J. Agric. Sci. Mansoura Univ., 25 (1): 297 - 304, 2000

ANTIOXIDATIVE EFFECTIVENESS OF USED BLACK TEA ON SUNFLOWER OIL DURING HEATING

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ABSTRACT

Antioxidative effectiveness (AE) % of used black tea and its extracts on sunflower oil (SO) during heating at 60°C for 20 days were investigated. Data revealed that addition of used tea powders at levels of 1,2,4,5, and 10 % to the SO showed only marginal AE % as compared to the synthetic antioxidant (BHT) used. The AE % increased as the level of tea powder increased. The used black tea was extracted with different solvents (ethanol, EE, methanol, ME, diethyl ether, DEE and methanol/diethyl ether, 2: 1, DEE). MDEE and DE gave the highest yields as compared to the other extracts. All extracts were added o SO at 200 ppm. Data revealed that the order of AE % for these extracts were ME (88.20)>M/DEE (87.06)>DEE (84.29)>BHT (79.50) and finally EE (40.24%) after heating at 60°C for 20 days. TBA values of SO increased progressively, with all extracts, with increasing the time of heating for 20 days. ME and M/DEE gave the lowest TBA values, respectively, as compared to the other extracts used. The control sample showed the highest TBA value. The antioxidant components in all extracts were tentatively identified using TLC to be phenolic compounds. The dominant compounds were identified in all extracts when reacted with ferric chloride to produce blue colour represents phrogallol, blue-black, represents trihydroxy phenolic compounds and green represents catechol.

Key words: Used black tea, Antioxidative Effectiveness, Sunflower oil, Heating, and Phenolic compounds.

INTRODUCTION

Lipid oxidation is one of the major deteriorative reactions in oils and fatty foods, and often results in a significant loss of quality (Alexander, 1978). It is well known that lipid oxidation can lead to changes in function, sensory, and nutritive values and even the safety of foods (Pearson, *et al*., 1983 and Wu and Nawar, 1986). Generally, these changes reduce consumer acceptance of oxidized products. These problems can be countered by the application of antioxidants.

Antioxidants are used as food additives in order to extent the lifetime of oils and fatty foods during storage and processing. The antioxidants to be used are determined by various factors including legislation, effectiveness and cost. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butyl hydroquinone (TBHQ) are presently used in the food industry to stabilize oils against oxidation (Giese, 1996). The commercial use of synthetic antioxidants is strictly controlled, and increasing consumer awareness of food additives and safety have promoted increased interest in the use of natural antioxidants as alternatives to synthetic compounds (Houlihan and Ho, 1985). Many herbs and spices have been shown to possess antioxidant activity (Chang *et al*., 1977; Jitoe *et al.,* 1992 and Adegoke and Krishna, 1998).

Tea is the most widely consumed beverage in the world and is prepared from the leaves of the *Camellia sinensis* plant. Consumption of green tea has been shown to be associated with a decrease in serum total cholesterol and triacylglycerols (Kono *et al.,* 1992 and Imai and Nakachi, 1995). It has been found an inverse association between tea consumption and coronary heart disease mortality after adjustment for age, diet and other risk factors (Hertog *et al*., 1993). The ingestion of both black and green tea produced a significant increase in human plasma antioxidant capacity *in vivo* (Serafini *et al.*, 1996). Tea extract may also offer an alternative in protecting fats and oils in foods from oxidative rancidity (Lunder, 1992 and Chen *et al*., 1998). Old used tealeaves are considered as one of the agricultural waste, which cause lots of problems to the environments. Therefore, the present study was conducted to examine the relative antioxiditive effectiveness of different solvent extracts from used black tealeaves on sunflower oil. In addition, to isolate and identify the compounds responsible for this antioxidative effect.

MATERIALS AND METHODS

Materials:

Black tea (Lipton tea bags, quality No 1) and sunflower oil were purchased from the local market, Alexandria, Egypt.

All solvents and chemicals used were of high grades.

