

Antioxidant Activity of Green Tea Extract in Soybean and Rice Bran Oils

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Abstract

Antioxidant activity of green tea extracts (GTE) was evaluated in soybean oil (SBO), rice bran oil (RBO) and winterized rice bran oil (WRBO) stored at 63°C for 36 days. Lipid oxidation of the oils was determined using the active oxygen method (AOM), peroxide value (POV), change in unsaturated free fatty acid concentrations and by sensory evaluation. SBO had a higher concentration of the polyunsaturated fatty acids, linoleic and linolenic acid than RBO and WRBO. WRBO and RBO were more stable against lipid oxidation than SBO. Addition of GTE (200 ppm) to the stored oils, increased the induction period (IP) in AOM, reduced the increase in POV, and lessened the change in unsaturated fatty acids. Furthermore, GTE prevented the development of rancid flavors resulting from storage, all of which demonstrate the protective antioxidative activity of GTE. However, oil color became darker in the GTE treated oils. The antioxidant protection of GTE was most effective in RBO.

Key words: green tea extract, antioxidant activity, soybean oil, rice bran oils, lipid oxidation

INTRODUCTION

Antioxidant addition to lipid-containing, commercially produced, foods is one of the most effective means of retarding fat oxidation and extending shelf-life. Synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone (TBHQ) have been widely used in many foods. However, the use of synthetic antioxidants has been questioned because of possible toxicity issues (1). Consequently, there is considerable interest in developing natural antioxidants that can provide potential nutritional and therapeutic properties (1-8).

The extract of rosemary leaves has been one of the most effective spice-derived antioxidants. Recently, it was reported that the antioxidative activity of green tea catechins is stronger than rosemary extract in canola oil, pork lard and chicken fat (9). Therefore, green tea catechins may be a viable alternative to rosemary and synthetic antioxidants for protecting fats and oils from oxidative rancidity (9-11). Catechin, an important constituent of green tea, is also known to have various physiological activities, including antimutagenicity, inhibition of platelet aggregation (12), prevention of dental caries (13) and cardiovascular disease (14), anticarcinogenicity (15), antibacterial activities (16) and protection against ethanol-induced oxidative stress in the brain (17). Although green tea extract is an effective antioxidant in oils, the effectiveness may vary depending on the type and processing of the oils (18).

Soybean is the most important oil seed in the global market. The high polyunsaturated fatty acid (PUFA) content of soybean oil (SBO) makes it an important dietary source of essential fatty acids. The PUFA in SBO, however, is susceptible to oxidative reaction (19).

Despite its similarities to other common vegetable oils, rice bran oil (RBO) offers several unique properties that make it very appealing as a specialty oil. It has been reported that foods fried in RBO have good stability and prolonged shelf-life, partly due to a low linolenic acid content (20). RBO also contains a unique complex of naturally occurring antioxidants, including tocopherols, tocotrienols and oryzanol compounds (21) that may also have health benefits, such as reducing cholesterol, preventing cancer and cardiovascular disease, and anti-aging protection (21,22). The ability of a green tea extract to act as an antioxidant in rice bran and soybean oils was evaluated under accelerated storage condition (63°C). Because green tea extract is water soluble, an ethanol-oil emulsion was used to facilitate suspension of the extract in the oils.

MATERIALS AND METHODS

Materials

Soybean oil (SBO) was provided by Cheil Jedang, Co. Ricebran oil (RBO) was obtained from Sinyang Rice & Oil Co., and divided into two portions, one for use without modification and the other winterized to produce

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WRBO, using an oil manufacturing process. Green tea extract (GTE), standardized to 85% total phenols, was obtained from Healthland Supplies Ltd. (China). Total phenol content of GTE was measured by the Sugiura method (23). General reagents were purchased from DUKSAN, Co.

Preparation of emulsion

Ethanol-oil emulsions containing green tea extract were prepared by the method of Chen et al. (9) and Jung et al. (24). Briefly, extracts were prepared by homogenizing (ULTRA-TURRAX T25, Janke & Kunkel GmbH & Co., KG., Germany) 200 mL of SBO, RBO or WRBO with 1 mL of ethanol, containing 0.04 mg of GTE, for 5 min at 18,000 rpm. The final concentration of GTE was 200 ppm.

Active oxygen method (AOM)

The induction period of the oils, defined as the time (to the nearest hour) required for the sample to attain a peroxide value (POV) of 100 meq/kg, was determined using a Rancimat (617, Methrom, Sweden) under the following conditions: 3.0 g oil sample, 18 L/h air flow rate, and 98°C temperature. The control was SBO without any antioxidant.

