Utilization of Flaxseed Cake Powder as a Natural Antioxidant in Flaxseed Oil

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ABSTRACT

Flaxseed cake powder (FCP) was evaluated as a natural antioxidant. Freshly pressed flaxseed oil (either alone or with added antioxidants) was stored at room temperature (24°C) and oven temperature (60°C) for 30 days. Antioxidant activity of FCP was compared with that of green tea powder (GTP) as a natural antioxidant and tertiary-butylhydroquinone (TBHQ) as a synthetic one. Peroxide values (PVs), anisidine values (AVs), totox values and free fatty acids (FFA%) were determined at 5 day intervals as criteria to assess the antioxidant activity of FCP, GTP and TBHQ. Fatty acid composition of flaxseed oil was determined at zero time as well as after 30 days of storage with or without added antioxidants. The results obtained for peroxide value revealed that adding FCP (400 ppm) decreased the rate of oxidation as comparing to the control sample (without antioxidants) as the initial PV was 3.2 and reached 38.00 and 24.86 meq/kg in the control sample and in that containing 400 ppm FCP, respectively after 30 days of storage at 24°C. With increasing FCP to 600 ppm the rate of oxidation became much lower as indicated by PV while with adding 800 ppm FCP the rate of oxidation became the lowest compared with GTP (800 ppm) and TBHQ (200 ppm). At 60°C the data of peroxide value clearly confirmed the same trend obtained at 24 °C which concluded that FCP (800 ppm) was much effective than GTP (800 ppm) or TBHQ (200 ppm). Similar results were obtained for anisidine value (AV) and totox value at 24 and 60°C up to 30 days of storage. Regarding the fatty acid composition, it was clear that The effect of different antioxidants on maintaining the unsaturation of the oil came in the following order: FCP (800 ppm) > TBHQ (200 ppm) > GTP (800 ppm) which proof the above mentioned results obtained for PV and AV. It was apparent that FCP (800 ppm) exhibited a strong antioxidant activity during storage of flaxseed oil which was almost equal to or sometimes higher than that of the synthetic antioxidant (TBHQ) (200 ppm). As FCP is a natural, safe, cheap and effective material, therefore, it is suggested that it can safely be used as a natural antioxidant in vegetable oils.

INTRODUCTION

Flaxseed oil is the richest natural source of α-linolenic $C_{18:3}$ ω-3 essential fatty acid, which presents about 50 – 60% of the oil fraction. The other essential fatty acid present in flaxseed oil is linoleic acid ($C_{18:2}$ ω-6) (Richard and Thompson, 1997). Owing to its high content of polyunsaturated fatty acids (PUFAs), flaxseed oil becomes susceptible to oxidative reactions, decreasing its shelf life. It is, therefore, used only as a salad oil and not preferred as cooking or frying oil as it is rapidly thermally degraded (Malcolmson *et al.*, 2001). The use of polyethyleneterephtalate packaging has also increased in the oil industry. However this packaging does not give a total

protection against light, oxygen and heat as the metallic packaging does (Oliveira and Regitano, 2004).

Lipid oxidation lowers the nutritive value and deteriorates the flavour and taste of foods (Addis and Warner, 1991). It also causes aging, heart diseases, stroke, emphysema, mutagenesis and carcinogensis (Barlow, 1990). Overall, it has been implicated in the pathogenesis of at least 50 diseases (Langseth, 1993 and Halliwell, 1994). It is necessary to suppress lipid oxidation for maintaining the safety and effectiveness of foods. Generally, the most common synthetic antioxidants used in the food industry are butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), propyl gallate (PG) and Tertiary-butylhydroquinone (TBHQ), but their safety has been questioned (Barlow, 1990; Tang *et al.*, 2001). Hence, there is a need to identify new natural antioxidants for prevention of lipid oxidation in the food industry.

According to Pokorny (1991) when compared to synthetic antioxidants, natural antioxidants have the following advantages: they are readily acceptable by the consumer, they are considered to be safe, and they not only stabilize the edible oils but also add to the nutraceutical value of these oils. Recently, natural antioxidants have become a part of the diet in human nutrition with the aim of decreasing the risk of diseases (McCune and Johns, 2002; Higdon and Frei, 2003). Moreover, natural antioxidants are reported to be more powerful than the synthetic ones, especially, rosemary, sage, and green tea extracts (Tang *et al.*, 2001; Yanishlieva and Marinova, 2001).

