric acid, catechin, xanthan gum, cumene hydroperoxide (CuOOH), Folin-Ciocalteu's phenol reagent, and ammonium thiocyanate were obtained from Sigma-Aldrich Co. Isooctane and ferrous sulfate were obtained from Junsei Co. Egg yolk lecithin was purchased from Goshen Biotech (Namyangju, Korea). HPLC grade *n*-hexane, methanol, water, acetone, ethanol, ethyl acetate, isopropanol, chloroform, and toluene were obtained from Mallinckrodt Baker Co. (Phillipsburg, NJ, USA). All other chemicals were analytical grade.

Preparation of emulsions and oxidation Herb extract was prepared using 80% ethanol; freeze-dried rosemary, peppermint, thyme, basil, and oregano were finely ground in an Essence HR 2084 blender (Philips, Amsterdam, Netherlands) and then mixed with 80% ethanol (1:10, w/v) at 25°C for 2 h. The mixture was filtered through paper (#42, Whatmann; GE Healthcare Life Sciences, Little Chalfont, UK) followed by complete removal of the solvent using a rotary evaporator (N-N series; Eyela, Tokyo, Japan) at 65°C. The acidic O/W emulsion basically comprised purified soybean oil and citrate buffer solution at pH 4.0 (4:6, w/w). The emulsifier used was egg yolk lecithin (350 mg/kg), which has been reported to have no effect on the iron-catalyzed oxidation of oil in an emulsion (21). The herb extract was added to the citrate buffer solution to obtain emulsions of 50, 100, 200, or 400 mg/kg; this was followed by the addition of 350 mg/kg xanthan gum and 5 mg/kg FeSO₄. After mixing the water-soluble ingredients, soybean oil and egg yolk lecithin were slowly added and then homogenized using an Ultra-Turrax T25 homogenizer (IKA Instruments, Staufen, Germany) at 10,000 rpm for 6 min. The emulsion (7 g) was transferred into 20 mL serum bottles, which were then tightly capped with rubber stoppers and aluminum caps. All the samples were placed in an LBI-250 incubator (Daihan Labtech Co., Seoul, Korea) at 25°C for 12 days. Light was excluded throughout the experiment for all the experiments. The control sample was prepared without herb extract. All samples were prepared in duplicates, and there was no phase separation observed throughout the period of oxidation.

Analysis of lipid oxidation in emulsion samples Lipid oxidation in the emulsion was evaluated based on the hydroperoxide production and *p*-anisidine values. The hydroperoxide content of the emulsion was determined using the ferric thiocyanate method (21). The emulsion was mixed with a solution of isooctane and 2-propanol (3:1, v/v), followed by centrifugation (Combi 514R; Hanil Science Industrial, Incheon, Korea) at 1,000×*g* for 2 min. The methanol and chloroform mixture (2:1, v/v) and ammonium thiocyanate (3.94 mol/L), BaCl₂ (0.132 mol/L), and FeSO₄ (0.144 mol/L) solutions were added to the organic layer in that order. After 20 min, the absorbance was recorded at 510 nm with an HP8453 UV-visible spectrophotometer (Hewlett Packard, Wilmington, DE, USA), and the hydroperoxide content was expressed as CuOOH, for which the calibration curve was linear between 0 and 1.5 mmol/kg ($r^2 = 0.9988$). The *p*-anisidine value was determined using the AOCS method Cd 18-90 (22) after

centrifugation (Avanti J; Beckman, Fullerton, CA, USA) of the emulsion dissolved in isooctane at 11,949×*g* and 4°C for 20 min.

Analysis of polyphenols in herb extracts and emulsion Composition of polyphenols in the herb extract was determined by HPLC (17) using a YL 9100 HPLC (Younglin Instrument Co., Ltd., Anyang, Korea) equipped with a UV detector (280 nm), YL 9150 autosampler, and symmetric C18 column (4.6 × 150 mm, 5 μm; Waters Corp., Milford, MA, USA). The mobile phase was a mixture of 2.5% formic acid and 100% methanol in a gradient system of 5% (0 min), 30% (15 min), 40% (40 min), 50% (60 min), 55% (65 min), and 100% (90 to 95 min) on methanol basis at 0.8 mL/min. Quantification was performed based on calibration curves ($r^2 > 0.9900$) of the standard polyphenols: rosmarinic acid, caffeic acid, gallic acid, *p*-coumaric acid, and catechin.

