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Spinach (*Spinacia oleracea*) Powder as a Natural Food-Grade Antioxidant in Deep-Fat-Fried Products

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The addition of spinach (*Spinacia oleracea*) powder in flour dough as a natural antioxidant was investigated, and oxidation of frying oil and the lipid in fried products during frying was also studied. Flour dough with spinach powder was rolled into sheets of 0.1 cm thickness and then cut into squares to be fried. Each frying was performed in 160 °C soybean oil for 1 min, repeated every 20 min for 20 h. Fried samples were analyzed immediately or after being stored at 60 °C for 12 days under dark. The lipid content of fried dough was lower in samples with the addition of spinach powder. Spinach in the dough decreased accumulation of the polar compounds in soybean oil during frying but had little effect on the fried dough. It also reduced conjugated diene and aldehyde formation in the lipid of fried dough during storage. Improvement in lipid oxidative stability, presumably due to pigments in spinach, was more noticeable in the fried products during storage than in the frying oil.

KEYWORDS: Lipid oxidation; frying; storage; spinach (*Spinacia oleracea*) powder

INTRODUCTION

Deep-fat frying is one of the most popular food preparation and processing practices. Oil is continuously exposed to air, light, and moisture for extended periods of time at an elevated temperature during frying. Under such conditions, the acceleration of both thermal and oxidative decomposition reactions occurs (*1*−*3*), causing fat deterioration. Lipid oxidation in the fried food also takes place during storage after frying. This, in turns, reduces the acceptability of the fried foods (*4*), and highly abused fat can result in adverse health effects such as colon carcinogenesis (*5*), reproductive disorders, mutagenicity, and growth depression (*6*).

Many efforts have been made to reduce or retard the undesirable lipid oxidation reactions, and use of antioxidants is proven to be useful. Several antioxidants are frequently used presently: butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), tocopherols, and so on. Because of safety concerns, natural antioxidants are preferred to synthetic compounds; therefore, alternative sources of antioxidants have been sought for food applications. Rosemary, defatted rice bran, and sage extracts were reported to be effective antioxidants in deep-fat-fried foods during frying and storage (*7*, *8*). Interest in plant sources became high due to their physiological functions, and many food products with added plant materials have been introduced to the market.

Spinach, which is cultivated throughout the year, provides color as well as many nutrients and is not expensive. There is a wide variety of foods with added spinach: bread, noodles, tofu, and cheese. Spinach contains about 5% lipids on a dry basis, and the lipid consists of palmitic (15.04%), hexadecatrienoic (7.69%), linoleic (11.84%), and linolenic (58.46%) acids (*9*). Because of the presence of highly unsaturated fatty acids and other components, such as 596 mg % chlorophyll and 42.8 mg % β-carotene (*10*), the addition of spinach as an ingredient to foods may affect food processing properties and the quality of the final products. Chlorophyll present in green tea has been reported to have an antioxidant activity during 60 °C storage or 180 °C frying of the oil (*11*). Pheophytins produced by chlorophyll decomposition during heating (*12*, *13*) have shown antioxidant activity in the oil stored in the dark (*14*). Chlorophyll was also suggested to protect β-carotene, a good free radical scavenger, from decomposition in the dried, oiled, and toasted laver during storage at 60 °C (*15*).

To extend the utilization of spinach to deep-fat-fried foods, it is thus necessary to evaluate lipid oxidation occurring both in frying oil and in fried foods. Therefore, this study was performed to investigate the effects of spinach powder added to flour dough on oxidation of frying oil and the lipid in fried products during frying and storage.

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Table 1. Compositions of Wheat Flour Dough Used for Deep Fat Frying

spinach content (%)	weight of ingredients (g)		
	water	wheat flour	spinach powder
0 (control)	35	100	0
5	35	95	5
15	35	85	15
25	35	75	25

MATERIALS AND METHODS

Samples and Chemicals. Wheat flour and soybean oil (refined, bleached, and deodorized) were provided by Daehan Flour Mills Co., Ltd. (Seoul, Korea) and Lotte Samkang Co. Ltd. (Seoul, Korea), respectively. Fresh spinach (*Spinacia oleracea* King) was purchased at a local supermarket in Incheon, South Korea, on the day of harvest and then trimmed, washed with distilled water, and drained. It was freeze-dried at −54° C for 24 h, ground to 5 mesh, and stored at −84° C in a freezer until further use.

Isooctane, 2-propanol, anisidine, 14% BF_3−methanol, and carotene were obtained from Sigma Chemical Co. (St. Louis, MO). Silica gel 60 (70−230 mesh ASTM) for column chromatography was a product of Merck Corp. (Darmstadt, Germany). Celite 545 was a product of Shinyo Pure Chemical Co., Ltd. (Osaka, Japan). All other chemicals were of reagent grade.

