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THE USE OF EXTRACTED PHENOLIC COMPOUNDS FROM GREEN AND BLACK TEA AS NATURAL ANTIOXIDANTS

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ABSTRACT

This study was carried out to separate and identify green and black tea phenolic compounds. These compounds were added to sunflower oil as natural antioxidants at levels 150, 200 and 250 ppm and stored in amber glass bottles at 40°C for nine weeks. The antioxidative effectiveness of these compounds was compared with pure catechin, butylated hydroxyanisole (BHA) and butylatedhydroxy toluene (BHT). The chemical properties of green and popular black tea collected from Egyptian local markets were also studied.

Green tea leaves contained higher percent of moisture, ether extract and protein than those of black tea leaves. In green tea 37.15% of the dry matter is soluble in hot water, while 37.65 - 44.50% of the dry matter is soluble in hot water depending on the origin of black tea leaves. Caffeine constitutes 2.45 - 2.78% of the dry matter of tea leaves. higher percent of tannin was found in green tea leaves (19.65%), while black tea leaves contained lower levels (13.29 - 14.22%). The data showed that, green tea contained higher amounts of polyphenolic compounds (32.75%) than those of black tea (2.7.20 - 31.12%). High performance liquid chromatography (HPLC) analysis indicated that both types of tea extracts contained nine phenolic compounds. Caffeic acid was the main phenolic acid in green tea extracts (36.97%), followed by p-hydroxybenzoic acid (18.18%), catechin (14.62%), ferullic acid (8.77%), gallic acid (8.20%) and chlorogenic acid (5.24%). While black tea contained higher amounts of tannic acid (35.26%) followed by caffeic acid (30.20%), catechin (10.33%) gallic acid (5.78%) and p-hybroxybenzoic acid (4.55%). Green tea extracts at 150 ppm possessed appreciable antioxidants activity greater than black tea extracts, pure catechin and BHA. Increasing the level added from 150 to 200 and 250 ppm lead to decreasing peroxide values (pv) and thiobarbituric acid (TBA) formed and retarding oxidative rancidity in sunflower oils. Green and black tea represent an excellent source of nature antioxidant. The antioxidative capacity of green tea is much higher than that of black tea. So, it could be recommended to use green tea rather than black tea because of the high contents of natural antioxidants as well as it low content of tannins.

Key words: Green tea, black tea, phenolic compounds, antioxidants.

INTRODUCTION

Tea is a widely consumed beverage throughout the world. The average consumption is 6 g per day for a 60 kg human (Chen, 1985 and Deng *et al*. 1997). Tea, a low cost beverage, which traditionally consumed in the far and Middle East without reported ill effects, was tested as a possible source of natural antioxidants. Tea has been used as a daily beverage and crude medicine in China for thousands of years. Polyphenols are the most abundant group of compounds in fresh tea leaves and are found in green tea beverage at 30-42% of the total dry matter (Graham, 1992). The pharmacological effects of tea are reviewed, including antioxidative activity (Matsuzaki and Hara, 1985), antimutagenic (Yen and Chen, 1994) and anti-cancer effects (Katiyar *et al*., 1992).

Tea beverages are very important, especially between diets because they can protect the human body from free radicals, which may cause various diseases. Aqueous tea extracts exhibit antiviral and antibacterial properties (Konowalchuk and Spierr, 1978). Green tea extracts are more effective than black tea extracts in the latter activity.

The characteristic taste of tea in known to be due to the unique combination of the various polyphenolic compounds, catechins, caffeine and soluble amino acids (Sanderson, *et al.* 1976). Polyphenols are secondary plant metabolites occurring widely in plant food (Harbone, 1989). They possess outstanding antioxidant and free radical scavenging properties, suggesting a possible protective role in man (Laughton *et al*., 1991 and Scott *et al*., 1993). Polyphenols possess powerful antioxidative properties (Serafini *et al.* 1994 and Maxwell *et al*. 1994) and are now considered to be potentially important for the prevention of chronic diseases in man. Green and black tea represent an excellent source of antioxidant polyphenols. *In vitro*, the antioxidative capacity of green tea is much higher than that of black tea (Serafini *et al*. 1996, Gerhard *et al*. 1989).