Total polyphenols of herb extracts were determined by spectrophotometry at 725 nm using the Folin-Ciocalteu reagent and a UV-visible spectrophotometer (HP 8453; Hewlett Packard) (23). Total polyphenol content in the emulsion was determined according to the method of Ahn and Choe (24) with a slight modification. A mixture of methanol and water (60:40, v/v) was added to the emulsion (0.5 g) dissolved in *n*-hexane, followed by centrifugation (Avanti J; Beckman) at 4°C and 11,872×*g* for 20 min. The Folin-Ciocalteu reagent was added to the bottom layer and the absorbance was read at 725 nm using a UV-visible spectrophotometer (HP 8453). The concentration of polyphenols was expressed as a caffeic acid equivalent with a calibration curve ($r^2 = 0.9599$).

Statistical analysis All the samples were prepared in duplicates, and each sample was measured twice. Data were statistically analyzed using SAS/PC (SAS 9.2, SAS Institute Inc., Cary, NC, USA) including regression analyses, *t*-test, and Duncan's multiple range test at a significance level of 5%.

Results and Discussion

Polyphenols of herb extracts The yield of 80% ethanol extracts of rosemary, basil, peppermint, thyme, and oregano were 22.9, 20.15, 20.18, 15.70, and 23.64%, respectively, based on a dry basis. The total polyphenol content of the peppermint extract (48.41±1.28 mg/g) was the highest ($p < 0.05$), followed by that of the basil extract (41.30±0.80 mg/g). There was no significant difference ($p > 0.05$) in the total polyphenol content among the rosemary (37.66±0.25 mg/g), thyme (35.82±1.30 mg/g), and oregano (35.14±1.49 mg/g) extracts. These values were lower than the values of the methanol extracts of herbs reported by Shan *et al.* (17), which could be because of the difference in hydrophilicity of the extracting solvent and genotype and/or cultivating conditions of the herbs (25-27).

Polyphenol compositions of the ethanol extracts of herbs obtained using HPLC are shown in Table 1. Among the HPLC peaks, rosmarinic acid, caffeic acid, gallic acid, *p*-coumaric acid, and catechin were

Table 1. Total contents and composition of polyphenols in herb extracts

Total content (mg/g)		Rosemary	Basil	Peppermint	Thyme	Oregano
		37.66±0.25	41.30±0.80	48.41±1.28	35.82±1.30	35.14±1.49
Relativity (%) ¹⁾	Rosmarinic acid	28.62	53.09	54.52	21.34	19.03
	Caffeic acid	0.86	1.56	2.40	2.56	3.26
	Gallic acid	0.74	0.58	0.64	1.57	0.57
	<i>p</i> -Coumaric acid	0.28	0.51	ND ²⁾	3.30	8.99
	Catechin	2.79	2.14	4.94	0.49	0.27
	Others	66.72	42.12	37.50	70.74	67.88

¹⁾Numbers represent % area of total peak areas²⁾Not detected **Fig. 1.** Effect of addition of herb extract on the hydroperoxide contents and *p*-anisidine values of the soybean oil-in-water (4:6, w/w) emulsion during iron-catalyzed oxidation at 25°C in the dark (◆, control without herb extract; □, rosemary; △, basil; ○, peppermint; ◇, thyme; and ×, oregano). Different letters on the line indicate significantly different values based on Duncan's multiple range test at 5%.

identified based on standard compounds. Rosmarinic acid showed the highest peak area, particularly high in the basil and peppermint extracts (>50%), which was similar to the result of others (10,17,28). Catechin was a relatively more important polyphenol in the rosemary, basil, and peppermint extracts than in the thyme and oregano extracts. The oregano extract showed *p*-coumaric and caffeic acid as relatively abundant compounds among polyphenols. The unidentified peaks corresponded to 66.72, 42.12, 37.50, 70.74, and 67.88% of the total peak areas in the rosemary, basil, peppermint, thyme, and oregano extracts, respectively, and require further studies. Polyphenol composition (types and concentrations) of herbs have been reported to be different depending on the harvest times, seasonal variations, and drying conditions (27-29).