Methods:

Antioxidant effectiveness (AE) of used tea powder:

Black tea was soaked in hot distilled water (80°C) and filtered. The used tea leaves was washed three times with hot water to remove the colour of the tea, then oven dried at 60°C. The dried tea leaves was cleaned of extraneous matter, ground and sieved. Tea powders were added separately at 1, 2, 4, 5 and 10 % to (50 g) sunflower oil (SO) in 100-ml beakers. Another beaker contained SO and BHT (200 ppm) as a synthetic antioxidant (food grade). Control sample contained only SO. After careful mixing, the beakers were transferred to an oven set at 60°C for up to 20 days. Peroxide values (PV) were determined every 5 days by AOCS official method Cd 8-53 (1989). The percentage antioxidant effectiveness (AE) was calculated from the equation reported by Adegoke and Gopala Krishna (1998):

$$\mathbf{AE} = \frac{\textbf{PV of control} - \textbf{PV of test sample}}{\textbf{PV of control}} \times 100\%$$

Extraction of antioxidative components:

The antioxidative components of used tea were extracted according to the method described by Adegoke and Gopala Krishna (1998) with some modification. The used tea (10 g each) was extracted, separately, with (100-ml) ethanol, methanol, diethyl ether, and methanol/ diethyl ether (2:1) for 1h, and filtered. The solvents were rotary-evaporated at room temperature to obtain the antioxidant extracts. The antioxidant extracts obtained were (Ethanol extract EE, Methanol extract ME, Diethyl ether extract DEE and Methanol/ diethyl ether extract, MDEE). Yields and colours of antioxidant extracts were determined. The extracts so obtained were added to the SO at 200 ppm, along with BHT as synthetic antioxidants. The percentage antioxidant effectiveness (AE) was carried out and calculated as described earlier. A thiobarbituric acid (TBA) test was carried out according to (Cervants and Martinez, 1984) to measure the extent of oxidation.

Isolation of antioxidant components:

The antioxidant components were isolated on silica gel TLC plates (type G). Each extract in the respective solvent (20 nl) was spotted. The plates were developed in chloroform/ethanol/acetic acid (98: 2: 2) Adegoke and Gopala Krishna (1998).

Identification of antioxidant components:

The extracted antioxidant components were identified as follows: The TLC plates were sprayed with a 1% potassium ferricyanide in water and a 1% ferric chloride in water, which gave a blue colour to indicate that these extracts probably phenolic compounds (Barton *et al.,* 1952). Furthermore, the plates were sprayed with ferric chloride (2 g), dissolved in 100 ml ethanol to produced blue colour for some spots, which indicates the probably presence of trihydroxy phenolic compounds (Reio, 1958). The TLC plates were exposed to iodine vapour to show the representative number of TLC spots in each extract.

RESULTS AND DISCUSSION

Antioxidant effectiveness (AE) of used tea powder:

Table (1) shows the antioxidative effectiveness percentages of black tea powders in SO during heating at 60°C for 20 days. Data revealed that addition of black tea powder at levels of 1; 2; 4; 5 and 10% to the SO showed only a marginal antioxidative effect as compared to a synthetic antioxidant BHT. The antioxidative effect of black tea increased as the level of tea powder added to the SO increased. The effect, however, increased with the lower levels only (1 and 2%) as the time of storage continued for 20 days. Nevertheless, the effects of higher levels decreased with the time of storage. These effects most probably due to that the antioxidants are known to be effective at low concentrations, while at higher levels they may become pro-oxidants (Rajalakshmi and Narasimhan, 1996). Another reason might be due to that the antioxidant components were not extracted, although the antioxidants are present. It is present inside the cellular compartments of the tea, and the oil could not reach inside and extract it. Also, for extraction of the antioxidant from the tea powder, a non-polar solvent such as petroleum ether (or a vegetable oil) may not be suitable (Adegoke and Gopala Krishna, 1998).

Table (1): The antioxidative effectiveness (AE) % of black tea powders on the sunflower oil during storage at 60°C for 20 days.

Storage time (Days)	Antioxidative effectiveness (AE)[a]					
	1%	2%	4%	5%	10%	BHT
5	14.54	15.02	23.14	25.24	35.78	83.33
10	15.52	16.53	14.21	19.52	32.17	80.25
15	16.32	17.70	10.49	16.47	25.36	79.91
20	17.56	18.95	5.10	10.76	20.56	79.53

(a) AE % = (PV of control - PV of test sample/ PV of control) x 100.

