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Determination of Main Tea Seed Oil Antioxidants and their Effects on Common Kilka Oil

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Abstract: To determine the main tea seed oil antioxidants, extraction, separation and determination of the β-carotene, vitamin E (8 compounds) and polyphenols (8 compounds) was done using HPLC method. Tea seed oil as natural antioxidants at 5 and 10% levels were added to common kilka (*Clupeonella cultiventris caspia*) oil the peroxide (PV) and thiobarbitoric acid (TBA) values after 13 days at 60°C were evaluated for antioxidant effects. The average contents of the β-carotene, vitamin E and polyphenols in tea seed oil were 251.3±2.5, 389.3±3.0 and 24.81±1.00 mg/kg, respectively. Results show that catechins are the most important antioxidant in this oil in comparison with other vegetable oils (which contain no catechins in other oils). The antioxidant effects of tea seed oil in fish oil system showed significant differences from the control for PV and TBA values and preserved well during storage. Tea seed oil could be important as a raw material and functional product.

Keywords: Tea seed oil, antioxidant effect, common kilka oil, β-carotene, tocopherols, catechins.

INTRODUCTION

Like other genera of *Camellia* (from Theaceae family), the tea plant (*C. sinensis*) produces large oily seeds. In some countries where tea seed oil is abundantly available, it has been accepted as edible oil (Sahari *et al.*, 2004). There are several reports that the oil content of tea seed is about 30-32% (when computing the kernel and seed ratio, the value was 20%), remaining a liquid even at refrigeration temperature, and has a high organoleptic acceptability (Ravichandran, 1993; Sahari *et al.*, 2004). The predominant fatty acid in tea seed oil, as determined by GC/MS, is monounsaturated fatty acid, e.g. oleic acid, followed by the poly unsaturated fatty acid (PUFA), linoleic acid. Tea seed oil is high quality cooking oil, like olive oil, and it can be stored well at room temperature. Tea seed oil is reputed to lower blood pressure and cholesterol level, to have a high content of antioxidants, and to be a rich source of emollients for skin care and to minimize signs of aging (Fattahi-Far *et al.*, 2006).

Five to ten percent of tea seed oil can affect the shelf life of sunflower seed oil. The high stability of the tea seed oil is probably due to the low content of glycerides of linolenic and linoleic acids and the presence of polyphenols and vitamin E as antioxidants. The intensely yellow oil remains clear and liquid during storage even at refrigeration temperatures which may be due to the presence of carotenoids (Sahari *et al.*, 2004).

Carotenoids, fat soluble nutrients found in plant and animal tissues, appear useful for their biological activity. The β-carotene is largely regarded as an important nutritional component and the need for its monitoring in various foods of plant origin, biological tissues and food derivatives has led to much activity in this area, especially owing to its possible role as a powerful antioxidant and protective factor against the cancers and precursor of vitamin A (Luterotti *et al.*, 2002).

Vitamin E is a general term used for designation of tocopherols and tocotrienols (α, β, γ, δ) which are natural antioxidants that prevent the rancidity of oils during storage and thus prolong its shelf life (Gimeno *et al.*, 2000; Sanagi *et al.*, 2005). Among all Vitamin E, α-tocopherol present the highest biological potency (Lopez Ortiz *et al.*, 2006). Tocopherols are being intensively studied owing to their medical, biological, and physicochemical significance (Pyka and Sliwiok, 2001).

Phenolic compounds have been reported as having beneficial biological activities including antioxidant and free radical scavenging activity and oxidative stability (Owen *et al.*, 2000; Tsanova-Savova *et al.*, 2005; Wolfe *et al.*, 2003).

This study was undertaken to determine the main tea seed oil antioxidants (such as carotenoids,

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vitamin E homologues and phenolic compounds) from Lahijan variety in Iran and to evaluate the antioxidative effect of this oil on fish oil model system.

MATERIALS AND METHODS

Preparation of Tea Seed Oil

The main material, tea seed (*Lahijan* variety), was obtained from Iranian farms in Lahijan located in the north of Iran. Five days after collection from the farms and transfer to the laboratory in baskets at ambient conditions, the tea seeds (*Camellia sinensis*) were oven-dried at 102°C (moisture= 15%). After the tea seeds were ground, tea seed oil was extracted by the solvent method (petroleum ether; b.p. range 40-60°C) and oil was clarified by passage through fine cheesecloth (Sahari *et al.*, 2004).