Effects of herb extract on oil autoxidation in the emulsion Degree of oil oxidation, as measured based on the hydroperoxide production and *p*-anisidine values, of the soybean O/W emulsions over 15 days of storage at 25°C in the dark is shown in Fig. 1. The hydroperoxide content of the emulsions increased with storage time because of the iron-catalyzed oil oxidation. The hydroperoxide content of the

control emulsion without herb extract was 0.02 mmol/kg before oxidation and increased to 0.26, 0.71, and 1.06 mmol/kg after 3, 9, and 15 days, respectively. The *p*-anisidine value of the control emulsion was 2.44 before oxidation and increased to 3.63, 3.81, 9.46, and 19.86 after 3, 6, 9, and 15 days, respectively. Addition of the herb extract significantly ($p < 0.05$) decreased the hydroperoxide content. The emulsion with the added rosemary, basil, peppermint, thyme, and oregano extracts at 100 mg/kg showed hydroperoxide contents of 0.08, 0.07, 0.07, 0.15, and 0.09 mmol/kg, respectively, after 3 days; 0.31, 0.20, 0.19, 0.26, and 0.29 mmol/kg, respectively, after 9 days; and 0.42, 0.35, 0.34, 0.43, and 0.39 mmol/kg, respectively, after 15 days. Hydroperoxide contents were significantly lower in the emulsion with the added basil or peppermint extract than in the emulsion with the added rosemary, thyme, or oregano extract ($p < 0.05$). This result shows that the addition of basil or peppermint extract significantly decreased the hydroperoxide production compared with the addition of rosemary, thyme, or oregano extract. The high antioxidant activity of basil and peppermint extracts could partly result from the relatively high proportion of rosmarinic acid in the extracts (30). *p*-Anisidine values of the emulsion also increased with

Table 2. Effect of herb extracts (100 mg/kg) on the regression analysis between time and the oil oxidation of soybean oil-in-water (4:6, w/w) emulsion during iron-catalyzed oxidation at 25°C for 15 days

Herb	Hydroperoxide content			<i>p</i> -Anisidine values		
	a ¹⁾	b ¹⁾	r ²	a ¹⁾	b ¹⁾	r ²
None (control)	0.0727	0.0785	0.9428	1.2353	0.1058	0.9027
Rosemary	0.0280* ²⁾	0.0299	0.9662	0.9835	0.2146	0.9095
Basil	0.0220*	0.0172	0.9860	0.9218*	0.0592	0.9279
Peppermint	0.0194*	0.0270	0.9532	0.9003*	0.5403	0.8573
Thyme	0.0261*	0.0616	0.9301	1.0023	0.5830	0.9035
Oregano	0.0246*	0.0299	0.9787	1.0225	0.1152	0.9219

¹⁾Hydroperoxide content (mmol/kg)/*p*-Anisidine values=a×oxidation time (day)+b, r²=determination coefficient

²⁾*; Significant difference between the control emulsion without herb extract addition and the emulsion with added herb extract by dummy variable regression analysis (*p*<0.05)

time, particularly after 6 days, because of the decomposition of the primary oxidation products of the emulsion lipid. Addition of the herb extract did not significantly affect the values up to 6 days; however, it significantly decreased the *p*-anisidine values upon 9 days of oxidation. The emulsion with added rosemary, basil, peppermint, thyme, and oregano extract at 100 mg/kg showed *p*-anisidine values of 3.02, 2.70, 2.73, 4.34, and 2.81, respectively, after 3 days; 6.12, 8.05, 4.90, 7.15, and 6.48, respectively, after 9 days; and 16.44, 15.56, 17.38, and 17.07, respectively, after 15 days. Among the herb extracts added, peppermint and basil extracts showed the highest reduction in the *p*-anisidine value. These results confirmed the higher antioxidant activity of the basil and peppermint extracts over the rosemary, thyme, and oregano extracts during the iron-catalyzed oxidation of soybean oil in the O/W emulsion. Gramza *et al.* (31) reported that polyphenols in the herb extract acted as antioxidants to decrease both the primary and secondary oxidation of lipids.