Sample Preparation. Wheat flour, spinach powder, and distilled water were mixed in the ratios shown in **Table 1** in a dough mixer (Kitchenaid, St. Joseph, MI). Spinach powder contents were 5%, 15%, and 25% on the basis of dry ingredients, and the control was made without any spinach powder. The dough was processed into sheets of 0.1 cm thickness by passing it between two rollers in a pasta maker (Atlas and Pastabike, OMC, Marcoto, Italy). The sheets were cut into squares (2 cm × 2 cm) and deep fried. Frying was done with 100 g of square-shaped dough in an electric fryer holding 3 L of soybean oil at 160 °C for 1 min. Frying was repeated every 20 min for 20 h. Fried dough and oil samples were collected every 4 h in glass bottles, and the heated oil in the fryer was replenished simultaneously. The bottles were tightly sealed after being flushed with nitrogen and placed in a −24 °C freezer for analyses. All samples were prepared in duplicate.

The samples used for the storage stability study were those fried in the oil whose heating time was less than 2 h. Fried dough samples (60.0 ± 0.5 g) were put into 460 mL glass bottles, and then the bottles were tightly sealed with Teflon-coated septa and aluminum caps. They were wrapped with aluminum foil to exclude light and stored at 60 °C in an incubator (Kukje, Seoul, Korea) for 12 days. Two bottles were taken out every other day for the analyses.

Moisture and Lipid Contents of Fried Samples. The moisture contents of fried samples were determined by the method of Choe et al. (*10*) using a moisture balance (HA-300; Precisa Instruments AG, CH-Dietikon, Switzerland). Lipid contents were measured gravimetrically after extraction by the Folch method (*16*) with chloroform and methanol (2:1, v/v).

Determination of Frying Oil Oxidation. The oxidation of soybean oil during repeated frying was evaluated by measuring the free fatty acid value (FFAV) by the AOCS method (*17*) as well as by determining the polar compounds content. Polar compounds were measured gravimetrically after the oil was passed through a glass column (2.1 cm × 45 cm) packed with silica gel 60 according to the AOAC method (*18*).

Analysis of Carotenes in Frying Oil. Carotenes in frying oil during frying were determined according to the AOAC method (*18*). Frying oil dissolved in hexane was applied to a glass column (1.25 cm × 30 cm) which was packed with a mixture of silica gel 60 and Celite 545 (1:1, w/w). The elution solvents were mixtures of hexane and acetone with volume ratios of 96:4, 90:10, and 80:20 for carotenes, monohydroxy xanthophylls, and dihydroxy xanthophylls, respectively. Other xanthophylls were eluted with a mixture of hexane, acetone, and methanol (80:10:10, v/v/v). Each carotenoid separated was analyzed by visible spectroscopy (*18*). A UV−vis spectrophotometer (HP 8453, Hewlett-Packard, Wilmington, DE) was used. Wavelengths for determination of carotene and all xanthophylls were 436 and 474 nm, respectively.

Table 2. Moisture and Lipid Contents of Fried Dough

spinach content (%)	moisture content (%)	lipid content (%)
0	4.31 ± 0.21	31.78 ± 0.04
5	5.91 ± 0.51	32.79 ± 0.01
15	7.39 ± 0.38	27.09 ± 0.88
25	6.24 ± 0.67	22.82 ± 0.17
spinach powder	9.96 ± 0.07	5.10 ± 0.14
wheat flour	12.68 ± 0.68	2.76 ± 0.90