Materials

The following materials namely (±)-α-tocopherol, (±)-β-tocopherol, (±)-γ-tocopherol, (±)-δ-tocopherol, (+)-gallocatechin [(+)-GC], (+)-catechin[(+)-C], (-)-epicatechin [(-)-EC], (-)-epigallocatechin [(-)-EGC], (-)-epigallocatechin gallate [(-)-EGCG], (-)-gallocatechin gallate [(-)-GCG], (-)-epicatechin gallate [(-)-ECG], (-)-catechin gallate [(-)-GC] and β-carotene were purchased from (Sigma Chemical Co. (MO, USA). Tocotrienols ($\alpha, \beta, \gamma, \delta$) were purchased from Apin Chemicals Limited (Oxen, UK). Methanol (≥99.5%), *n-hexane* (≥99.5%), tert-butyl methyl ether, tetrahydrofuran (THF), water (≥99.5%), chloroform and acetonitrile were of HPLC grade and petroleum benzene (b.p. 40-60°C) and orthophosphoric acid and other chemical reagents were of analytical grade with highest purity from Merck (Germany).

HPLC Analysis

All HPLC analyses were performed at 25°C with Waters 600E high performance liquid chromatograph (Waters, Millford, MA, USA) equipped with Separon SGX NH_2 C_{18} (250 mm × 4 mm) fitted with Separon SGX NH_2 C_{18} 5 µm (13mm × 4 mm) guard column (Tessek, Prague, Republic of Czech) for tocopherols and equipped with µBondapak C_{18} 10 µm (250 mm × 4.6 mm, 5 µm, Waters, Millford, USA) fitted with μBondapak C_{18} cartridge guard column (Waters, Millford, MA, USA) for polyphenols and β-carotene.

The stock solutions of tocopherols, catechins and β-carotene standards (50 ppm) were prepared by dissolving appropriate amounts of each standard (17 compounds) in hexane, water and methanol: THF (90: 10, v/v), respectively.

For determination of β-carotene, the mobile phase was methanol: THF (90: 10, v/v) and the elution was performed at a flow rate of 1 ml/min. Detection was accomplished with a UV detector and the chromatogram was recorded at 450 nm. The injection volume was 20 μl. Peaks were identified by comparing their retention times and with authentic standards.

For determination of tocopherols in tea seed oil, a mobile phase consisting of *n*-hexane: tert-butyl methyl ether: THF: methanol (79: 20: 1: 0.1, v/v) was used at a flow rate of 1ml/min. Injection volume was 20 μl and the eluate was detected using a Waters 2475 scanning fluorescence detector set at emission and excitation wavelengths of 326 nm and 294 nm, respectively. Peaks were identified by comparing their retention times and fluorescence spectra with authentic standards.

For determination of phenolic compounds, the mobile phase consisted of 0.1% ortho-phosphoric acid in water (v/v; eluent A) and 0.1% orthophosphoric acid in methanol (v/v; eluent B). The gradient program was as follows: 0-5 min, 20% B, 5-7 min, linear gradient from 20 to 24% B; 7-10 min, 24% B; 10-20 min, linear gradient from 24 to 40% B; 20-25 min, linear gradient from 40 to 50% B. Post-run time was 5 min. Elution was performed at a solvent flow rate of 1 ml/min. Detection was accomplished with a UV detector and chromatograms were recorded at 210 nm. The sample injection volume was 20 μl. Peaks were identified by comparing their retention times and UV spectra with authentic standards. Phenolic compounds were identified by comparing their retention times with those of corresponding standards. β-carotene, tocopherol, tocotrienol and phenolic compounds were quantified using external calibration standard method.

Fatty Acid Analysis

Fresh common kilka (five kg) was purchased from Caspian Sea (Babolsar, Mazandaran Province, and north of Iran). Common kilka oil (from one source) was extracted using the method of Bligh and Dyer (1959).

The fatty acid profile of common kilka oil was determined by GC. A BPX70 fused-silica capillary column with 30 m x 0.25 mm x 0.22 μm film thickness, from SGE, Melbourne, Australia at 190°C and helium as the carrier gas (50 psi) were used to separate the fatty acids. A split injector (1.2 μl injection) at 240°C and a FID at 250°C were also used during the separation. Fatty acids quantification was done using the internal standard method (C_{15} as an internal standard).