Green tea leaf extracts are becoming increasingly important as a functional food because of their high polyphenol contents (Manzocco *et al.*, 1998). Its polyphenol contents can increase up to 36% (dry basis) due to climate, season or variety (Wanasundara and Shahidi, 1998). The major polyphenolic compounds causing the antioxidative properties of green tea are catechins. The four most abundant naturally occurring tea catechins are: (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG). They are free radical scavengers, metal chelators, inhibitors of transcription factors, and enzymes (Zandi and Gondon, 1999; Higdon and Frei, 2003). Therefore green tea extracts have been used as natural antioxidant, antibacterial, antiviral, anticarcinogenic and antimutagenic agents (Manzocco *et al.*, 1998; Yang *et al.*, 2000; Tang *et al.*, 2001; Higdon and Frei, 2003). Green tea extract is the most effective and extensively used natural antioxidant. It has been used in canola oil (Chen *et al.*, 1996), marine oil (Wanasundara and Shahidi, 1998), rapeseed oil (Zandi and Gondon, 1999), sunflower oil (Ziada, 2002) and corn oil (Yanishlieva and Marinova, 2001). Wanasundara and Shahidi (1998) reported that crude tea catechins have been found to be more effective to reduce the lipid oxidation than α-tocopherol or BHA.

Lignans are phytoestrogens comprising a group of plant-derived diphenolic compounds structurally similar to the estrogens produced in humans (IUPAC, 1999). Bakke and Klosterman (1956) isolated SDG 1 from a fat free extract of linseed meal with a 3% yield. SDG 1 ($C_{32}H_{46}O_{16}$, MW= 686.3) was found to be very soluble in water and alcohol. They also isolated SECO 2 (2,3-di-{methoxy-4-hydroxybenzyl} butane-1,4-diol) by acid hydrolysis of SDG 1. SECO 2 ($C_{20}H_{26}O_6$, MW= 362.3) is the major lignan present in flaxseed, (Ford *et al.*, 1999). It is the non-sugar portion of SDG 1. Both SDG 1 and SECO 2 have a UV absorption maximum at 280 nm, which is characteristic for lignans. Flaxseed also contains small amounts of the lignans matairesinol, pinoresinol, pinoresinol diglucoside, and isolariciresinol (Whiting, 1987; Mazur *et al.*, 1996; Meagher *et al.*, 1999; Ford *et al.*, 2001). Among foods, flaxseed is the richest source of lignans (7 mg/g SDG 1 or 3.7 mg /g SECO 2). It contains 75-800 times more SDG 1 than any other food depending on the variety, location and year (Mazur *et al.*, 1996; Westcott and Muir, 1996). Whole and ground flaxseed typically contain between 0.7 and 1.9% SDG 1, which is approximately 77–209 mg SDG 1/tbsp of whole seed or 56–152 mg SDG 1/tbsp of ground flaxseed (Morris, 2004).

Lignans have been shown to exhibit significant antioxidant (Kitts *et al.*, 1999), antibacterial, antifungal and antiviral activity (Serraino and Thompson, 1991). In Chinese traditional medicine lignans are used for treatment of viral hepatitis and protection of the liver (USDA, 1999). The flaxseed lignans scavenge certain free radicals like the hydroxyl ion (•OH) (Parasad, 1997). The antioxidant action of flaxseed lignans was reported to be greater than that of vitamin E (Bhathena and Velasquez, 2002).

TBHQ is a solid antioxidant that is readily soluble at use levels in fats and oils and in a number of food-grade solvents, but is practically insoluble in water. It has a melting point range of 126.5 – 128.5°C. TBHQ is especially effective for highly unsaturated vegetable oils and many animal fats and carries advantage over other previously approved antioxidants in its ability to extend the storage stability of vegetable oils. TBHQ is intended to be used at a level of up to 200 mg/kg fat or oil (WHO, 1999; Williams *et al.*, 1999).

The objective of this work was to study the effect of flaxseed cake powder as an antioxidant in flaxseed oil.

MATERIALS AND METHODS

Materials:

Fresh cold pressed flaxseed oil (Eliaza variety, 10 kg) and flaxseed cake (2 kg) were obtained from a private commercial flaxseed press mill, El-Mahalla El-Kobra, Gharbia Governorate, Egypt. Green tea (*Camellia*

sinensis) was obtained from a local grocery. TBHQ was obtained from Extracted oils and Derivatives Company, Alexandria, Egypt.

Green tea (rinsed with distilled water) and flaxseed cake were dried overnight at 40 ± 2°C in an air-draft drying oven (WT-binder labortechnic GMBH). The samples were cleaned, ground, sieved through 60-mesh and kept at -18°C until using. Flaxseed oil samples (200 g each) were placed in 500 ml obscure glass bottles.

Methods:

Flaxseed cake was characterized according to AOAC (1990) in terms of moisture content. crude oil content, crude protein content, crude fiber content, and ash content. Carbohydrate content was calculated by difference.