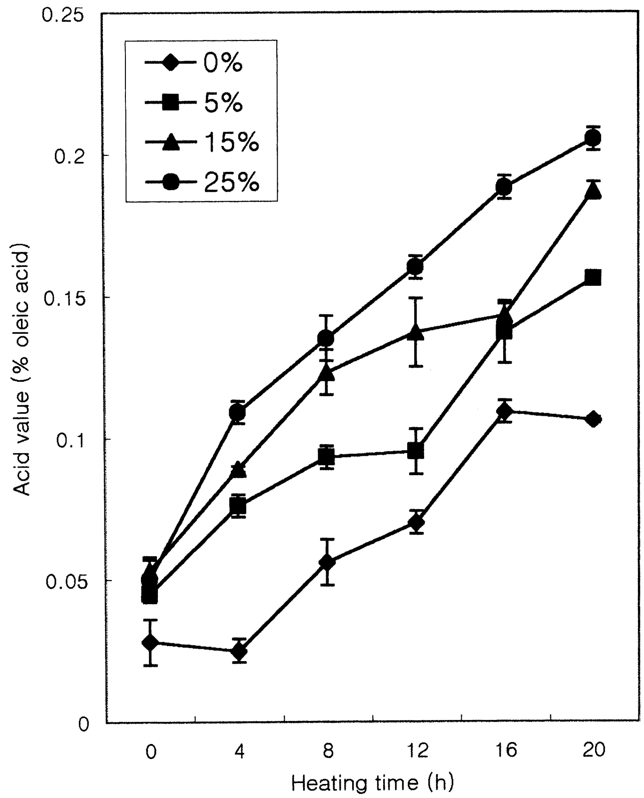

Figure 1. Effects of spinach powder added to flour dough on the free fatty acid values of soybean oil during frying at 160 °C. (For zero heating time, samples were taken from the oil heated to 160 °C and used to fry one batch of dough for 1 min.)

Analysis of Lipid Oxidation of Fried Dough. Lipid in fried dough was extracted before the analyses, as described above. Oxidation of lipid in fried dough was evaluated by determining conjugated dienoic acid (CDA) and *p*-anisidine values (PAVs) by the AOCS method (*17*). The fatty acid composition was determined by gas chromatography (GC) with a Younglin M600L gas chromatograph (Younglin Co., Seoul, Korea) equipped with a Supelcowax capillary column (30 m × 0.53 mM, 1.0 μM thick; Supelco Inc., Bellefonte, PA) and a flame ionization detector after esterification with 14% BF_3−methanol solution (*7*). The temperatures of the oven, the injector, and the detector were 230, 280, and 280 °C, respectively. The nitrogen flow rate was 5 mL/min, and the split ratio was 33:1.

Statistical Analysis. One-way analysis of variance (*19*) was used to analyze the data. A *p* value of 0.05 or less was considered significant.

RESULTS AND DISCUSSION

Moisture and Lipid Contents of Fried Dough. The moisture and lipid contents of the dough fried in soybean oil with different heating times are shown in **Table 2**. The moisture contents of the fried samples ranged from 4.31% to 7.39%, while the lipid

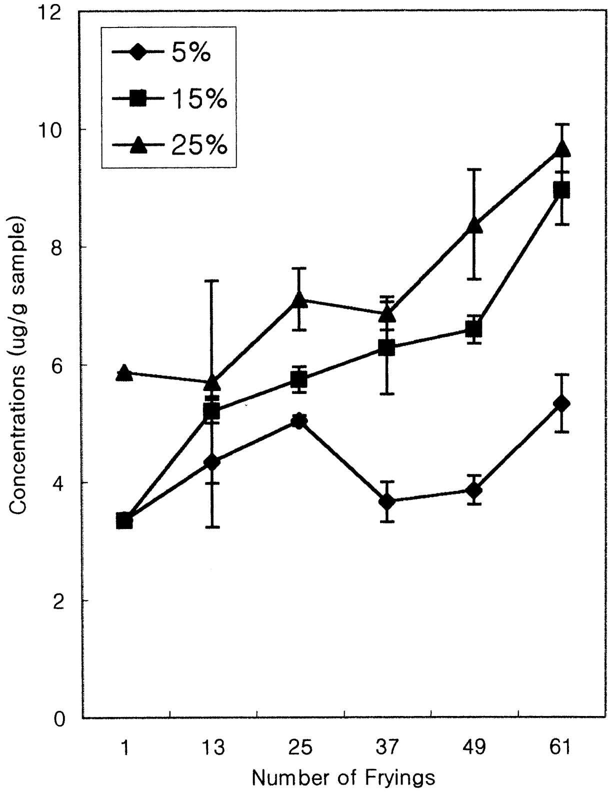

Figure 2. Carotene contents in soybean oil during frying of flour dough containing spinach powder at 160 °C for 20 h.

contents ranged from 22.82% to 32.79%. The moisture content of fried dough was lower than that of spinach powder (9.96%) or wheat flour itself (12.68%). On the other hand, fried dough contained more lipid than spinach powder (5.10%) or wheat flour (2.76%) due to oil absorption from the frying medium. The higher the moisture content, the less lipid the fried dough contained. The total lipid content of deep-fat-fried foods has been reported to be influenced by the original fat and moisture contents and by the type of breading or batter used (*20*, *21*). Fried dough containing spinach powder tended to have more moisture and less lipid than the control, although spinach powder contained more lipid and less moisture than wheat flour. This might be due to higher hydrophilicity introduced by nitrate and oxalate present in spinach (*22*). Spinach also contains hydrophilic sulfolipid and phospholipids at about 3% and 22% of total lipid (*9*), respectively.