Fish Oil Stability

The extracted oils (tea seed with petroleum benzene) at 5 and 10% levels were added to fish oil system (common kilka oil). The oven test method at 60°C was used to evaluate stability (Egan *et al.*, 1987). The PV of oils stored under accelerated oxidation conditions were determined periodically (0, 4, 8 and 12 days) by the iodometric method according to AOCS guidelines (AOCS, 1989) and TBA values on 0, 5, 9 13 days were determined as described by Sidewell *et al.* (1954). A control sample was prepared under the same condition, without adding tea oil. All the experiments were carried out in triplicate and results were averaged.

Statistical Analysis

Statistical analysis was performed using SPSS software. Means separation test for significant treatment differences for parameters studied ($P< 0.05$) were done using the Fisher's Least Significant difference (LSD) and Duncan methods.

RESULTS AND DISCUSSION

HPLC Analysis

A typical HPLC profile of β-carotene in tea seed oil is presented in Figure 1. The results show that the β-carotene content (251.3 ± 2.5 mg/kg) in tea seed oil was similar, higher and lower compared with other oils (Table 1) such as palm (180-700 mg/kg), sesame seed (140 mg/kg), safflower seed (310 mg/kg) and olive (596 mg/kg), respectively, (Luterotti *et al.*, 2002; Chuang and Brunner, 2006). Many studies showed that, for antioxidant activity, β-carotene must be used together with citrate and/or ascorbate as a synergist reagent (Pokonery, 2001), which suggests that the high antioxidant activities of the tea seed oil cannot depend only on β-carotene.

A typical HPLC profile of tocopherols and the contents of these compounds in the tea seed oil are presented in Figure 2 and Table 2. The results show that the total content of tocopherols and tocotrienols were 376.0 ± 3.0 and 13.40 ± 0.34 mg/kg, respectively, and in vitamin E, α-tocopherol content was higher (210.0 ± 2.3) than the others. According to our results and other reports, the α-tocopherol content of tea seed oil is approximately similar to that of other vegetable oils such as olive (1-240), palm (180-260), peanut (80-330) and maize (80-260), mg/kg. However, its β-tocopherol content is lower than for other vegetable oils (soybean and sunflower) and is similar with that of olive and maize grain oil. Also, the content of γ-tocopherol in tea seed oil (23.60 ± 0.45 mg/kg) was lower than that of safflower seed (70- 190 mg/kg) and peanut (130-590 mg/kg) oils and was higher than that of olive and coconut oils. Tea seed oil's δ-tocopherol content (11.20 ± 0.33 mg/kg) was also similar with that of rape seed and peanut oils (10-20 mg/kg). The tocotrienols content of tea seed oil was higher than for other vegetable oils (except maize grain and palm oils) (Madhavi *et al.*, 1996; Luterotti *et al.*, 2002; Chuang and Brunner, 2006).

A representative chromatogram of phenolic compounds and the content of these compounds, in tea seed oil, are shown in Figure 3 and Table 3, respectively. The results show that the total content of phenolic compounds is 24.81 ± 1.0 mg/kg and the major component was EGCG (12.93 ± 0.97 mg/kg). Although tocopherols and carotenoids compounds can be found in other vegetable oils, the phenolic compounds are found only in tea seed oil. It can therefore be concluded that the main antioxidant activities of tea seed oil are due to phenolic and tocopherol compounds. Since these compounds have native polyphenolic they show high antioxidant activities (Shahidi and Naczk, 2004; Tsimogiannis and Oreopoulou, 2004).

Therefore, it can be suggested that the main antioxidant activities of tea seed oil are dependent on phenolic and tocopherol compounds especially EGCG, α-tocopherol and tocotrienols, respectively. In Table 3 the contents of tocopherol compounds in some vegetable oils, without any interpretation, are presented.