Antioxidants in foods have recently attracted special interest because they can protect the human body from free radicals, which may cause various diseases (including carcinogenesis) and aging. (Osawa *et al*., 1987 and Cutler, 1992).

Recent papers have provided the first evidence of on epidemiological link between polyphenol intake and risk of cordiovascular disease in man (Hertog *et al*., 1993). Evidence of an in vivo antioxidant effect in man after ingestion of polyphenol rich beverages, such as tea (Serafini *et al*., 1994) and wine (Maxwell *et al*., 1994), has also been recently published. Addition of synthetic antroxidants can control lipid oxidation in foods. However, use of such compounds has been related to health risks resulting in strict regulations over their use in food products (Hettiarachechy *et al*., 1996). Ito *et al*. (1982) reported that, BHA to be carcinogenic in experimental animals. Serafini *et al*. (1996) were surprised to find that black tea produced a response of the same antioxidative intensity of green tea *in vivo*. Yen and Chen (1995) studied the antioxidative activity of various tea extracts in relation to their antimutagenicity and reported that all tea extracts (green tea, pouchong tea, oolong tea, and black tea) exhibited marked antioxidative activity and reducing power. They cleared also that, antioxidative effect of tea extracts was well correlated to their antimutagenicity in some cases but varied with the mutagen and antioxidative properties. Drinking tea has beneficial effects on serum lipids and blood pressure (Bozidar, 1994)

The present work was carried out to determine the gross chemical composition of green and popular black tea collected from Egyptian local markets, which could be useful in assessing the amounts of tea extracts ingested through drinking tea beverage. This study was aimed also to isolate and identify the phenolic compounds in methanolic extracts of green and black tea. These methanolic extracts were used as natural antioxidants and its effectiveness was studied during storage of sunflower oil compared with synthetic antioxidants.

MATERIALS AND METHODS

Materials:

Green and the most popular marketable of black tea samples were purchased from retail markets of Alexandria and Kafr El-Sheikh governorates.

The tea samples used in this study were one green tea sample imported from China and six brands (1, 2, 3, 4, 5 and 6) of black tea imported from different tea producing countries. Black tea of brand 1 and 2 were imported blended from India, Kenya and Ceylon tea. Black tea of brand 3 and 4 were imported from Kenya, while black tea of brand 5 represented Indian tea. Black tea of brand 6 was imported from Ceylon.

About 500 gm of all tea samples (green tea and each brands of black tea were milled in a cycloteic mill (cycloteic 1093 sample Mill tecator Abbox 70, Hoganas, Sweden), then passed through a 50 mesh screen. The produced tea powders were stored until analysis. All analysis was carried out in triplicate.

Antioxidants and oils :

Butylated hydroxy toluene (BHT) was produced by Tokyo Kasei Kogyo Co. (Tokyo, Japan). Butylated hydroxy anisole (BHA) and pure catechin were products from Sigma Company (England). Free of antioxidant sunflower oil was obtained from Tanta Company for Oils and Soaps, Tanta, Egypt.

Methods:

Chemical analysis of green and black tea samples:

Moisture, protein, total nitrogen, total ash, water soluble ash, extractive, caffeine, tannin and fibre of green and black tea samples were determined according to A.O.A.C. (1990). Ether extract was determined according to A.O.C.S. (1984)

- **Extraction of phenolic compounds from green and black tea :**

  Phenolic compounds from green and black tea powders were extracted according to the methods of Rodriguez de **Sotillo *et al*. (1994)** using methanol alcohol 95% under cooling (4°C) as follows: five grams of green and black tea (powder) were homogenized for 4 min (in a dual-range Osterizer blender) with 29 ml of cold methanol. The resulting slurry was centrifuged (Hettich, Mikro Rapid/K type 1306) at 3000 $\times$ g for 10 min at 5°C. The supernatant liquid was filtered through Whatman No. 4, filter paper the filtrate was collected for quantitative analysis.