Table 2 shows high correlations (*r*²>0.85) between time and oil oxidation of the emulsion during storage for 15 days at 25°C. The rates of hydroperoxide production in the emulsion with the added rosemary, basil, peppermint, thyme, and oregano extracts were 0.0280, 0.0220, 0.0194, 0.0261, and 0.0246 mmol/kg/day, respectively, which were significantly lower (*p*<0.05) than that of hydroperoxide production in the control emulsion (0.0727 mmol/kg/day). The rates of the *p*-anisidine value increase in the emulsion with the added basil and peppermint extracts were 0.9218 and 0.9003/day, respectively, which were significantly lower (*p*<0.05) than that of the *p*-anisidine value increase in the control emulsion (1.2353/day). There was no significant difference (*p*>0.05) in the rate of *p*-anisidine value increase among the control emulsion and the emulsions with the added rosemary (0.9835/day), thyme (1.0023/day), or oregano (1.0225/day) extract. These results strongly suggest that basil and peppermint extracts at 100 mg/kg improve the oxidative stability of lipids in a soybean O/W emulsion by decreasing the production of primary and secondary oil oxidation products. It was shown that the ethyl ether extracts of peppermint, rosemary, and basil decreased the oxidation of lard (32). The antioxidant activity of the ethanol extract of basil was reported to be through scavenging of reactive oxygen species,

Fig. 2. Polyphenol content of the soybean oil-in-water (4:6, w/w) emulsion with added herb extracts during iron-catalyzed oxidation at 25°C in the dark (□, rosemary; △, basil; ○, peppermint; ◇, thyme; and ×, oregano). Different letters on the line indicate significantly different values based on Duncan's multiple range test at 5%.

including superoxide anion radicals, hydrogen peroxides, and metal chelating (18). Rosmarinic acid, a major antioxidant compound in sweet basil, can capture 1.52 radicals (10). The ethanol (95%) extract of peppermint at 10 mg/mL showed low metal chelating activity, approximately 14.3% of that shown by EDTA (33). The antioxidant activity of oregano in O/W emulsion is also related to the radical scavenging capacity but not to the iron-chelating ability (34); thus, its antioxidant activity in our iron-catalyzed oxidation system may have been low.

Polyphenol contents during the oxidation of the emulsion Total content of polyphenols in the emulsions with the added herb extracts significantly decreased (*p*<0.05) during the 15 days of oxidation at 25°C, as shown in Fig. 2. The total contents of polyphenols in the emulsions with the added rosemary, basil, peppermint, thyme, and oregano extracts at 100 mg/kg were initially 8.13, 10.71, 11.00, 7.45, and 7.69 mg/kg, respectively, before oxidation and decreased to 7.37, 9.76, 10.02, 6.43, and 7.09 mg/kg, respectively, after 3 days; to 5.41, 7.30, 7.96, 5.01, and 5.21 mg/kg,

Table 3. Degradation of polyphenols in the soybean oil-in-water (4:6, w/w) emulsion with added herb extracts during iron-catalyzed oxidation at 25°C for 15 days

Herb added	Regression parameters ¹⁾		
	k	t _{1/2}	r ²
Rosemary	0.0272	25.48	0.9341
Basil	0.0265	26.16	0.9302
Peppermint	0.0259	26.76	0.9597
Thyme	0.0236	29.37	0.9068
Oregano	0.0240	28.88	0.8810

¹⁾Estimated by regression assuming 1st-order kinetics, $\ln([A]/[A]_0) = -k \times \text{time (day)}$, where $[A]$ and $[A]_0$ are the total content of polyphenols (mg/kg) at times t and 0 , respectively; $t_{1/2}$, half-life; r^2 , determination coefficient

respectively, after 9 days; and 5.06, 6.85, 6.94, 4.66, and 5.13 mg/kg, respectively, after 15 days. This clearly indicates degradation of polyphenols derived from herb extracts during the oxidation of the emulsion. Degradation of polyphenols during lipid oxidation is related to their role as antioxidants: polyphenols can donate hydrogen to peroxy radicals of lipids, and/or the radicals of polyphenols can directly form a linkage with peroxy radicals (15).