Fatty Acid Analysis

The FA profile of common kilka oil and tea seed oils are presented in Table 4. The FA composition of oils showed that common kilka oil had high amounts of EPA (5.21%) and DHA (5.73%). Furthermore, (EPA+DHA)/C16:0 ratio was 0.579 in common kilka oil. The total content of ω-3 and PUFA fatty acids in common kilka was 11.80±0.23 and 18.35±0.30%, respectively. The suitable content of these FA make fish oil an important functional food but because of its lower oxidation stability, natural antioxidants such as tea seed oil need to be added (Frankel, 1980; Sahari *et al.*, 2004).

Fish Oil Stability

The PV and TBA values of the common kilka oil (without addition of tea seed oil; control) and samples containing 5 and 10% extracted tea seed oil, during 13 days storage at 60°C, are presented in Table 5.

The results show that all the treated samples (with 5 and 10% of tea seed oil, as natural antioxidants) were significantly different from the control in PV and TBA values in fish oil system.

Table 1: Comparison of the contents of tocopherol and β-carotene compounds in some vegetable oils (mg/kg)

Vegetable oils	Tocopherols*				Tocotrienols*				β-carotene**
	α	β	γ	δ	α	β	γ	δ	
Coconut	5–10	—	5	5	5	Trace	1-20	—	—
Cottonseed	40–560	—	270–410	0	—	—	—	—	—
Maize grain	60–260	0	400–900	1-50	—	0	0-240	0	—
Maize germ	300–430––	1-20	450–790	5-60	—	—	—	—	—
Olive	1–240	0	0	0	—	—	—	—	596
Palm	180–260	Trace	320	70	120-150	20-40	260-300	70	180-700
Peanut	80–330	––	130-590	10-20	—	—	—	—	—
High oleic peanut***	14.59–16.17	0.70-1.03	6.90-10.62	4.61-4.99	––	—	—	—	—
Rapeseed	180–280	––	380-590	10-20	—	—	—	—	—
Safflower	340–450	––	70-190	230-240	—	—	—	—	310
Soybean	30–120	0-20	250-930	50-450	0	0	0	—	—
Sunflower	350–700	20-40	10-50	1-10	—	—	—	—	—
Walnut	560	––	590	450	—	—	—	—	—
Wheat germ	560–1200	660-810	260	270	20-90	80-90	—	—	—
Sesame	––	––	—	—	—	—	—	140	

*Madhavi *et al.*, 1996
**Luterotti *et al.*, 2002; Chuang and Brunner, 2006
***Jonnala *et al.*, 2006

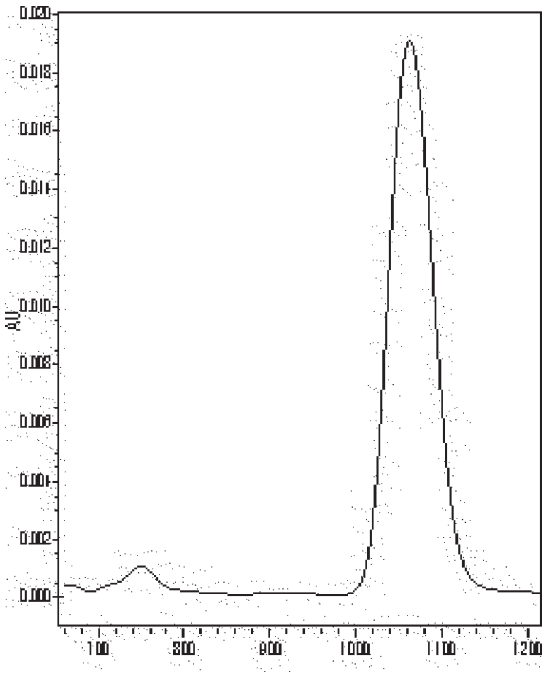

Figure 1: HPLC profile of β–carotene in tea seed oil

Table 2: Contents of vitamin E compounds in tea seed oil

No. peak	Component	Retention time	Content (mg/kg)
1	α-tocopherol	13.85	210 ± 2.3
2	β-tocopherol	14.54	ND
3	γ-tocopherol	22.06	23.60± 0.45
4	δ-tocopherol	24.27	11.20± 0.33
5	α-tocotrienol	31.05	119.0± 1.9
6	β-tocotrienol	35.71	2.20± 0.08
7	γ-tocotrienol	48.16	23.30± 0.25
8	δ-tocotrienol	57.79	ND