- **Quantitative determination of total polyphenolic compounds:**

  A suitable aliquot of methanolic extracts of green and black tea under investigation containing not more than 0.5 ml of acetone was diluted with distilled water to about 7 ml, in 10 ml graduated test tube. The samples were well mixed and 0.5 ml of Folin-Denis reagent was added. The test tubes was thoroughly shaken again for 4 min. About 1 ml of saturated sodium carbonate solution was added and the mixture was made up to 10 ml with good mixing. After one hour of absorption (Spectrophotometer JENWAY 6100) determined in 1 cm cells at 725 nm using a blank (water and reagents only). Calibration required for evaluation was carried out using standard chlorogenic acid solution in the concentration ranged from 10 to 100 μg (Swain and Hillis, 1959).

- **HPLC determination of phenolic compounds from green and black tea:**

  Phenolic compounds of methanolic extracts from green and black tea were determined using HPLC according to the method described by Andersen and Pedersen, (1983).

- **Antioxidative activity of both green and black tea extracts:**

  The antioxidative activities of methanolic extracts from green and black tea were assayed at concentrations 0, 150, 200, and 250 ppm to free of antioxidant sunflower oil samples (control). The treated sunflower oils were stored at 40°C for 9 weeks in amber glass bottles and the degree of oxidation was weekly determined using peroxide values (PV's) (Leonard *et al*. 1987) and thiobarbituric acid (TBA) (Tarladgis *et al*. 1960) and modified by Rhee (1978).

RESULTS AND DISCUSSION

Gross chemical composition of green and black tea samples :

The chemical composition of tea leaves varies greatly depending on their origin and the type of processing as presented in Table (1). In green tea, 37.15% of the dry matter is soluble in hot water, while 37.65-44.50% of the dry matter is soluble in hot water depending on the origin of black tea leaves (Table 1). The moisture content of green tea leaves (8.56%) was higher than that of black tea leaves. Variation in moisture content of black tea samples (7.63-8.41%) could be related to the level of fermentation, condition of drying and packing, storage period and the technology of processing (Yamanishi *et al*. 1992). All samples of tea have low content of moisture, which makes them not highly susceptible to microorganism attack. This might be advantageous in terms of the shelf life of tea samples.

Table (1) indicated that oil was present only at low levels. The ether extract (oil content) of green tea leaves was higher (2.55%) than that of black tea leaves, which ranged from 1.27 to 2.20%. These results are in agreement with those given by Nematalla (1996). On the other hand, Belitz and Grosch (1987) found that, lipid content of fresh and fermented tea leaves was 7%.

The total nitrogen content of green and black tea samples showed slight differences and ranged between 3.99 and 4.52 % (Table 1). On the other hand, the protein content of black tea samples was ranged from 9.60 to 11.12% depending on the origin, nitrogen and caffeine contents of tea leaves. Green tea leaves contained 12.18% protein (as dry weight basis).

Green tea contained the lowest ash content (5.87) and the highest content of total ash was found in Indian black tea (6.22%). It was within the range given by Yamanishi *et al*. (1992). The average of the total ash content of black tea was around 5.96% of the dry matter. On the other hand, the water soluble ash in both types of tea samples was ranged between 3.06 to 3.50% (Table 1). The results indicate that, green tea contained the higher levels of fibre (13.82%) than those of all types of black tea samples, which ranged from 10.94 to 13.35%.

Table (1): Chemical composition of green and black tea samples (on dry weight basis).

Constituents %	Green tea*	Black tea brands**					
		1	2	3	4	5	6
Moisture	8.56	8.41	7.86	7.63	8.12	8.37	7.92
Ether extract	2.55	1.85	1.79	1.27	1.44	1.80	2.20
Protein	12.18	11.12	10.12	10.93	10.44	9.60	10.31
Total nitrogen	4.48	4.28	4.52	4.35	4.22	3.99	4.12
Total ash	5.87	5.95	5.70	5.90	6.07	6.22	5.98
Water soluble ash	3.06	3.32	3.17	3.28	3.40	3.50	3.27
Fibre	13.82	11.42	10.94	13.35	12.08	11.13	11.62
Extractives	37.15	44.50	43.60	37.65	38.20	42.68	38.72
Caffeine	2.53	2.50	2.78	2.60	2.55	2.45	2.47
Tannin	19.65	13.35	14.22	13.87	13.70	13.46	13.29
Polyphenols	32.75	31.12	29.89	27.20	28.85	30.21	29.10

*** Green tea : from China**
**** Black tea : brands 1 and 2 blend from India, Kenya and Ceylon.**
: brands 3 and 4 blend from Kenya.
: brand 5 blend from India.
: brand 6 blend from Ceylon.