Yields and colours of antioxidant extracts:

Yields and colours of antioxidant extracts of used black tea are given in Table (2). MDEE gave a dark-brown powder with the highest yield (17.65%), DE gave (15.32%) of reddish-yellow powder, ME gave a dark-brown powder (12.45%), and finally EE gave a yellow powder (7.25%).

Table (2): Yields and colours of antioxidant extracts of used black tea.

Extract	Yield (% dry basis)	Appearance and colours of extract
Ethanol extract	7.25	Yellow liquid
Methanol extract	12.45	Dark-brown powder
Diethyl ether extract	15.32	Reddish-yellow liquid
Meth./ diethyl ether extract	17.65	Dark brown powder

Antioxidant effectiveness of tea extracts:

The antioxidative effectiveness of the various black tea extracts on SO during heating at 60°C for 20 days is given in Table (3). Data revealed that the order of AE% of extracts, when added to the SO at 200 ppm, were ME (88.20)> M/DEE (87.06)> DEE (84.29)> BHT (79.50) and finally EE (41.25) after 20 days of heating. The effect of EE, comparable to BHT was considerable lower than for BHT, although a significant antioxidant effect was observed for the EE as compared to the control. Similar results were found for the spice *Aframomum danielli* (Adegoke and Gopala Krishna, 1998). The differences found for the AE% between different extracts used could be explained as follows. The antioxidants present in the black tea are soluble in slightly polar solvents, such as diethyl ether and methanol. Therefore, diethyl ether and methanol, which are more polar solvents, extracted the antioxidants effectively. Ethanol extract exhibited little antioxidative effect as compared to the other extracts and BHT used. The antioxidative properties of various tea ethanol extracts were previously examined (Chen, *et al.,* 1996). They reported that green and white tea (which is the product of unfermented tender or unopened leaf buds) extracts were found to exhibit a stronger inhibition on lipid oxidation in canola oil than did BHT. They added that, In contrast, the extract from black and dark-green tea showed little or no antioxidative activity. The present study agreed with their results concerning the ethanol extract, however, the extracts from black tea using methanol or diethyl ether as solvents disagreed with their results. They showed stronger inhibition on lipid oxidation in sunflower oil as compared to BHT when used at

Table (3): The antioxidative effectiveness (AE) % of different black tea extracts on of the sunflower oil during storage at 60°C for 20 days.

Storage time (Days)	Antioxidative effectiveness (AE)[a]				
	Ethanol extract	Methanol extract	Diethyl ether extract	Meth./ diethy ether extract	BHT
5	37.29	83.15	80.65	82.05	83.33
10	39.12	85.00	82.46	83.00	80.25
15	40.54	87.50	84.25	85.38	79.91
20	41.25	88.20	85.29	87.06	79.53

a) AE % = (PV of control - PV of test sample/ PV of control) x 100.

200 ppm. Crude ethyl acetate extracts from green teas (longjing and jasmine) at 200 ppm exhibited similar results as antioxidative activity against lipid oxidation in canola oil (Chen, *et al.,* 1998).

Effect of different used black tea extracts on the TBA values of sunflower oil during heating at 60°C for 20 days is given in Table (4). The TBA test has been widely used as an objective measure of secondary products of oils. It was assumed that accumulation of these products during consecutive days of storage affected the oil quality and was responsible for the development of rancid odour and off-flavour of the oil. The TBA values of all extracts increased progressively with heating time. Control sample progressively had the highest TBA values, while ME and M/DEE gave the lowest TBA values, respectively, as compared to other extracts used. The effectiveness of these extracts as lipid antioxidants has been attributed mainly to their ability to remain stable for long time at high temperature (Cuvelier and Berset, 1994). The primary phenolic antioxidants react with lipid or hydroxyl radicals and convert them into stable products (Gordon, 1990).

Table (4): Effect of different black tea extracts on of the TBA value (n moles malondialdehyde/ kg oil) of sunflower oil during storage at 60°C for 20 days.