ND= not detected

Table 3: Contents of phenolic compounds in tea seed oil

No. peak	Component	Retention time	Content (mg/kg)
1	Gallocatechin	5.85	1.01 ± 0.07
2	Catechin +	10.09	6.32 ±0.21*
3	Epigallocatechin	16.90	12.93 ±0.97
4	Epigallocatechin gallate	18.28	1.724 ±0.07
5	Epicatechin	22.31	0.79 ±0.08
6	Gallocatechin gallate	22.93	1.76 ±0.06
7	Epicatechin gallate	26.02	0.28 ±0.03
8	Catechin gallate		

*On the basis of epigallocatechin

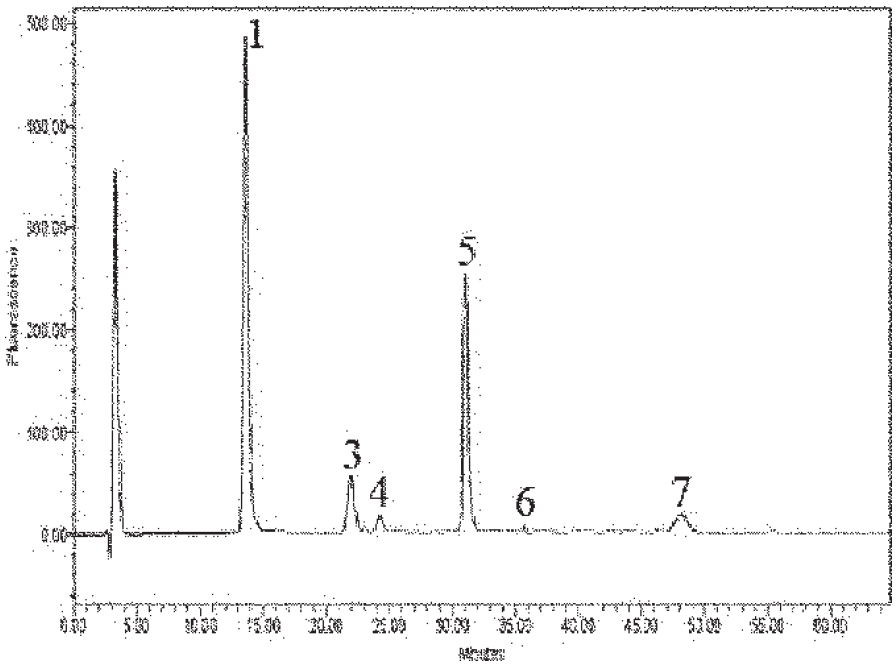

Figure 2: HPLC profile of tocopherol compounds in tea seed oil
1=α-Tocopherol; 3=γ-Tocopherol; 4=δ-Tocopherol; 5=α-Tocotrienol; 6=β-Tocotrienol; 7=γ-Tocotrienol