Caffeine constitutes 2.45-2.78% of the dry matter of tea leaves. Caffeine plays an important part in determining the tea taste and "briskness" of the tea beverage. The obtained data was in agreement with the results of Belitz and Grosch (1987). The results presented in Table (1) showed that, the highest percent of tannin was found in green tea leaves (19.65%). Black tea leaves contained lower levels of tannin which ranged from 13.29 to 14.22%. This was within the results given by Takeda (1994). Variation in tannin could be attributed to the level of fermentation and storage of tea. However, it is well known that some of the reaction occuring during fermentation are enzyme-catalyzed and of these the more important are the oxidation of tea flavonols by polyphenol oxidose, which lead to the development of colour, strength and the occurrence of reactions responsible for producing the characteristic flavour of black tea. Total polyphenols make up 27.20 and 32.75% of the dry matter content of black and green tea leaves, respectively. The data indicated that green tea contained higher amounts of polyphenolic compounds (32.75%) than those in black tea samples. Table (1) indicated also, little variation in the content of polyphenolic compounds in black tea samples. Among the six samples of black tea, Brand 1 contained the highest amount of polyphenolic compounds, whereas Brand 3 had the lowest amount.

It was generally observed that, when the total polyphenol content of tea samples was greater, the catechin amount was also greater (Table 1 and 2). Balentine, (1992) found that, tea leaves contain > 35% of their dry weight in polyphenols, which differ depending on manufacturing procedure.

Isolation and identification of phenolic compounds of green and black tea extracts :

High-performance liquid chromatography (HPLC) was used for separation and identification of phenolic compounds in both types of green and black tea (Brand 1) extracts. The results in Fig. (1) and Table (2)

Time (min)

Fig. (1): HPLC chromatograms of the phenolic compounds extracted from green (A) and black (B) tea leaves.

Table (2): Separation and identification of phenolic compounds from green and black tea extracts using HPLC.

Phenolic compounds	Green tea		Black tea	
	R.T. (min)	%	R.T. (min)	%
Tannic acid	2.14	0.40	2.13	35.26
Ferulic acid	4.60	8.77	4.40	0.59
Chlorogenic acid	6.26	5.24	6.59	3.81
P-hydroxybenzoic acid	6.87	18.18	6.90	4.55
Caffeic acid	7.37	36.97	7.38	30.28
Gallic acid	7.82	8.20	7.83	5.78
Catechin	8.77	14.62	9.08	10.33
Unknown	9.03	4.06	9.67	6.17
Cinnamic acid	9.33	3.56	10.12	2.55

indicated that, methanolic extracts of green and black tea leaves contained nine phenolic compounds. The data cleared that, caffeic acid was the main plenolic acid in green tea extract, representing about 36.97% of the total phenolic compounds, followed by p-hydroxy benzoic acid (18.18%) and catechins (14.62%). Green tea extract contained also higher amounts of ferulic acid (8.77%), chlorogenic acid (5.24%) and gallic acid (8.20%). On the other hand, methanolic extracts of black tea contained higher amounts of tannic acid (35.26%), caffeic acid (30.28%), gallic acid (5.78%) and catechin (10.33%). Both tea leaves extracts contained also cinnamic acid in small amounts, which represented about 3.56% and 2.55% in green and black tea extracts, respectively.