Table 3. Changes in Contents of Polar Compounds of Soybean Oil during Frying at 160 °C of Flour Dough with Various Levels of Added Spinach Powder

	polar compounds (%)[a]			
heating time (h)	0% spinach powder added	5% spinach powder added	15% spinach powder added	25% spinach powder added
0[b]	6.86 ± 0.16^{A}	3.38 ± 0.01^{D}	4.55 ± 0.24^{C}	5.56 ± 0.52^{B}
4	7.46 ± 0.11^{A}	5.35 ± 0.47^{C}	5.87 ± 0.08$^{B C}$	6.74 ± 0.64$^{A B}$
8	8.77 ± 0.57^{A}	8.60 ± 0.34^{A}	7.86 ± 0.05^{A}	8.70 ± 0.57^{A}
12	9.73 ± 0.93^{A}	9.51 ± 0.71^{A}	8.74 ± 0.02^{A}	10.86 ± 1.31^{A}
16	10.91 ± 0.44^{B}	13.02 ± 0.46^{A}	11.58 ± 0.58^{B}	13.99 ± 0.03^{A}
20	16.15 ± 0.64^{A}	14.78 ± 2.09^{A}	12.87 ± 0.67^{A}	15.12 ± 0.98^{A}

[a] Different superscripts mean significant differences as analyzed by Duncan's multiple range test among samples within the same row at $\alpha = 0.05$. [b] For zero heating time, samples were taken from the oil heated to 160 °C and used to fry one batch of dough for 1 min.

Effects of Spinach Powder Added to Flour Dough on the Oxidation of Soybean Oil during Frying. The effects of spinach powder added to flour dough on the FFAV of soybean oil during frying at 160 °C for 20 h are shown in **Figure 1**.

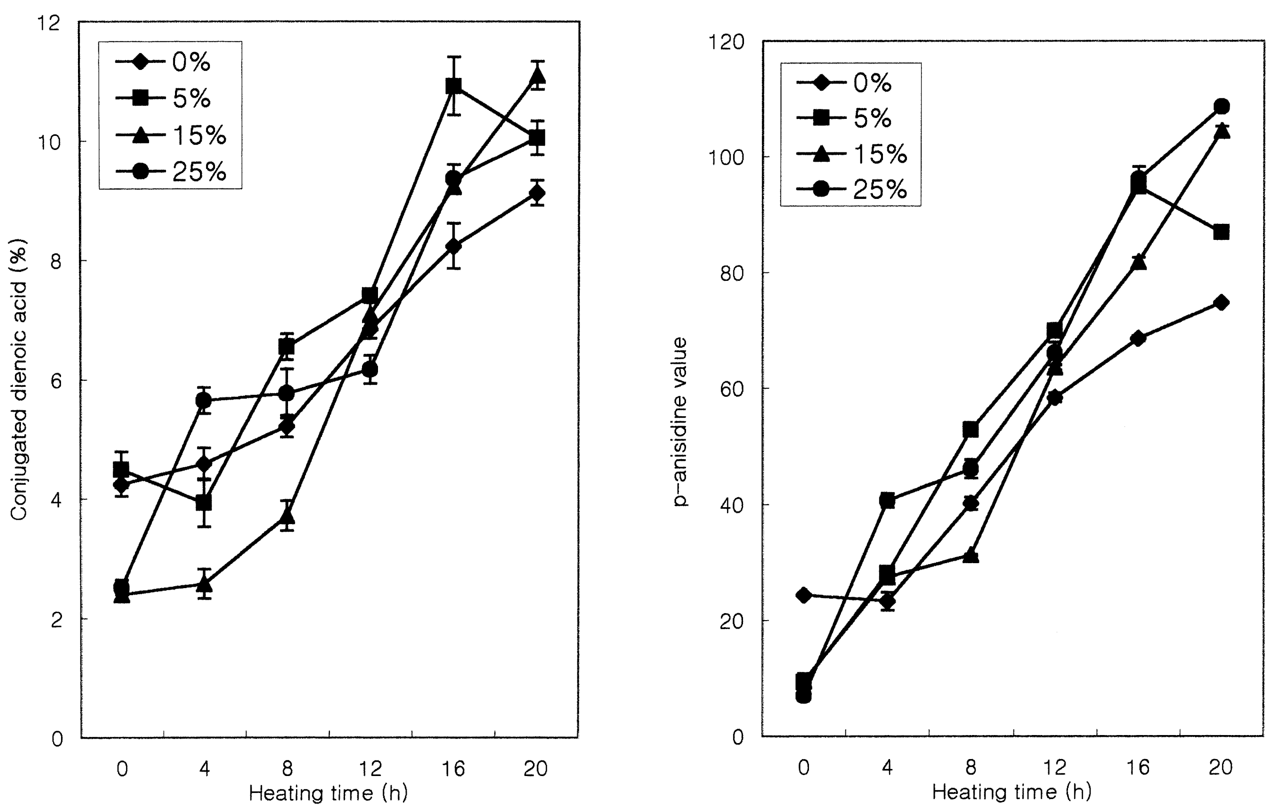

Figure 3. Effects of spinach powder added to flour dough on conjugated dienoic acid content and *p*-anisidine values of the fried dough in soybean oil at 160 °C. (For zero heating time, samples were taken from the oil heated to 160 °C and used to fry one batch of dough for 1 min.)