Natural antioxidants (Flaxseed cake powder FCP and green tea powder GTP) were individually added to the flaxseed oil samples in concentrations of 400, 600 and 800 ppm. TBHQ was added to the flaxseed oil samples in concentration of 200 ppm. A total of eight samples including the control sample (without any additives) were prepared. Each sample was divided into two parts; one was stored at room temperature (24 ± 2°C) while the other at oven temperature (60 ± 2°C) up to 30 days. Samples were taken regularly every five days for analysis.

Physicochemical characteristics of the oil were investigated in terms of peroxide value (PV) Method (Cd 8-53), free fatty acid (FFA%) method (Cd 3a-94) according to AOCS (1989). Anisidine value (AV) was determined by colorimetric method as described by Egan *et al.*, (1981). The total oxidation value (Totox value, TV) was calculated according to Hamilton and Rossel (1986) using the following equation: Totox value = 2PV + AV.

Fatty acid compositions of the fresh and stored oils were determined according to the procedure of Radwan (1978) using a gas chromatography (Shimadzu GC-4CM-PFE) equipped with stainless steel column packed with 3% di-ethylene glycol succinate on chromosorb W 80/100 and flame ionization detector (FID). The oven and detector temperatures were 180°C isothermal and 270°C, respectively. N_2 was used as a carrier gas at a flow rate of 20 ml/min.

RESULTS AND DISCUSSION

Characterization of flaxseed cake:

The proximate chemical composition of flaxseed cake (Table 1) was 7.10, 6.00, 39.00, 4.80, 7.00 and 36.10% for moisture, crude oil, crude protein, crude fiber, ash and carbohydrate contents, respectively.

Table (1). Proximate chemical composition (%) of flaxseed cake:

Moisture	Crude oil	Crude protein	Crude fiber	Ash	Carbohydrate
7.10	6.00	39.00	4.80	7.00	36.10

These results are in accordance with those of Batterham *et al.*, (1991), Oomah and Mazza (1993) and Jenkins (1995).

Oxidative stability of flaxseed oil:

From the fatty acid composition of flaxseed oil (Table 11) it was observed that the oil contained high amount of unsaturated fatty acids (more than 88%). These are more liable to epoxide formation by reaction with oxygen in air. This was experimentally proved by the sharp increase in peroxide values (Table 2 and 3) especially at oven temperature which indicated that flaxseed oil has an extremely high tendency to be oxidized. The initial PV of flaxseed oil was 3.2 meq./kg and reached to 38.00 and 112.80 meq./kg after 30 days of storage at 24 and 60°C, respectively.

Effect of adding flaxseed cake powder (FCP) on the oxidative stability of flaxseed oil as monitored by different oxidation criteria:

Oxidative stability of flaxseed oil with either synthetic (TBHQ) or natural (GTP and FCP) antioxidants was studied at different temperatures.

A. Peroxide value (PV):

Since hydroperoxides are the primary products of lipid oxidation, measurement of peroxide value is an indicator of initial oxidation (Sherwin, 1968). Peroxide values of flaxseed oil containing antioxidants (TBHQ, GTP or FCP) (Table 2 and 3), were lower than that of the control oil.

Table (2). Peroxide value (meq/kg) of flaxseed oil with added powder of FCP, GTP and TBHQ stored at room temperature (24°C) for 30 days.

Storage time (days)	Control	TBHQ	Green tea powder (GTP)			Flaxseed cake powder (FCP)		
		200 ppm	400 ppm	600 ppm	800 ppm	400 ppm	600 ppm	800 ppm
0	3.20	3.20	3.20	3.20	3.20	3.20	3.20	3.20
5	6.00	3.24	3.76	3.26	3.24	3.90	3.28	3.22
10	12.00	5.42	7.50	5.88	3.84	7.80	6.10	3.64
15	16.00	7.30	10.00	7.80	5.16	10.50	8.20	4.88
20	21.00	9.50	13.20	10.20	7.44	13.70	11.00	6.40
25	30.00	13.52	19.00	14.40	9.15	20.00	15.50	9.00
30	38.00	17.20	23.70	18.86	12.60	24.86	19.60	11.60

After 5 days of storage at room temperature (24°C), the PVs were 3.24, 3.76 and 3.90 meq./kg for flaxseed oil containing TBHQ (200 ppm), GTP (400 ppm) and FCP (400 ppm), respectively. These values reached 17.20, 23.70 and 24.86 meq./kg after 30 days of storage. By increasing the natural antioxidants level, the PVs tended to decrease gradually and reached 18.86 and 19.60 meq./kg when adding 600 ppm GTP and FCP, respectively. Increasing the level of GTP and FCP to 800 ppm decreased the PVs to 11.60 meq./kg.