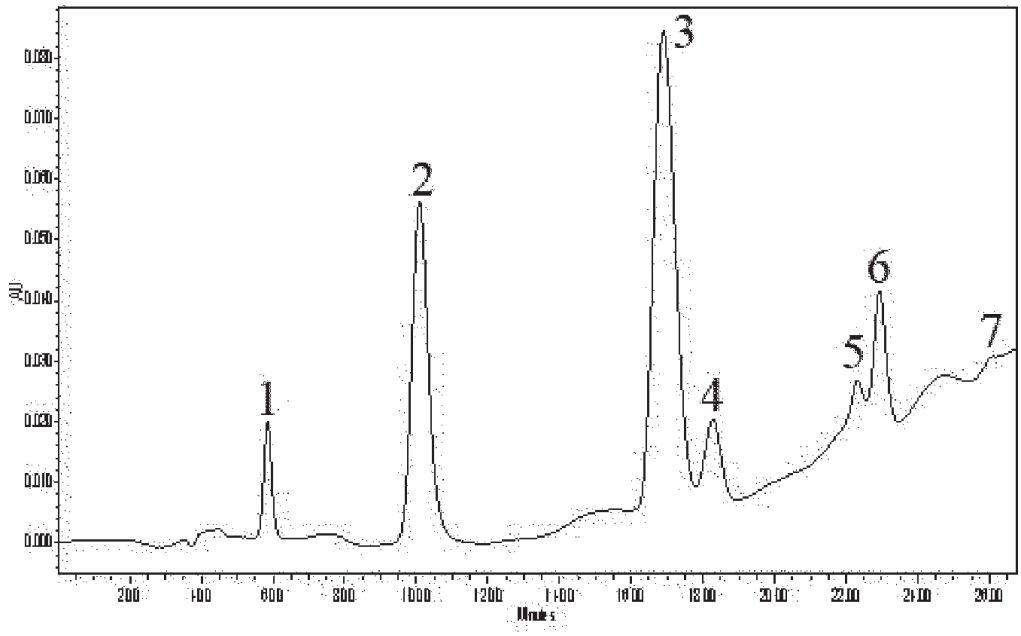

Figure 3: HPLC profile of phenolic compounds in tea seed oil.
1=Gallocatechin; 2=Catechin +Epigallocatechin; 3=Epigallocatechin Gallate; 4=Epicatechin; 5=Gallocatechin Gallate; 6=Epicatechin Gallate; 7=Catechin Galla.

Table 4: FA profiles of carp, common kilka fish and tea seed oils

FA	Common kilka	Tea seed oil**
C14:0	7.01 ± 0.19	—
C16:0	18.90 ± 0.43	16.50
C16:1	11.04 ± 0.27	—
C17:0	1.10 ± 0.07	—
C18:0	3.14 ± 0.11	3.33
C18:1	30.69 ± 0.66	65.97
C18:2	6.31 ± 0.18	22.17
C18:3	0.86 ± 0.07	0.30
C20:0	1.25 ± 0.08	0.53
C20:4	0.24 ± 0.05	—
C20:5 (EPA)	5.21 ± 0.15	—
C22:6 (DHA)	5.73 ± 0.16	—
ω-3*	11.80 ± 0.23	0.30
PUFA	18.35 ± 0.30	22.47
(EPA+DHA)/C16:0	0.579	—

*(C18:3 n-3 + C20:5 n-3 + C22:6 n-3)
**Sahari *et al.*, 2004

However, no significant difference was observed between the fish oil treated with 5 and 10% tea seed oil. As shown in Table 5, the PV and TBA values of treated samples are lower than that of the control; this means that tea seed oil at 5 and 10% can extend the shelf life of fish oil (common kilka). Similar results were reported by Sahari *et al.* (2004) in sunflower oil system.

PV is a chemical indication of how much of the oil is in the early stages of oxidation, and it reflects the degree of oxidation (Pokonery *et al.*, 2001; Madhavi *et al.*, 1996). The addition of antioxidants certainly reduces the rate of oxidation and this reduction rate in fish oil is evidence of the potential of tea seed oil as natural antioxidant. They are inhibited measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. Aldehydes (especially malonaldehyde) produce off-flavors in oxidize oils that can be quantified through their reaction with TBA (Rossel, 1994).

According to the results obtained in this study, tea seed oil exhibited strong antioxidant activity and the high stability of the tea seed oil was probably due to the low content of linolenic and linoleic acids and to the presence of polyphenols and vitamin E as antioxidants (Sahari *et al.*, 2004; Ravichandran, 1993). In conclusion, these results suggest that tea seed oil possess antioxidant properties and could be used as an alternative natural antioxidant. No single compound can be considered responsible for this stability.

Table 5: Comparison of the PV and TBA of common kilka oil and that mixed with 5 (T-5) and 10% (T-10) tea seed oil

Characteristics	PV*				TBA*			
	Storage time at 60 °C (day)							
Sample	0	4	8	12	0	5	9	13
Control**	2.7 ± 0.6	16.3 ± 1.2	53.6 ± 2.5	132 ± 6.8	0.055 ± 0.016	0.201 ± 0.012	0.661 ± 0.053	1.507 ± 0.107
T-5	2.7 ± 0.6	11.9 ± 1.1*	36.6 ± 1.1*	96.3 ± 2.3*	0.055 ± 0.016	0.177 ± 0.006*	0.463 ± 0.011*	1.159 ± 0.035*
T-10	2.7 ± 0.6	8.7 ± 0.9*	32.5 ± 1.7*	79.9 ± 2.1*	0.055 ± 0.016	0.161 ± 0.007*	0.390 ± 0.017*	0.969 ± 0.033*

Asterisk (≠) indicate significant different from control sample at ± = 0.05.
**Control= Sample without any antioxidant.

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