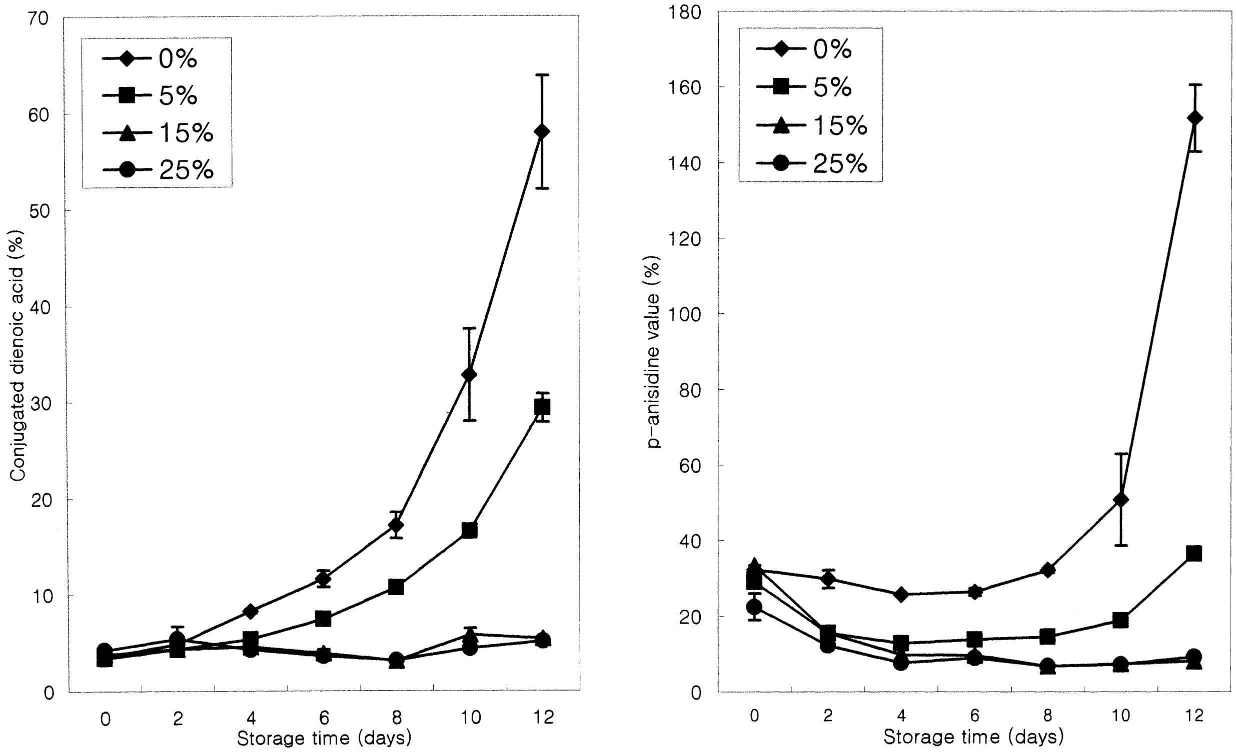

Figure 4. Effects of spinach powder added to flour dough on conjugated dienoic acid content and *p*-anisidine values of the fried dough during storage at 60 °C for 12 days.

Table 4. Fatty Acid Compositions of the Fried Dough with Various Levels of Added Spinach Powder during Storage at 60 °C