At oven temperature (60°C), there was a higher increase in PVs than that at room temperature during storage up to 30 days. After 5 days, the PVs reached 10.10, 7.00 and 6.80 meq./kg when adding TBHQ, GTP (800 ppm) and FCP (800 ppm), respectively.

Table (3). Peroxide value (meq/kg) of flaxseed oil with added powder of of FCP, GTP and TBHQ stored at oven temperature (60°C) for 30 days.

Storage time (days)	Control	TBHQ	Green tea powder (GTP)			Flaxseed cake powder (FCP)		
		200 ppm	400 ppm	600 ppm	800 ppm	400 ppm	600 ppm	800 ppm
0	3.20	3.20	3.20	3.20	3.20	3.20	3.20	3.20
5	20.25	10.10	12.90	10.50	7.00	14.30	11.10	6.80
10	33.26	17.00	22.00	17.60	11.50	23.40	19.00	11.40
15	43.38	22.10	29.20	22.80	16.20	30.37	23.90	15.20
20	65.08	32.60	43.68	34.80	23.00	45.60	35.90	22.90
25	85.32	43.00	57.20	46.10	32.64	59.80	46.20	32.00
30	112.80	56.00	75.70	60.40	42.15	78.96	62.00	41.60

This increment in PVs continued with increasing the storage time but with higher increment rates than that at room temperature. The PVs reached 56.00, 40.15 and 41.60 meq./kg, respectively after 30 days of storage at 60°C. It was clear that adding 800 ppm FCP was more effective in reducing the peroxide values than TBHQ (200 ppm) at different temperatures up to 30 days of storage.

B. Anisidine value (AV):

The peroxides in an oxidized oil are transitory intermediates, which decompose readily during storage and heating into carbonyls and other compounds. This decomposition accelerates as the temperature is raised, and the PV of an oil may therefore be reduced or eliminated. The peroxide decomposition products in the oil may catalyze further oxidation, or alternatively, decompose and react further giving rise to new off-flavor compounds. The ρ-anisidine values have been measured to determine the total amounts of carbonyl compounds and aldehydes (especially 2-alkenals) in oxidized oils.

Since the peroxide value is non-linear in nature, meaning that it does not increase in a linear fashion over time, the anisidine value is a good indication of the freshness of an oil. The analysis is based on the reaction of ρ-anisidine and unsaturated aldehydes forming a yellowish pigment measured at 350 nm. For fresh refined oils, the AV should be less than 10 (Rossell, 1986). McMullen (1988) reported a good correlation between AV and sensory attributes of oils subjected to shallow-pan frying and deep fat frying.

Tables 4 and 5 show the AV of the control flaxseed oil and that with added antioxidants during storage for 30 days at different temperatures.

Table (4). Anisidine value (AV) of flaxseed oil with added powder of of FCP, GTP and TBHQ stored at room temperature (24°C) for 30 days.

Storage time (days)	Control	TBHQ	Green tea powder (GTP)			Flaxseed cake powder (FCP)		
		200 ppm	400 ppm	600 ppm	800 ppm	400 ppm	600 ppm	800 ppm
0	1.60	1.60	1.60	1.60	1.60	1.60	1.60	1.60
5	2.60	1.61	1.92	1.62	1.61	1.94	1.61	1.61
10	5.40	2.56	3.94	3.20	3.02	3.95	3.21	2.58
15	9.00	4.10	6.70	4.98	4.52	6.70	5.18	4.15
20	17.60	9.92	14.40	11.36	10.60	14.30	11.62	9.92
25	23.86	13.11	19.10	14.92	14.02	19.05	15.30	13.14
30	33.16	17.20	26.00	20.00	19.00	26.00	20.80	17.30

Data indicated that the initial AV of flaxseed oil was 1.60. This value reached 33.16 (20.7 folds) and 64.42 (40.3 folds) after 30 days of storage at room and oven temperatures, respectively.

Table (5). Anisidine value (AV) of flaxseed oil with added powder of of FCP, GTP and TBHQ stored at oven temperature (60°C) for 30 days.

Storage time (days)	Control	TBHQ	Green tea powder (GTP)			Flaxseed cake powder (FCP)		
		200 ppm	400 ppm	600 ppm	800 ppm	400 ppm	600 ppm	800 ppm
0	1.60	1.60	1.60	1.60	1.60	1.60	1.60	1.60
5	8.80	5.70	5.88	5.78	3.68	5.91	5.81	5.70
10	14.30	8.10	8.40	8.20	7.99	8.60	8.20	8.03
15	21.50	11.60	12.15	11.80	11.40	12.31	11.86	11.48
20	34.60	19.20	19.99	19.40	18.88	20.20	19.50	19.05
25	48.80	25.70	26.90	25.95	25.14	27.20	26.22	25.60
30	64.42	32.98	34.52	33.30	32.20	35.00	33.50	32.44

At room temperature, AV reached 17.20, 26.00, 20.00, 19.00, 26.00, 20.80 and 17.30 when adding TBHQ, GTP (400 ppm), GTP (600 ppm), GTP (800 ppm), FCP (400 ppm), FCP (600 ppm) and FCP (800 ppm), respectively. The corresponding values were 32.98, 34.52, 33.30, 32.20, 35.00, 33.50 and 32.44, respectively when storing at oven temperature (60 ± 2°C) for 30 days (Table 5).