From results presented in Table (2), it could be seen that green tea extracts contain higher amounts of ferulic, p-hydroxybenzoic, caffeic, gallic, chlorogenic and cinnamic acids than those of black tea extracts. The results indicated also that, green tea extracts contained higher amounts of catechin (14.62%) than those of black tea extracts (10.33%). On the other hand, black tea extracts contained higher amounts of tannic acid (35.26%) than those of green tea extracts (0.40%). Generally, the results in Table (2) are in agreement with those reported by Rabe *et al*. (1994) and Ferreira *et al*. (1995). They reported that the phenolic acids in tea leaves extracts consist of caffeic acid, p-hydroxy benzoic acid, vanillic acid, ferulic acid and catechin. Most of these compounds are widely distributed in nature and have been shown to possess antioxidative properties (Pratt and Hudson, 1990, Ho *et al*., 1992 and Kanner *et al*., 1994). The presence of phenolic hydroxylgroups increases the antioxidative activity of phenolic acids, while methoxylation of hydroxylgroups causes a decrease in activity (Marinova and Yanishlieva, 1992).

Use of green and black tea methanolic extracts as natural antioxidant for sunflower oil during storage :

Antioxidative activities of green and black tea methanolic extracts were assayed during storage of sunflower oil, which contained high percentage of polyunsaturated fatty acids (88.70%) Maiti *et al*. (1988).

The addition of green tea extract at various concentrations did not affect the colour or the appearance of sunflower oil. On the other hand, addition of black tea extract at higher concentrations slightly affected the colour or the appearance of the tested oil.

Measurements of peroxide value (PV) and thiobarbituric acid value (TBA) are a suitable potent methods to characterize oxidative changes in sunflower oils stored at 40°C for nine weeks. The development of rancidity was estimated and compared with those of untreated sunflower oil (control sample).

Changes in peroxide values of oils treated with tea extracts and synthetic antioxidants :

Peroixide values (PV,s) were estimated after 0, 7, 14, 21, 28, 35, 42, 56 and 63 days to follow the oxidation or stability of sunflower oil samples during storage at 40°C in amber glass bottles. The results given in Fig. (2) indicate that PV of control (sunflower oil without antioxidants) was increased rapidly to 36.10 meq./kg after 63 days, while the oils treated with 150 ppm of black tea extract was 18.20 meq./kg after 63 days. The oil treated with 150 ppm of green tea extract reached only to 13.18 meq./kg after 63 days. Similarly, increases were found in the case of BHA (15.35 meq./kg) and pure catechin (13.21 meq./kg). The highest antioxidative activity was found in the case of BHT (150 ppm) where PV was 11.42 meq./kg after 63 days.

From the result in Fig. (2) we can notice that green tea extracts at 150 ppm possessed appreciable antioxidant activity greater than black tea extracts, pure catechin and BHA.

The results in Fig. (2) indicated that increasing the levels added from 150 to 200 and 250 ppm lead to decreasing PV's formed and retarding oxidative rancidity in sunflower oils. The oil treated with 250 ppm of pure catechin was the best natural antioxidants in decreasing PV (9.17 meq./kg) followed by, those treated by the same level of green tea extract (11.00 meq./kg), then samples treated by 250 ppm of black tea extract (11.60 meq./kg). The highest antioxidative activity was found in the oil treated by 250 ppm of BHT (8.39 meq./kg). Antioxidant activity of the phenolic acids was decreased in the order vanillic acid > ferulic acid > syringic acid > p-hydroxybenzoic acid > p-coumaric acid > protocatechuic acid > caffeic acid (Gadow, *et al.* 1997). They reported also that, benzoic acid derivatives were more effective as antioxidants than their cinnamic acid analogues (vanillic acid > ferulic acid; p-hydroxy benzoic acid > p-coumaric acid; protocatechuic acid > caffeic acid).