spinach content (%)	storage time (days)	relative content (%)					U/S[a]
		C16:0	C18:0	C18:1	C18:2	C18:3	
0	0	11.32 ± 0.22	5.05 ± 0.33	23.79 ± 0.48	53.01 ± 0.77	6.67 ± 0.19	5.104
	2	11.38 ± 0.46	4.95 ± 0.25	24.25 ± 0.47	52.46 ± 0.42	6.55 ± 0.08	5.107
	4	11.66 ± 0.10	5.11 ± 1.23	24.33 ± 0.47	54.19 ± 1.63	4.67 ± 0.60	4.981
	6	11.60 ± 0.23	5.45 ± 0.09	24.80 ± 0.12	52.25 ± 0.18	5.89 ± 0.27	4.863
	8	11.81 ± 0.21	5.25 ± 0.13	24.64 ± 0.23	52.58 ± 0.44	5.72 ± 0.13	4.863
	10	12.22 ± 0.68	5.54 ± 0.13	25.69 ± 0.24	51.42 ± 0.74	5.14 ± 0.40	4.638
	12	14.82 ± 0.10	6.78 ± 0.20	28.78 ± 1.12	44.90 ± 0.41	4.21 ± 0.13	3.605
5	0	11.46 ± 0.24	5.46 ± 0.10	24.81 ± 0.05	52.76 ± 0.53	5.52 ± 0.35	4.910
	2	11.68 ± 0.27	5.36 ± 0.20	24.98 ± 0.17	52.71 ± 0.32	5.27 ± 0.48	4.869
	4	11.48 ± 0.07	5.21 ± 0.20	24.60 ± 0.12	53.06 ± 0.29	5.66 ± 0.14	4.992
	6	11.61 ± 0.13	5.03 ± 0.21	24.60 ± 0.21	53.34 ± 0.26	5.47 ± 0.15	5.014
	8	11.41 ± 0.20	5.02 ± 0.14	24.97 ± 0.05	53.79 ± 0.14	4.81 ± 0.11	5.086
	10	11.47 ± 0.25	4.94 ± 0.12	24.90 ± 0.31	53.54 ± 0.21	5.16 ± 0.45	5.097
	12	11.97 ± 0.16	5.24 ± 0.03	25.27 ± 0.03	52.21 ± 0.11	5.31 ± 0.03	4.810
15	0	11.54 ± 0.17	5.12 ± 0.08	24.34 ± 0.05	52.99 ± 0.18	6.01 ± 0.25	5.002
	2	11.45 ± 0.09	5.14 ± 0.14	24.72 ± 0.35	52.85 ± 0.21	5.85 ± 0.21	5.031
	4	11.26 ± 0.06	4.96 ± 0.11	24.18 ± 0.04	53.70 ± 0.32	5.90 ± 0.25	5.166
	6	11.43 ± 0.18	4.81 ± 0.08	24.31 ± 0.40	53.98 ± 0.14	5.48 ± 0.46	5.160
	8	11.39 ± 0.09	4.71 ± 0.24	24.08 ± 0.24	52.29 ± 0.68	5.54 ± 0.59	5.215
	10	11.26 ± 0.10	4.82 ± 0.15	24.09 ± 0.06	53.92 ± 0.48	5.90 ± 0.35	5.217
	12	11.48 ± 0.31	4.76 ± 0.31	24.13 ± 0.10	53.87 ± 0.57	5.77 ± 0.66	5.161
25	0	11.49 ± 0.05	4.96 ± 0.09	24.15 ± 0.08	53.50 ± 0.38	5.90 ± 0.43	5.080
	2	11.45 ± 0.13	5.10 ± 0.10	24.26 ± 0.10	53.04 ± 0.10	6.16 ± 0.04	5.044
	4	11.53 ± 0.10	4.89 ± 0.05	24.15 ± 0.17	53.65 ± 0.28	5.79 ± 0.31	5.091
	6	11.37 ± 0.10	4.83 ± 0.28	24.11 ± 0.19	53.67 ± 0.73	6.02 ± 0.20	5.175
	8	11.43 ± 0.12	4.65 ± 0.12	23.73 ± 0.10	54.20 ± 0.15	5.99 ± 0.13	5.219
	10	11.55 ± 0.21	4.68 ± 0.33	23.73 ± 0.46	53.84 ± 0.50	6.19 ± 0.20	5.163
	12	11.65 ± 0.20	4.87 ± 0.25	24.31 ± 0.25	53.69 ± 0.60	5.57 ± 0.30	5.061

[a] Ratio of unsaturated fatty acids to unsaturated fatty acids.

The FFAV of soybean oil increased progressively with the heating time of the frying oil. In addition, the FFAV of the oil used to fry the dough containing spinach powder was significantly higher than that of the oil used to fry the dough without spinach powder. This might be due to hydrolysis of triacylglycerols by thermostable phospholipase or galactolipase present in spinach powder (*23*).

Changes in polar compounds in soybean oil during frying are shown in **Table 3**. The contents of polar compounds increased with the heating time due to accumulation of polar compounds in the oil as a result of degradation of hydroperoxides and/or dimerization or polymerization. This tendency was also reported by Cuesta et al. (*24*). In most countries, the limit of heat abuse is set at 25% polar compounds (*25*). Addition of spinach powder to the dough helped to reduce the accumulation of polar compounds in oil, especially in the early stage of frying.