C. Totox value:

Total oxidation value, the so-called Totox value, calculated from twice the peroxide value plus the *p*-anisidine value, is a useful indicator of measuring the onset of progressive deterioration in oil and provides information regarding progression of the formation of primary and secondary oxidation products. It is used as a measure of the precursor non-volatile carbonyls present in a processed oil, plus any further oxidation compounds developed during storage (Hamilton and Rossell, 1986).

Tables 6 and 7 show the totox values of the control flaxseed oil as well as that with added antioxidants during storage for 30 days at different temperatures. Data clarified that the initial totox value of flaxseed oil was 8.00. This value reached 109.16 (13.65 folds) and 290.02 (36.25 folds) after 30 days of storage at room and oven temperatures, respectively. At room temperature, the totox values were 51.60, 73.40, 57.72, 42.20, 75.72, 60.00 and 40.50 when adding TBHQ, GTP (400 ppm), GTP (600 ppm),

GTP (800 ppm), FCP (400 ppm), FCP (600 ppm) and FCP (800 ppm), respectively.

Table (6). Totox value of flaxseed oil with added powder of of FCP, GTP and TBHQ stored at room temperature (24°C) for 30 days.

Storage time (days)	Control	TBHQ	Green tea powder (GTP)			Flaxseed cake powder (FCP)		
		200 ppm	400 ppm	600 ppm	800 ppm	400 ppm	600 ppm	800 ppm
0	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
5	14.60	8.09	9.44	8.14	8.09	9.74	8.17	8.05
10	29.40	13.40	18.94	14.96	10.70	19.55	15.41	9.86
15	41.00	18.70	26.70	20.58	14.84	27.70	21.58	13.91
20	59.60	28.92	40.80	31.76	25.48	41.70	33.62	22.72
25	83.86	40.15	57.10	43.72	32.32	59.05	46.30	31.14
30	109.16	51.60	73.40	57.72	44.20	75.72	60.00	40.50

The corresponding values were higher when storing at oven temperature (60 ± 2°C) as they reached 144.98, 185.92, 154.10, 112.50, 192.92, 157.50 and 115.64, respectively for 30 days (Table 7).

Table (7). Totox value of flaxseed oil with added powder of of FCP, GTP and TBHQ stored at oven temperature (60°C) for 30 days.

Storage time (days)	Control	TBHQ	Green tea powder (GTP)			Flaxseed cake powder (FCP)		
		200 ppm	400 ppm	600 ppm	800 ppm	400 ppm	600 ppm	800 ppm
0	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
5	49.30	25.90	31.68	26.78	17.68	34.51	28.01	19.30
10	80.82	42.10	52.40	43.40	30.99	55.40	46.20	30.83
15	108.26	55.80	70.55	57.40	43.80	73.05	59.66	41.88
20	164.76	84.40	107.35	89.00	64.88	111.40	91.30	64.85
25	219.44	111.70	141.30	118.15	90.42	146.80	118.62	89.60
30	290.02	144.98	185.92	154.10	116.50	192.92	157.50	115.64

It was clear from these results that increasing the antioxidant level up to 800 ppm had reduced the total oxidation of flaxseed oil at either room or oven temperature.

The effect of different used antioxidants in reducing total oxidation of flaxseed oil was in the following order: GTP (800 ppm) > FCP (800 ppm) > TBHQ (200 ppm) > GTP (600 ppm) > FCP (600 ppm) > GTP (400 ppm) > FCP (400 ppm). The effect of TBHQ was higher than that of GTP and FCP at 400 or 600 ppm but it was lower than that of the two natural antioxidants when used at 800 ppm.

D. Free fatty acids content (FFA%):

Free fatty acids contents (FFA%) of the control flaxseed oil as well as that containing antioxidants and stored at different temperatures for 30 days are shown in tables 8 and 9. The initial FFA% of flaxseed oil was 0.80%. Data indicated a graduate increase in free fatty acids with increasing the storage time. The FFA% reached 1.85 and 3.30% after 30 days of storage at room and oven temperatures, respectively. Application of synthetic and natural antioxidants reduced the development of free fatty acids. Data showed that increasing the levels of GTP and FCP caused a consequent reduction in FFA% at different temperatures up to 30 days of storage. FFA% reached 1.06, 1.22, 1.12, 1.08, 1.22, 1.14 and 1.08% when adding TBHQ, GTP (400 ppm), GTP (600 ppm), GTP (800 ppm), FCP (400 ppm), FCP (600 ppm) and FCP (800 ppm), respectively.