Storage time (Days)	TBA values (n moles malondialdehyde/ kg oil)					
	Control	Ethanol extract	Methanol extract	Diethyl ether extract	Meth./ diethy ether extract	BHT
0	12.34	12.34	12.34	12.34	12.34	12.34
5	50.42	40.27	13.58	32.17	15.11	12.44
10	104.27	70.15	15.92	40.91	18.72	17.57
15	140.96	90.58	20.39	45.64	24.84	25.18
20	170.65	108.47	22.45	50.83	28.55	31.34

Tentative identification of black tea antioxidants components:

TLC patterns of the components of antioxidant extracted from the black tea are shown in Figure (1). The antioxidant components in the extracts were tentatively identified to be phenolic compounds as they gave the identical colours for the specific spray reagents used. Methanol extract showed 10 spots, while ethanol extract showed 7 spots. Diethyl ether and

M/diethyl ether extracts showed 12 spots. The main phenolic compounds were identified in all extracts when reacted with ferric chloride to produce blue, blue-back, green, and yellow, and brown colours. According to Van Sumere, (1989), blue colour represents phrogallol derivatives, blue-black represents trihydroxy phenolic compounds, and green represents catechol. Although the method cannot be used for positive identification, it may indicate the presence of catechols, phrogallol, and trihydroxy phenolic compounds. Shahidi and Wanasundra (1992) reported that natural antioxidant extracts are primarily plant polyphenolic compounds that may occur in several parts of the plant.

The present findings of the antioxidative effects of used black tea needs more research into the application of such natural waste food materials as antioxidants for lipid stability.

Figure (1): TLC pattern of antioxidants extracted from used black tea leaves using different solvents.

(a) Plate was sprayed with ferric chloride. (b) Plate was exposed to iodine vapour.

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التأثير المضاد للأكسدة لتفل الشاي الأحمر المستعمل علي زيت عباد الشمس أثناء التسخين

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تم دراسة التأثير المضاد للأكسدة لتفل الشاي الأحمر المستعمل ومستخلصاته علي زيت عباد الشمس أثناء التسخين علي درجة حرارة ٦٠°م لمدة ٢٠ يوم. ولقد أوضحت النتائج أن إضافة بودرة تفل الشاي المستعمل بمعدلات ١،٢،٤،٥ و ١٠% إلى الزيت أظهر تأثيرا مضاد للأكسدة قليل إذا ما قورن بتأثير مادة مضاد الأكسدة الصناعية المستخدمة (BHT) ، وقد زاد هذا التأثير بزيادة معدل الإضافة. هذا وقد تم استخلاص تفل الشاي المستعمل بواسطة مذيبات مختلفة (الأيثانول، الميثانول، داي ايثيل أيثر وخليط من الميثانول / داي ايثيل ايثر بنسبة ١:٢). وقد أوضحت النتائج ان المستخلص بواسطة ميثانول/ داي ايثيل ايثر ثم مستخلص داي ايثيل ايثر قد أعطي أعلي كمية بالمقارنة بالمستخلصات الأخرى. وعند إضافة هذه المستخلصات إلى زيت عباد الشمس بنسبة ٢٠٠ ppm أثناء التسخين، كانت النسبة المئوية للتأثير المضاد للأكسدة لها كالأتي: مستخلص الميثانول (٨٨,٢٠)>مستخلص ميثانول/ داي ايثيل ايثر(٨٧,٠٦)>مستخلص داي ايثيل أيثر(٨٤,٢٩)>BHT (٧٩,٥٠) وأخيرا مستخلص الأيثانول (٤٠,٢٤) وذلك بعد التسخين عند درجة حرارة ٦٠°م لمدة ٢٠ يوم. وقد زادت قيم TBA للزيت بزيادة زمن التسخين لكل هذه المستخلصات. وقد أظهرت العينات المضاف إليها مستخلص الميثانول وكذا الميثانول/داي ايثيل ايثر أقل قيم لاختبار TBA علي التوالي بالمقارنة بالمستخلصات الأخرى. وقد تم التعرف علي مكونات مضادات الأكسدة الموجودة في كل المستخلصات باستعمال TLC ووجد إنها مركبات فينولية. وقد تم التعرف مبدئيا علي هذه المركبات عند تفاعلها مع كلوريد الحديديك (لون أزرق) وتمثل phrogallol، المركبات ذات اللون الأزرق الداكن تمثل trihydroxy phenol وتلك الخضراء تمثل catechol .