Fatty acid composition

Fatty acids were periodically analyzed by gas chromatography (model 5890 series, Hewlett Packard, USA) during the 66 day storage period at 63°C. The injector and detector (flame ionization detector) temperatures were 230°C and 240°C, respectively, and the column temperature was 250°C (SP 2330, SUPELCO, USA). Fatty acid concentrations were calculated from their relative percentages by the area of each peak.

Oven test

A 200 g aliquot of each oil sample was placed in an uncapped Erlenmeyer flask and stored for 36 days in incubator (LBI-150M, LABTECH, Korea) set at 63°C. The peroxide values (POV) were measured in triplicate using the AOCS method (25).

Color value

The colors of oil samples were measured using a digital color difference meter (CM-3500d, Minolta spectrophotometer, Japan). Color values were recorded as L (lightness; 0 = black, 100 = white), a (-a = greenness, +a = redness), b (-b = blueness, +b = yellowness) and ΔE values ($\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$). The instrument was standardized with distilled water (L = 100.03, a = -0.01, b = -0.02).

Sensory evaluation

A nine-member panel was selected from departmental graduate students with experience in sensory evaluation to evaluate the rancidity of oil emulsions during the stor-

age period at 63°C. The control emulsion was stored in a refrigerator (4°C). Prior to evaluation, the samples were placed in 50 mL beakers and allowed to equilibrate to room temperature for 1 hr. The panel evaluated the emulsions at the beginning of the storage period and at 4 day intervals throughout the study. Rancidity was evaluated on a 7 point scale (0: equal to control, 3: beginning to lose acceptability, 6: unacceptability) (26).

Statistical analysis

All the collected data were analyzed by analysis of variance (ANOVA) and Duncan's Multiple Range Test using the programmed SPSS (Statistical Package, SPSS Inc.). Significant level is $p < 0.05$ unless otherwise indicated (27).

RESULTS AND DISCUSSION

Active oxygen method (AOM)

The AOM induction periods (IP) of control and GTE-treated oils are shown in Table 1. The IP of RBO (18.5 hrs) and WRBO (19 hrs) appeared to be longer than that of SBO (13.5 hrs). The use of GTE in the three oils increased the IP, indicating a delay in lipid oxidation; probably due to the high content of phenolic compounds in green tea, which are known to have antioxidant effects (10). Equal concentrations of GTE (200 ppm) elicited a more pronounced increase in the antioxidative index (AI) in RBO and WRBO than SBO, proving that oil type is a determinant of the relative effectiveness of GTE as an antioxidant. These results are in concordance with Evans (18) who postulated that tea extracts provide a positive response in all oils, but that relative response varies.

Peroxide value (POV)

The primary products of lipid oxidation are hydroperoxides or peroxides. Therefore, it seems reasonable to determine the concentration of peroxides as a measure of the extent of oxidation. Measuring the POV of oils monitored the formation of primary oxidation products. Changes

Table 1. Changes in induction period (IP) from active oxygen method (AOM) of various oils with 200 ppm green tea extract (GTE)

Sample	IP (hrs)	AI ¹⁾
SBO	13.5	1.0
SBO+GTE (200 ppm)	16.0	1.2
RBO	18.5	1.4
RBO+GTE (200 ppm)	23.5	1.7
WRBO	19.0	1.4
WRBO+GTE (200 ppm)	25.0	1.9

¹⁾Antioxidative index=induction period of oil with antioxidants /induction period of SBO.

Abbreviation: SBO=soybean oil; RBO=rice bran oil; WRBO=winterized rice bran oil; IP: induction period.

in POV during the storage period are shown in Fig. 1. POV increased with storage time in all three oils, but the greatest increase was in SBO; probably due to higher concentrations of linoleic and linolenic acid, which are susceptible to lipid oxidation. During the 36 day storage period, the increase of POV was higher in oils containing no GTE than those with GTE, demonstrating that GTE had a positive antioxidant effect in all three oils. Other investigators (10,11) have reported that the use of water-soluble extracts of green tea in soybean oil lowers POV during storage at 55~60°C. The antioxidant activity of GTE was most effective in RBO, followed by SBO and WRBO. These results were probably due to in part the substrate-specific nature of free radical interceptor reactions with antioxidants (18). Although the origins of the two rice bran oils, RBO and WRBO, were the same, there was impressively less change in the POV in RBO. One explanation for the reduced oxidation in RBO is that winterization removes naturally occurring antioxidant compounds that act synergistically with GTE. Alternatively, winterization might remove some of the saturated fatty acids. Others have sug-