Table (8). Free fatty acids (FFA%) of flaxseed oil with added powder of of FCP, GTP and TBHQ stored at room temperature (24°C) for 30 days.

Storage time (days)	Control	TBHQ	Green tea powder (GTP)			Flaxseed cake powder (FCP)		
		200 ppm	400 ppm	600 ppm	800 ppm	400 ppm	600 ppm	800 ppm
0	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
5	0.85	0.80	0.82	0.82	0.80	0.85	0.82	0.80
10	0.90	0.83	0.86	0.85	0.82	0.88	0.86	0.83
15	1.00	0.85	0.91	0.90	0.84	0.92	0.91	0.86
20	1.15	0.90	1.00	0.96	0.90	1.00	0.98	0.91
25	1.40	1.01	1.10	1.06	1.01	1.10	1.06	1.02
30	1.85	1.06	1.22	1.12	1.08	1.22	1.14	1.08

When storing at oven temperature (60°C) for 30 days (Table 9), the corresponding FFA values were 1.92, 2.20, 2.14, 1.96, 2.18, 2.05 and

1.90%, respectively. Results clarified that the antioxidant effect of FCP was almost equal to that of TBHQ at room temperature and slightly higher at oven temperature.

Table (9). Free fatty acids (FFA%) of flaxseed oil with added powder of of FCP, GTP and TBHQ stored at oven temperature (60°C) for 30 days.

Storage time (days)	Control	TBHQ	Green tea powder (GTP)			Flaxseed cake powder (FCP)		
		200 ppm	400 ppm	600 ppm	800 ppm	400 ppm	600 ppm	800 ppm
0	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
5	1.05	0.86	1.10	0.90	0.86	0.90	0.88	0.85
10	1.35	0.98	1.30	1.12	0.99	1.15	1.10	0.96
15	1.70	1.12	1.47	1.34	1.15	1.38	1.30	1.10
20	2.10	1.38	1.68	1.60	1.40	1.58	1.54	1.32
25	2.60	1.65	1.92	1.86	1.70	1.82	1.78	1.60
30	3.30	1.92	2.20	2.14	1.96	2.18	2.05	1.90

Effect of adding different antioxidants on the reduction of flaxseed oil oxidation:

Table (10) shows the reduction percentages in PVs, FFAs%, AVs and totox values of flaxseed oil with added antioxidants during storage at different temperature for 30 days.

Table (10). Effect of adding different antioxidants* to flaxseed oil on the reduction (%) in oxidation rate after 30 days of storage at different temperatures.

		TBHQ	GTP			FCP		
		200 ppm	400 ppm	600 ppm	800 ppm	400 ppm	600 ppm	800 ppm
Peroxide value	(24°C)	54.74	37.63	50.37	66.84	34.58	48.42	69.47
	(60°C)	50.35	32.89	46.45	62.63	30.00	45.04	63.12
F. fatty acids	(24°C)	42.70	34.05	39.46	41.62	34.05	38.38	41.62
	(60°C)	41.82	33.33	35.15	40.61	33.94	37.88	42.42
Anisidine value	(24°C)	48.13	21.59	39.69	42.70	21.59	37.27	47.83
	(60°C)	48.80	46.41	48.31	50.02	45.67	48.00	49.64
Totox value	(24°C)	52.73	32.76	47.12	59.51	30.63	45.03	62.90
	(60°C)	50.01	35.89	46.87	59.83	33.48	45.69	60.13

Data revealed that adding 200 ppm TBHQ at 24°C caused a reduction of 54.74, 42.70, 48.13 and 52.37% in PVs, FFAs, AVs and totox values, respectively. Although TBHQ was reported to exhibit higher thermal stability than other natural (ajowan: *Carum copticum*) and synthetic (BHA and EQ) antioxidants (Bera *et al.*, 2006), these percentages were reduced to 50.35, 41.82, 48.80 and 50.01%, respectively when storing at 60°C.