Figure 2 shows the carotene content in the frying oil during continuous frying of dough. As frying was repeated, the carotene content in the oil increased. This indicates that carotene in the dough containing spinach powder was dissolved into the frying oil during frying. Also, the more spinach added to the dough, the more carotene was dissolved into the oil.

The results clearly suggest that spinach powder added to flour dough reduced the oxidation of frying oil, which was attributed to dissolution of carotene into the oil from spinach powder. It has been reported that carotene is relatively stable during heating, and it can act as a free radical scavenger to reduce lipid oxidation (*26*, *27*).

Effects of Spinach Powder Added to Flour Dough on the Lipid Oxidation of Fried Dough during Frying. CDA contents and PAVs of the lipid extracted from samples fried in soybean oil with different heating times are shown in **Figure 3**. CDA contents and PAVs of the lipid in the dough fried in oil that had been heated longer tended to be higher. This is due to the higher stability of the conjugated double bond than of a nonconjugated system and the increase in aldehyde compounds formed from hydroperoxide decomposition, respectively. However, significant differences over heating time of the oil among fried dough containing different concentrations of spinach powder were not found, which indicates that spinach added to the dough did not affect the lipid oxidation of the fried dough during frying.

Effects of Added Spinach Powder on Lipid Oxidation of Fried Dough during Storage. CDA contents and PAVs of the lipid extracted from fried dough during storage at 60 °C for 12 days are shown in **Figure 4**. As the storage time increased from 0 to 12 days, CDA contents and PAVs of fried dough containing no spinach powder increased at a rate faster than those of fried dough containing spinach powder. Fried dough with 15% or 25% spinach powder did not show any changes in CDA content and PAV during the storage time. Fried dough with 5% spinach powder showed values lower than those of the control but higher than those of fried dough containing 15% or 25% spinach powder.

Changes in the fatty acid composition of fried dough during storage at 60 °C in the dark are shown in **Table 4**. Lipids in fried dough consisted of palmitic, stearic, oleic, linoleic, and linolenic acids, and their composition was very similar to that of the frying oil, despite the very high content (52.01%) of linolenic acid in spinach (*9*). This is due to a very low contribution from spinach to the lipid of the fried products. It was reported that the frying oil composition exerted the major influence on the composition of the lipids in deep-fat-fried foods (*20*). A higher decrease in the linolenic and linoleic acid contents and U/S ratio, and a significant increase in the palmitic acid content, were found in fried dough containing no spinach powder during storage. On the other hand, these changes were negligible with the fried dough containing spinach powder over the storage period.

The results clearly indicate that spinach powder can reduce lipid oxidation of fried products remarkably during storage in the dark. This might be partly due to the antioxidant activity of chlorophyll, pheophytin, and carotenes in spinach powder, as reported by Endo et al. (*14*) and Goulson (*26*), respectively.

ABBREVIATIONS USED

FFAV, free fatty acid value; CDA, conjugated dienoic acid; PAV, *p*-anisidine value; GC, gas chromatography; U/S, ratio of unsaturated fatty acids content to saturated fatty acids content.

LITERATURE CITED

(1) Chang, S. S.; Peterson, R. J.; Ho, C. T. Chemical reaction involved in the deep-fat frying of foods. *J. Am. Oil Chem. Soc.* **1978**, *55*, 718−727.

(2) Grandgirard, A.; Sebedio, J. L.; Hemy, J. Geometrical isomerization of linoleic acid during heat treatment of vegetable oils. *J. Am. Oil Chem. Soc.* **1984**, *61*, 1563−1568.

(3) Sebedio, J. L.; Prevost, J.; Grandgirard, A. Heat treatment of vegetable oils. II. GC-MS and GC−FTIR spectra of some isolated cyclic fatty acid monomers. *J. Am. Oil Chem. Soc.* **1987**, *64*, 1324−1333.

(4) Jacobson, G. A. Quality control in deep-fat frying operations. *Food Technol.* **1991**, *45*, 72−74.

(5) Yang, C. M.; Kendall, C. W. C.; Stamp, D.; Medline, A.; Archer, M. C.; Bruce, W. R. Thermally oxidized dietary fat on colon carcinogenesis in rodents. *Nutr. Cancer*. **1998**, *30*, 69−73.

(6) Clark, W. L.; Serbia, G. W. Safety aspects of frying fats and oils. *Food Technol.* **1991**, *45*, 84−90.

(7) Choe, E.; Lee, Y.; Choi, S. Effects of antioxidants in the frying oil on the flavor compound formation in the ramyon during storage. *Korean J. Food Sci. Technol.* **1993**, *25*, 444−448.