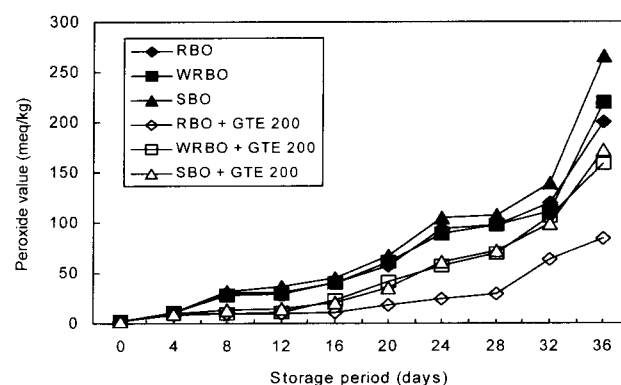


Fig. 1. Changes in peroxide value by various oils emulsified with green tea extract (GTE) during 36 day storage at 63°C.

gested that naturally occurring antioxidants may be lost during the winterization process (28).

Fatty acid composition of oils

The changes in fatty acid composition of oils during the storage period are shown in Table 2. RBO and WRBO had less unsaturated fatty acid, especially linoleic (18:2) and linolenic acids (18:3), than SBO. The loss of unsat-

Table 2. Changes of fatty acid compositions (FAC) of various oils containing 200 ppm green tea extract (GTE) during 66 day storage at 63°C (unit: %)

Oils	FAC	Control				GTE			
		Storage day				Storage day			
		0	17	32	66	0	17	32	66
RBO	C 14:0	0.42	0.41	0.39	0.46	0.41	0.40	0.41	0.44
	C 16:0	19.44 ^c	19.1 ^d	19.77 ^b	21.8 ^a	19.25 ^b	18.99 ^c	19.06 ^c	19.77 ^a
	C 16:1	0.22 ^b	0.25 ^a	0.24 ^a	0.06 ^b	0.16 ^{ab}	0.23 ^a	-	0.12 ^{ab}
	C 18:0	2.05 ^c	2.25 ^b	2.07 ^c	2.41 ^a	2.03 ^b	2.06 ^b	2.10 ^b	2.24 ^a
	C 18:1	42.09 ^c	42.04 ^c	43.35 ^b	46.20 ^a	42.08 ^b	41.88 ^{bc}	41.72 ^c	43.06 ^a
	C 18:2	33.23 ^a	33.14 ^a	31.35 ^b	27.09 ^c	33.31 ^b	33.49 ^b	33.74 ^a	31.65 ^c
	C 18:3	1.45 ^a	1.57 ^a	1.47 ^a	0.98 ^b	1.56 ^{ab}	1.60 ^{ab}	1.74 ^a	1.38 ^b
	C 20:0	1.20	1.11	1.21	0.99	1.14	1.22	1.19	1.29
	C 20:1	-	0.09	-	-	-	0.12	-	-
	UR	3.33	3.37	3.26	2.90	3.38	3.41	3.39	3.21
WRBO	C 14:0	0.30 ^{bc}	0.28 ^c	0.31 ^{ab}	0.32 ^a	0.30	0.30	0.31	0.30
	C 16:0	18.08 ^c	17.81 ^d	18.48 ^b	19.79 ^a	18.03 ^{bc}	17.8 ^c	18.20 ^b	19.5 ^a
	C 16:1	0.21	0.23	0.28	0.04	0.21	0.16	0.22	0.05
	C 18:0	1.88 ^b	1.81 ^b	1.89 ^{ab}	1.99 ^a	1.85 ^c	1.83 ^c	1.87 ^b	2.05 ^a
	C 18:1	41.78 ^c	41.86 ^c	42.93 ^b	45.09 ^a	41.81 ^c	41.98 ^c	42.66 ^b	45.02 ^a
	C 18:2	35.13 ^a	35.25 ^a	33.81 ^b	30.36 ^c	35.02 ^a	35.26 ^a	34.01 ^b	30.66 ^c
	C 18:3	1.64	1.69	1.26	1.35	1.67 ^a	1.77 ^a	1.84 ^a	1.31 ^b
	C 20:0	1.03	0.96	1.13	1.01	1.03	0.78	1.09	1.06
	C 20:1	-	-	-	-	0.15	0.08	-	-
	UR	3.70	3.79	3.59	3.32	3.72	3.83	3.67	3.36
SBO	C 14:0	0.07	0.04	0.08	0.06	-	0.06	-	-
	C 16:0	11.06 ^c	11.18 ^c	11.53 ^b	14.04 ^a	11.17 ^c	11.18 ^c	11.37 ^b	12.24 ^a
	C 16:1	-	-	-	0.22	-	-	-	-
	C 18:0	4.03 ^b	3.96 ^b	4.08 ^b	5.21 ^a	3.90 ^c	3.87 ^c	4.00 ^b	4.36 ^a
	C 18:1	24.22 ^c	24.24 ^c	25.22 ^b	29.55 ^a	24.34 ^c	24.18 ^c	24.92 ^b	26.57 ^a
	C 18:2	52.76 ^a	53.04 ^a	51.49 ^b	46.29 ^c	53.21 ^a	52.87 ^b	52.6 ^c	50.78 ^d
	C 18:3	6.40 ^a	6.38 ^a	6.01 ^b	4.14 ^c	6.45 ^a	6.52 ^a	6.03 ^b	5.19 ^c
	C 20:0	0.99	0.95	0.90	0.89	0.93	0.91	0.94	0.86
	C 20:1	0.52 ^a	0.26 ^b	0.41 ^{ab}	-	-	0.38	0.14	-
	UR	5.20	5.20	5.01	3.97	5.25	5.24	5.13	4.28