The effect of GTP and FCP was almost the same on reducing the previous oxidation criteria. The effect of TBHQ was higher than that of either GTP or FCP at 400 or 600 ppm but lower when increasing their levels to 800 ppm. At 800 ppm, GTP and FCP caused a reduction of 66.84 and 69.47% in PVs, 41.62% in FFAs, 42.70 and 47.83% in AVs and 59.51 and 62.90% in totox values, respectively. A same trend was observed at 60°C. FCP (800 ppm) caused the highest reduction in the total oxidation of flaxseed oil at oven temperature. It may be clarified on the fact that lignan is more stable at elevated temperatures. It was reported that the flaxseed lignan is stable to baking (Muir and Westcott, 2000). In a study of SDG stability, there were no differences in the SDG content of the crust and crumb of baked bread, despite there being a difference in exposure to heat between the crust and the internal loaf (Muir and Westcott, 1996).

Effect of adding flaxseed cake powder (FCP) on fatty acid composition of flaxseed oil stored at different temperatures up to 30 days:

Table (11) shows the mean values for fatty acid composition (%) of flaxseed oil. Data revealed that the oil consisted of 0.12% ($C_{14:0}$), 6.20% ($C_{16:0}$), 0.98% ($C_{16:1}$) 5.00% ($C_{18:0}$), 18.90% ($C_{18:1}$), 14.80% ($C_{18:2}$), and 54.00% ($C_{18:3}$). Storage of the oil for 30 days at 24°C and 60°C reduced the total unsaturated fatty acids from 88.68 to 78.40% and 61.48%, respectively. Adding different antioxidants had increased the unsat./sat. percent at different temperatures. At room temperature, this percent was 4.98, 4.95 and 5.28 when adding TBHQ (200 ppm), GTP (800 ppm) and FCP (800 ppm), respectively. When storing at 60°C, the corresponding values were 2.79, 2.71 and 2.90, respectively. At either room or oven temperature, the effect of different antioxidants on maintaining the unsaturation of the oil came in the following order: FCP (800 ppm) > TBHQ (200 ppm) > GTP (800 ppm). FCP (800 ppm) caused the least reduction in total unsaturation (5.20 and 16.15%) compared to that of GTP (800 ppm) (6.20 and 17.60%) and TBHQ (200 ppm) (6.10 and 17.00%) at 24 and 60°C, respectively. Out of these results, it was apparent that FCP (800 ppm) had the highest antioxidant effect among the studied antioxidants. It

may be explained on the base that lignans and other phenolic antioxidants of flaxseed are present in the seed hull and stay in the cake after oil extraction. This explain why whole or ground flaxseeds can be stored at room temperature for at least 1 – 2 years indicating the presence of a strong protective system against oxidation (Malcolmson *et al.*, 2000; Przybylski and Daun, 2001). It is most likely because it is rich in lignans which are powerful antioxidants. This study provided useful evidence that flaxseed cake can be used as natural antioxidants.

CONCLUSION

In view of some likely bad effects of synthetic antioxidants, some efforts are being made to add natural substance or their extracts to control or lessen the oxidative deterioration. The results of our study clearly indicate that there is a possibility of achieving useful effect of natural antioxidants. It was apparent that FCP exhibited a strong antioxidant activity in flaxseed oil during storage which was almost equal to or sometimes higher than that of the synthetic antioxidant (TBHQ). However, the level of FCP needed was 4 times higher than that of TBHQ. Therefore, it is suggested that FCP can safely be used as a natural cheap source of antioxidants instead of the synthetic antioxidants to suppress lipid oxidation and prolong the shelf life of the highly susceptible flaxseed oil.

Table (11). Effect of adding different antioxidants' on fatty acid composition of flaxseed oil stored at different temperatures up to 30 days.

		After 30 days of storage							
		Room temperature (24 ±2°C)				Oven temperature (60 ±2°C)			
Fatty acids	Control (zero time)	Without antioxidants	TBHQ (200 ppm)	GTP (800 ppm)	FCP (800 ppm)	Without antioxidants	TBHQ (200 ppm)	GTP (800 ppm)	FCP (800 ppm)
$C_{14:0}$	0.12	0.60	0.33	0.40	0.22	3.10	2.80	2.88	2.90
$C_{16:0}$	6.20	8.40	7.00	7.68	7.00	13.40	11.20	11.80	11.50
$C_{16:1}$	0.98	1.20	1.10	1.12	1.10	1.80	1.52	1.50	1.60
$C_{18:0}$	5.00	12.60	9.40	9.40	8.20	22.02	18.00	18.60	17.30
$C_{18:1}$	18.90	22.18	19.97	20.20	19.28	23.88	21.68	21.24	20.70
$C_{18:2}$	14.80	12.80	13.40	13.20	14.00	10.40	12.00	11.98	14.20
$C_{18:3}$	54.00	42.22	48.80	48.00	50.20	25.40	32.80	32.00	31.80
Total Sat.	11.32	21.60	16.73	17.48	15.42	38.52	32.00	33.28	31.70
Total Unsat.	88.68	78.40	83.27	82.52	84.58	61.48	68.00	66.72	68.30
UnSat. / Sat.	7.83	3.63	4.98	4.72	5.49	1.60	2.60	2.00	2.15
$C_{18:3}$ / $C_{18:2}$	3.65	3.30	3.64	3.64	3.59	2.44	2.73	2.67	2.24
Reduction (%) in total unsat.	0.00	11.59	6.10	6.20	5.20	30.67	17.00	17.60	16.15