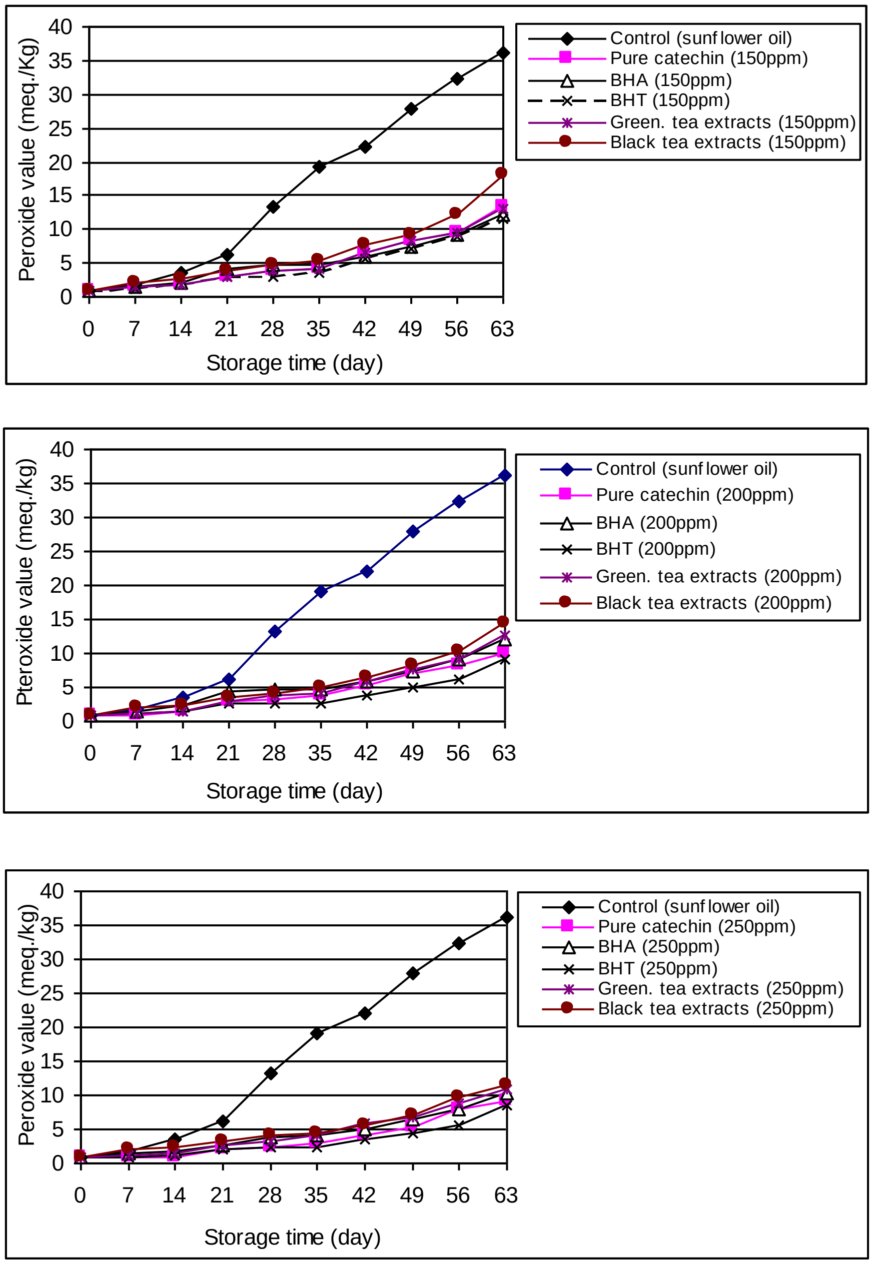

Fig. (2): Antioxidative activities of methanolic extracts from green and black tea leaves at different concentrations (150, 200 and 250 ppm) on sunflower oil during storage at 40°C (measured by proxide value).

Changes in thiobarbituric acid values (TBA) of oils treated with tea extracts and synthetic antioxidants :

Tthiobarbituric acid (TBA) value is frequently used to measure the oxidation degree of oils, fats and fatty foods. A red pigment is used by reaction of TBA with malondialdehyde. Therefore, the TBA-value is often expressed as mg of malondialdehyde/kg of fat or oil. Pearson *et al*. (1983) reported that the TBA assay measured the release of malonaldehyde, the secondary oxidation products of poly-unsaturated fatty acids (Gray, 1978).

Methanolic extracts of green and black tea were used as natural antioxidants and compared with pure catechin BHA and BHT. These compounds were added to sunflower oil at levels 150, 200 and 250 ppm and stored in amber glass bottles at 40°C.