Abbreviation: SBO=soybean oil; RBO=rice bran oil; WRBO=winterized rice bran oil; UR=unsaturated ratio.

urated fatty acids is one characteristic of oxidative deterioration of oils (29). As oils deteriorate during storage there is a time-dependent decrease in the proportion of palmitoleic acid (16:1), linoleic acid (18:2) and linolenic acid (18:3); and a simultaneous increase in palmitic acid (16:0) and oleic acid (18:1). RBO and WRBO without GTE had less loss of unsaturated linoleic and linolenic acids than SBO. The loss of unsaturated fatty acids was reduced in RBO and SBO containing GTE, but here was little effect on WRBO. GTE was most effective in preventing unsaturated fatty acid loss in SBO. These results were not consistent with data from AOM and POV, probably due to the difference in the thermal stability of the oils.

Changes in oil color

The changes in oil color are shown in Table 3. SBO and RBO became darker in color during the storage period, while WRBO remained unchanged. The "L" and "a" values decreased, and "b" and ΔE value increased in SBO and RBO. With GTE, first day value of "L" was lower, and "a" and "b" values were higher than those without GTE, showing that the oils became darker. The increased degree of "b" and ΔE was large in oils with GTE during the storage period. This could be due to

the browning reaction in which phenolic compounds in GTE were oxidized (30). Of the three GTE treated oils, SBO had the greatest change in color, which was consistent with the higher increase in POV, indicating that SBO is the least stable and most easily oxidized of the oils.

Sensory evaluation

Since peroxides are vulnerable to further reaction, the complete oxidation history of an oil may not be revealed by POV. Therefore, the oil samples were periodically examined by sensory evaluation to detect any development of rancid odors or flavors (31). Rancidity was defined as objectionable flavors resulting from the accumulation of oxidative decomposition products. Sensory evaluation results are shown in Table 4. The starting point of rancid flavor was scored over 3 points by panels. Without GTE, rancid flavors in SBO, WRBO and RBO were detected after 20, 28 and 32 days, respectively; the addition of 200 ppm GTE delayed rancidity by 4~8 days. Therefore, these results demonstrate that GTE can retard the development of rancid flavors in oils.

In summary, WRBO and RBO were more stable over time against lipid oxidation than SBO, as measured by AOM, POV, fatty acid composition, and rancidity de-

Table 3. Changes of color in various oils containing green tea extract (GTE) 200 ppm during 32 day storage at 63°C