(8) Jaswir, I.; Che Man, Y. B.; Kitts, D. D. Effects of natural antioxidants in controlling alkaline contaminant materials (ACM) in heated palm olein. *Food Res. Int.* **2000**, *33*, 75−81.

(9) Lee, J.; Park, K.; Lee, S.; Choe, E. Lipid changes of freeze-dried spinach by various kinds of oxidation. *J. Food Sci.* **2000**, *65*, 1290−1295.

(10) Choe, E.; Lee, J.; Park, K.; Lee, S. Effects of heat pretreatment on lipid and pigments of freeze-dried spinach. *J. Food Sci.* **2001**, *66*, 1074−1079.

(11) Zandi, P.; Gordon, M. H. Antioxidant activity of extracts from old tea leaves. *Food Chem.* **1999**, *64*, 285−288.

(12) Schwartz, S. J.; Woo, S. L.; Von Elbe, J. H. High performance liquid chromatography of chlorophylls and their derivatives in fresh and processed spinach. *J. Agric. Food Chem.* **1981**, *29*, 533−535.

(13) Schwartz, S. J.; Von Elbe, J. H. Kinetics of chlorophyll degradation to pyropheophytin in vegetables. *J. Food Sci.* **1983**, *48*, 1303−1306.

(14) Endo, Y.; Usuki, R.; Kaneda, T. Antioxidant effects of chlorophyll and pheophytin on the autoxidation of oils in the dark. I. Comparison of the inhibitory effects. *J. Am. Oil Chem. Soc.* **1985**, *62*, 1375−1378.

(15) Choe, E.; Cha, J. Lipid oxidative stability of dried, oiled and toasted laver as affected by β-carotene and chlorophyll. *Food Sci. Biotechnol.* **1998**, *7*, 60−65.

(16) Folch, J.; Lees, M.; Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipid from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497−502.

(17) AOCS. *Official Methods and Recommended Practices of the American Oil Chemists' Society*; AOCS: Champaign, IL, 1990.

(18) AOAC. *AOAC methods of analysis*, 16th ed.; AOAC: Arlington, VA, 1990.

(19) Ott, L. Introduction to the analysis of variance. In *An Introduction to Statistical Methods and Data Analysis*, 2nd ed.; PWS Publishers: Boston, MA, 1984; pp 325−360.

(20) Smith, L. M.; Clifford, A. J.; Creveling, R. K.; Hamblin, C. L. Lipid content and fatty acid profiles of various deep fat fried foods. *J. Am. Oil Chem. Soc.* **1985**, *62*, 996−999.

(21) Romero, A.; Cuesta, C.; Sanchez-Muniz, F. J. Trans fatty acid production in deep fat frying of frozen foods with different oils and frying modalities. *Nutr. Res.* **2000**, *20*, 599−608.

(22) Gupta, K.; Wagle, D. S. Nutritional and antinutritional factors of green leafy vegetables. *J. Agric. Food Chem.* **1988**, *36*, 472−474.

(23) Kim, M.; Oh, J.; Cheon, S.; Cheong, T.; Lee, A., Choi, E., Lee, H.; Park, C., Park, K. Thermal inactivation kinetics and application of phospho- and galactolipid-degrading enzymes for evaluation of quality changes in frozen vegetables. *J. Agric. Food Chem.* **2001**, *49*, 2241−2248.

(24) Cuesta, C.; Sanchez-Muniz, F. J.; Garrido-Polonio, C.; Lopez-Varela, S.; Arroyo, R. Thermoxidative and hydrolytic changes in sunflower oil used in fryings with a fast turnover of fresh oil. *J. Am. Oil Chem. Soc.* **1993**, *70*, 1069−1073.

(25) Hein, M.; Henning, H.; Isengard, H.-D. Determination of total polar parts with new methods for the quality survey of frying fats and oil. *Talanta* **1998**, *47*, 447−454.

(26) Goulson, M. J.; Warthesen, J. J. Stability and antioxidant activity of beta carotene in conventional and high oleic canola oil. *J. Food Sci.* **1999**, *64*, 996−999.

(27) Beutner, S.; Bloedorn, B.; Frixel, S.; Blanco, I. H.; Hoffmann, T.; Martin, H.-D.; Mayer, B.; Noack, P.; Ruck, C.; Schmidt, M.; Schulke, I.; Sell, S.; Ernst, H.; Haremza, S.; Seybold, G.; Sies, H.; Stahl, W.; Walsh, R. Quantitative assessment of antioxidant properties of natural colorants and phytochemicals: carotenoids, flavonoids, phenols and indigoids. The role of β-carotene in antioxidant functions. *J. Sci. Food Agric.* **2001**, *81*, 559−568.

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