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الملخص العربي

استخدام مسحوق كسب الكتان كمضاد أكسدة طبيعي في زيت بذرة الكتان

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تم إجراء هذه الدراسة بهدف تقييم مسحوق كسب الكتان كمضاد أكسدة طبيعي لتحسين الثبات التأكسدي لزيت بذرة الكتان. تم تخزين زيت بذرة الكتان المعصور حديثاً (سواء الزيت بمفرده أو المضاف إليه مضادات الأكسدة) على درجة حرارة الغرفة (٢٤°م) وكذلك على درجـة حرارة

الفـــرن (٦٠°م) لمدة ٣٠ يوم. تم مقارنة النشاط المضاد للأكسدة لمسحوق كسب الكتان بنظيره لمسحوق الشاي الأخضر (كمضاد أكسدة طبيعي) ومادة الـ TBHQ (كمضاد أكسدة صناعي). تم تقدير قيم البيروكسيد، قيم الأنسيدين، قيم التوتكس ومحتوى الأحماض الدهنية الحرة كل ٥ أيام كمعايير لتقييم النشاط المضاد للأكسدة للمواد المستخدمة. تم تقدير تركيب الأحماض الدهنية في الزيت قبل التخزين وبعد التخزين لمدة ٣٠ يوم سواء مع أو بدون إضافة مضادات الأكسدة. أوضحت النتائج المتحصل عليها من قياس رقم البيروكسيد أن إضافة مسحـوق كسب الكتان (٤٠٠ جزء في المليون) قلل معدل أكسدة الزيت بالمقارنة بالزيت الغير مضاف إليه مضادات الأكسدة (الكنترول) حيث كانت قيمة البيروكسيد الأولية للزيت ٣,٢ وصلت إلى ٣٨,٠٠ و ٢٤,٨٦ ملليمكافئ/كجم في الزيت الكنترول والمضاف له ٤٠٠ جزء في المليون من مسحوق الكسب على التوالي بعد التخزين لمدة ٣٠ يوم على درجة حرارة الغرفـــة (٢٤°م). بزيادة تركيز مسحوق كسب الكتان المضاف إلى ٦٠٠ جزء في المليون زاد انخفاض معدل الأكسدة، كما أنه بوصول التركيز إلى ٨٠٠ جزء في المليون كان الانخفاض في معدل الأكسدة أعلى ما يمكن مقارنةً باستخدام مسحوق الشاي الأخضر (٨٠٠ جزء في المليون) أو الـ TBHQ (٢٠٠ جزء في المليون). عند التخزين على درجة حرارة ٦٠°م أكدت نتائج تقدير قيم البيروكسيد نفس الاتجاه الملاحظ عند التخزين على ٢٤°م مما يؤكد أن استخدام مسحوق كسب الكتان (٨٠٠ جزء في المليون) كان أكثر فعالية في خفض معدل الأكسدة عن مسحـوق الشـاي الأخضـر (٨٠٠ جزء في المليون) والـ TBHQ (٢٠٠ جزء في المليون). أظهر قياس قيم الأنسيدين وحساب قيم التوتكس نفس الاتجاه الملاحظ مع قيم البيروكسيد عند التخزين على درجة حرارة ٢٤°م أو ٦٠°م لمدة ٣٠ يوم. فيما يخص تركيب الأحماض الدهنية جاء تأثير مختلف مضادات الأكسدة المستخدمة على الحفاظ على نسبة عدم التشبع في الزيت في الترتيب التالي: مسحوق كسب الكتان (٨٠٠ جزء في المليون) >الـ TBHQ (٢٠٠ جزء في المليون) > مسحوق الشاي الأخضر (٨٠٠ جزء في المليون) وهو ما يؤكد النتائج المشار إليها عالياً بخصوص قيم البيروكسيد والأنسيدين. اتضح من الدراسة أن مسحوق كسب الكتان أظهر تأثيراً قوياً كمادة مضادة للأكسدة والذي يساوي تقريباً بل يزيد أحياناً عن تأثير مضاد الأكسدة الصناعي (TBHQ). حيث أن مسحوق كسب الكتان مادة طبيعية آمنة منخفضة التكلفة وفعالة ضد الأكسدة كما أظهرته نتائج الدراسة الحالية، لذلك نوصي باستخدامه كمضاد أكسدة في الزيوت النباتية.