TBA values found in Fig. (3) reflected that, by storage for nine weeks for untreated oil, TBA values were increased gradually to 15.80 mg MDA/kg. The oil treated by 150 ppm of pure catechin showed TBA value to reach 12.57 mg MDA/kg, while in the case of sunflower oil treated by tea extracts (150 ppm) were 12.90 and 13.10 mg MDA/kg for green and black tea extracts, respectively. The results in Figure (3) indicate that, the oils treated with pure catechin showed slightly lower in TBA values than the other oil samples treated with tea extracts. Antioxidative activity of catechin and tea extracts (green and black) was increased proportionally with increasing their concentration (Fig. 3). The highest effect was recorded with the highest concentration (250 ppm). The results in Fig (3) are in agreement with those reported by Farag *et al*. (1989), they found that some spice extracts (Caraway, clove, cumin, rosemary, sage and thyme) possessed an antioxidant effect and this phenomenon was increased by increasing their concentration. Furthermore, the present tea extracts were more effective in retarding oxidative changes in sunflower oil. The extent of activity of both green and black tea extracts was equal to those of commercial antioxidants, i.e. TBA and BHT.

Tea extracts were reported elsewhere to contain some polyphenolic compounds, which can act as antioxidants (Thanaraj and Seshadri, 1990). Antioxidants can protect the human body from free radical, which may cause various diseases including carcinogenesis (Cutlar, 1992). Sources of natural antioxidants are primarily plant phenolics that may occur in all parts of the plants, especially in the leaves and seeds (Pratt and Hudson, 1990).

Green and black tea extracts contained many types of polyphenolic compounds and their derivatives (Thanaraj and Seshadri, 1990). Phenolic compounds make up 25-35% of the dry matter content of fresh tea leaves (Belitz and Grosch, 1987). Green and black tea represent an excellent source of antioxidant polyphenols. In vitro, the antioxidative capacity of green tea is much higher than that of black tea (Serafini *et al.* 1996 and Gerhard *et al*. 1989)

Generally, Figs. (2 and 3) and Table (2) cleared that green tea extract was more effective as antioxidants than those of the case of black tea extract. The highest amounts of caffic acid, p-hydroxybenzoic acid, catechin and ferulic acid in green tea extracts posses the highest antioxidative activity of green tea extracts.

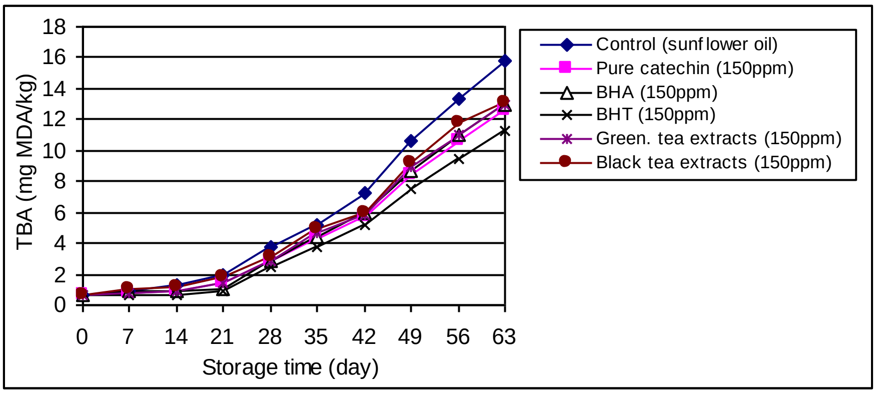

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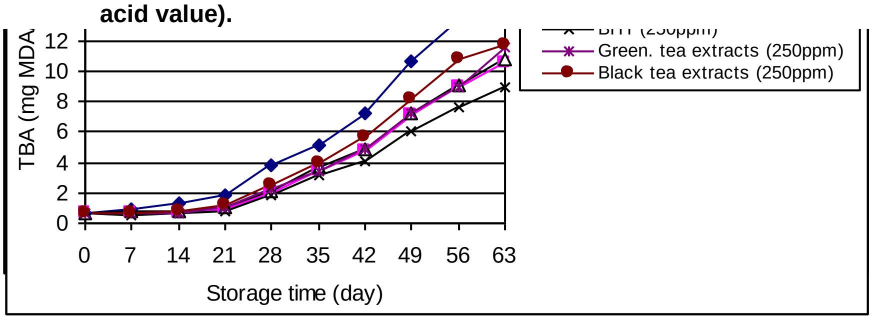
Fig. (3): Antioxidative activities of methanolic extracts from green and black tea leaves at different concentrations (150, 200 and 250 ppm) on sunflower oil during storage at 40°C (measured by thiobarbituric acid value).