	Day	SBO		RBO		WRBO	
		-	GTE 200	-	GTE 200	-	GTE 200
L ¹⁾	0	99.60 ^a	97.14 ^a	98.09 ^a	96.35 ^a	95.90 ^c	94.07 ^a
	8	98.86 ^b	97.10 ^b	97.12 ^d	95.22 ^b	95.32 ^c	93.96 ^b
	16	98.75 ^c	96.81 ^d	97.32 ^c	94.90 ^c	95.79 ^d	93.80 ^d
	24	98.72 ^d	96.69 ^e	97.08 ^e	94.60 ^d	96.07 ^b	93.62 ^e
	32	98.87 ^b	96.98 ^c	97.54 ^b	94.48 ^c	96.41 ^a	93.87 ^c
a	0	-2.26 ^d	-2.12 ^e	-5.20 ^d	-4.83 ^a	-6.29 ^d	-5.68 ^a
	8	-3.63 ^c	-3.30 ^d	-5.18 ^d	-4.42 ^b	-5.97 ^c	-5.08 ^c
	16	-3.85 ^b	-3.36 ^c	-5.49 ^b	-4.27 ^c	-6.44 ^c	-4.98 ^d
	24	-4.24 ^a	-3.43 ^b	-5.36 ^c	-4.14 ^d	-6.76 ^b	-4.88 ^e
	32	-4.26 ^a	-3.59 ^a	-5.69 ^a	-4.08 ^c	-6.88 ^a	-5.24 ^b
b	0	7.50 ^e	10.08 ^e	22.79 ^e	24.95 ^e	36.42 ^d	37.53 ^e
	8	11.97 ^d	16.73 ^d	26.89 ^b	28.32 ^d	38.45 ^a	39.79 ^d
	16	12.48 ^c	17.82 ^c	26.60 ^d	29.08 ^c	37.52 ^b	40.32 ^c
	24	13.93 ^a	18.44 ^a	28.43 ^a	30.42 ^b	36.98 ^c	41.93 ^a
	32	13.28 ^b	18.81 ^b	26.81 ^c	31.08 ^a	36.21 ^e	41.33 ^b
ΔE	0	7.86 ^e	10.71 ^e	23.47 ^c	25.70 ^c	37.21 ^d	38.44 ^e
	8	12.58 ^d	17.33 ^d	27.56 ^b	29.09 ^d	39.21 ^a	40.59 ^d
	16	13.13 ^c	18.43 ^c	27.31 ^d	29.84 ^c	38.31 ^b	41.11 ^c
	24	14.62 ^a	19.41 ^a	29.08 ^a	31.17 ^b	37.80 ^c	42.69 ^a
	32	13.96 ^b	19.01 ^b	27.49 ^c	31.81 ^a	37.01 ^e	42.09 ^b

Abbreviation: See footnote of Table 2.

¹⁾L: Measures lightness and varies from 100 for perfect white to zero black.

a: Measures redness when plus and greenness when minus.

b: Measures yellowness when plus and blueness when minus.

ΔE : $\sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$, color difference.

^{a-c}Means with the same letter in column of each sample are not significantly different with increasing storage period.

Table 4. Results of the difference from control test for rancid flavor of various oils with addition of 200 ppm GTE during 36 day storage at 63°C

Day	RBO		WRBO		SBO	
	-	GTE	-	GTE	-	GTE
4	D ^{0.75} a ¹⁾	D ⁰ b	E ^{0.63} ab	E ⁰ b	D ^{0.5} ab	E ^{0.75} a
8	CD ^{1.38} a	D ^{0.13} b	E ¹ ab	DE ^{0.88} ab	C ^{1.88} a	DE ¹ ab
12	CD ^{1.5} ab	CD ¹ b	DE ^{1.63} ab	CDE ^{1.13} b	BC ^{2.5} a	CDE ^{1.5} ab
16	BCD ^{1.88} ab	CD ^{1.13} b	CDE ^{1.75} ab	CD ^{1.25} b	BC ^{2.63} a	CDE ^{1.88} ab
20	BCD ² ab	BC ^{1.75} b	BCD ^{2.5} ab	BCD ^{1.88} ab	AB ^{3.5} a	BCD ^{2.25} ab
24	BC ^{2.25} b	BC ^{1.88} b	ABC ^{2.75} ab	BC ^{2.25} b	AB ^{3.75} a	ABC ^{2.75} ab
28	AB ^{2.88}	AB ^{2.75}	ABC ³	AB ^{2.63}	AB ^{3.75}	AB ^{3.63}
32	AB ^{3.13}	AB ^{2.88}	AB ^{3.63}	AB ^{2.88}	A ^{4.13}	A ^{3.75}
36	A ^{3.75}	A ^{3.38}	A ^{3.88}	A ^{3.5}	A ^{4.5}	A ^{3.88}

Abbreviation: See footnote of Table 2.

¹⁾Means of difference score from control; 0: equal to control, 1: slight difference, 2: more distinct difference but still acceptable, 3: beginning to lose acceptability, 4: more distinct loss of acceptability, 5: very distinct loss of acceptability, 6: unacceptability.

^{a-b}Means with the same letter in each row among samples are not significantly different ($p < 0.05$).

^{A-E}Means with the same letter in column of each sample are not significantly different with increasing storage period.

velopment. Addition of 200 ppm GTE extended the AOM induction period, decreased the POV, lessened the changes in unsaturated fatty acids, and retarded the development of rancid flavors during storage; demonstrating that GTE is an effective antioxidant in oil. However, GTE-treated oil became darker over time than untreated. GTE provided greater antioxidant protection in RBO than WRBO or SBO, as measured by changes in POV and rancidity. Therefore, we conclude that green tea extract is most effective as an antioxidant in rice bran oil, as compared to soybean oil and winterized rice bran oil.

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