The rate of polyphenol degradation, as determined from the slope of a regression line between oxidation time and polyphenol retention ($r^2 > 0.88$), was not significantly different among the emulsions with the different herb extracts ($p > 0.05$), ranging from 0.0236 to 0.0272/day (Table 3). This indicates no significant difference in the degradation rate of total polyphenols among the emulsions by the addition of different herb extracts. This result suggests the contribution of polyphenol composition to the antioxidant activity of herb extracts because significantly different antioxidant activity was observed among the herb extracts with polyphenols that did not show significantly different degradation rates as aforementioned. It has been reported that the antioxidant activity of the herbs in lard did not correlate with total phenols (12,32). A systematic study on the oil oxidation affected by polyphenol composition is required.

Concentration dependence of the basil and peppermint extracts on oil oxidation in the emulsion The effect of herb extract concentration on the oil oxidation in the emulsion was studied using the basil and peppermint extracts (0, 50, 100, 200, and 400 mg/kg), which showed a high antioxidant activity. Degree of oil oxidation, measured based

Fig. 3. The hydroperoxide content and *p*-anisidine values of oil in soybean oil-in-water (4:6, w/w) emulsion with added basil or peppermint extract at different addition levels during iron-catalyzed oxidation at 25°C in the dark. Different letters on the bar indicate significantly different values based on Duncan's multiple range test at 5%.

on the hydroperoxide production and *p*-anisidine values, of the emulsions stored at 25°C in the dark for 6 and 12 days is shown in Fig. 3. As the addition level of the basil and peppermint extracts increased, oil oxidation decreased. The hydroperoxide contents of the emulsions with the added basil and peppermint extracts at 50, 100, 200, and 400 mg/kg were 0.33 and 0.27, 0.23 and 0.27, 0.21 and 0.14, and 0.17 and 0.12 mmol/kg, respectively, after 6 days of oxidation; the *p*-anisidine values were 4.21 and 3.73, 3.48 and 2.95, 2.75 and 2.35, and 2.54 and 2.32, respectively. After 12 days of oxidation, the hydroperoxide contents of the emulsions with the added basil and peppermint extracts at 50, 100, 200, and 400 mg/kg were 0.74 and 0.75, 0.53 and 0.62, 0.30 and 0.30, and 0.38 and 0.30 mmol/kg, respectively, and the *p*-anisidine values were 9.12 and 9.69, 8.46 and 7.79, 6.90 and 5.62, and 4.18 and 3.58, respectively. All the values were significantly lower ($p < 0.05$) than those of the control emulsion without the added herb extracts (0.62 mmol/kg and 5.68 after 6 days and 1.02 mmol/kg and 13.18 after 12 days). The radical scavenging activity of the basil extract was reported to be

Table 4. Regression between addition level of herb extracts and oil oxidation during iron-catalyzed oxidation of the soybean oil-in-water emulsion (4:6, w/w) at 25°C in the dark for 6 or 12 days

Herb	Oxidation days	Hydroperoxide production			<i>p</i> -Anisidine values		
		a ¹⁾	b ¹⁾	r ²	a ¹⁾	b ¹⁾	r ²
Basil	6	0.003	0.417	0.6837	0.002	4.696	0.7804
	12	0.002	0.772	0.5982	0.003	11.720	0.9600
Peppermint	6	0.004	0.409	0.7581	0.002	4.287	0.6656
	12	0.003	0.854	0.8059	0.003	11.566	0.9606

¹⁾ Hydroperoxide contents (mmol CuOOH/kg)/*p*-Anisidine values = $b \times e^{-a \times \text{addition level of herb in mg/kg}}$, r^2 = determination coefficient

concentration dependent within 50 mg/kg (18). The regression for the evaluation of concentration dependence of the basil and peppermint extracts is shown in Table 4. The hydroperoxide contents and *p*-anisidine values showed relatively good exponential relationships with the addition level of the basil ($r^2 > 0.59$) and peppermint extracts ($r^2 > 0.66$). Peppermint essential oil at 0.1% decreased generation of secondary oxidized products by 76.9% in a model linoleic acid emulsion system (35), although its role as a prooxidant was reported in lard on a cellulose matrix (31).

In conclusion, the ethanol extracts of rosemary, basil, peppermint, thyme, and oregano significantly improved the oxidative stability of oil in soybean O/W (4:6, w/w) emulsions by decreasing the generation of primary and secondary oxidation products. The antioxidant activity of the basil and peppermint extracts was higher than that of the rosemary, thyme, and oregano extracts and showed concentration dependence within 400 mg/kg.

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