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استخدام المركبات الفينولية المستخلصة من الشاى الأخضر والأسود كمضادات أكسدة طبيعية

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تم فى هذا البحث استخلاص وفصل المركبات الفينولية من أوراق الشاى الأخضر والأسود باستخدام كحول الميثانول واستخدامها كمضادات أكسدة طبيعية لحماية الزيوت من عمليات الأكسدة ومقارنتها بمضادات الأكسدة الطبيعية مثل الكاتشين والصناعية مثل بيوتلاتيدهيدروكسى أنيسول (BHA) وبيوتلاتيدهيدروكسى تولوين (BHT) بعد إضافة كل منها لزيت عباد الشمس بتركيزات مختلفة 150، 200، 250 جزء فى المليون وتخزينها فى زجاجات غامقة على درجة 40 درجة مؤية لمدة تسعة اسابيع. كما تم دراسة الخواص الكيميائية للشاى الأخضر والأسود الأكثر تداولاً فى السوق المصرى.

وقد أظهرت النتائج ما يلى :

زيادة محتوى أوراق الشاى الأخضر من الرطوبة والمستخلص الأيثيرى والبروتين والتانين والمركبات الفينولية بالمقارنة بأوراق الشاى الأسود بينما أحتوت هذه العينات على نسب متقاربة من المستخلص المائى والكافين.

أدى استخدام التحليل الكروماتوجرافى السائل عالى الكفاءة (HPLC) لمستخلص الميثانول للمركبات الفينولية للشاى الأخضر والأسود الى فصل تسعة مركبات فينولية أهمها فى الشاى الأخضر : حمض الكافيك 37.97% ، حمض البارا هيدروكسى بنزويك 18.18%، الكاتشين 14.62%، الفريوليك 8.77%، حمض الجاليك 8.20%، حمض الكلوروجينيك 5.24% وأهمها فى الشاى الأسود: حمض التانيك 35.26%، حمض الكافيك 30.28%، الكاتشين 10.33%، حمض الجاليك 5.78%، حمض البارا هيدروكسى بنزويك 4.55%.

أثبتت النتائج أن مستخلص الميثانول للمركبات الفينولية المفصولة للشاى الأخضر والأسود ذات نشاط مضاد لأكسدة زيت عباد الشمس يماثل مضادات الأكسدة الطبيعية مثل الكاتشين والصناعية مثل BHA ، BHT حيث أدت إضافتها بتركيزات مختلفة 150، 200، 250 جزء فى المليون الى انخفاض رقمى البيروكسيد (PV) وحمض الثيوباربتيوريك (TBA) وبالتالى انخفاض مقدار التزنخ التأكسدى لهذا الزيت بينما فى حالة زيت عباد الشمس (الكنترول) الذى لم يعامل بأى من هذه المستخلصات ومضادات الأكسدة الطبيعية مثل الكاتشين والصناعية مثل BHA ، BHT كانت قيم رقمى البيروكسيد وحمض الثيوباربتيوريك عالية.

أثبتت الدراسة أن مستخلص الميثانول للمركبات الفينولية المفصولة من الشاى الأخضر أكثر نشاطاً كمضاد لأكسدة زيت عباد الشمس عنها فى حالة مستخلص الشاى الأسود وكذا مضادات الأكسدة الطبيعية مثل الكاتشين والصناعية مثل BHA ، BHT. لذلك يمكن التوصية باستخدام الشاى الأخضر لاحتوائه على مضادات الأكسدة الطبيعية بنسبة عالية.