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Using Egyptian Caper Seeds Oil (*Capparis spinosa* L) as a Natural Antioxidant to Improving Oxidative Stability of Frying Oils During Deep Fat Frying

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Abestract: The addition of natural antioxidants in food plays an important role to avoid toxic effects of synthetic antioxidants. This work was aimed to evaluate the effect of natural antioxidants addition in the protection of sunflower and soybean oils as frying oils during the deep frying of potato fingers at 180°C for 180 min, since caper seed oil (CSO) as a rich source of natural antioxidants. The CSO was added to sunflower and soybean oils separately at ratio as follows: 200, 400 and 600 ppm. Besides, 200 ppm of tertiary butylhydro quinone (TBHQ) served as a control sample. From the obtained results it could be concluded that the CSO contain higher phenolic compounds content (858.54 mg/100g), total tocopherols (758.12 mg/100 gm as vitamin E), total carotenoids (675.8 mg/100 g) and the DPPH activity was recorded (152.40 %). The induction period (IP) in hrs was taken as an index for the oxidative stability of the oil samples. However, the addition of CSO to oil samples at different mentioned levels led to increase its stability against oxidation. The higher IP of sunflower and soybean oil samples was found at addition ratio of the CSO 600 ppm was (9.89 and 8.11 hrs, respectively) compared with the TBHQ (6.67 hrs, as control sample). During frying process of potato finger, the results showed that the addition of CSO at levels 400 and 600 ppm to sunflower and soybean oils reduced the refractive index, free fatty acids, peroxide, TBA values and polymer content as compared to oil samples treated with TBHQ under the same conditions. Although the soybean and sunflower oils had the highest amounts of polyunsaturated fatty acids, but the CSO was exhibited superior ability in protecting the oil from oxidation during frying process. Finally, it can be recommended that the caper seed oil (CSO) suggests as a good natural antioxidant alternative to TBHQ as antioxidant in frying oils.

Key words: Natural antioxidant · Caper seed oil · Deep fat frying · Sunflower oil · Soybean oil · Fatty acids profile · Oil quality

INTRODUCTION

In recent years, there has been a significant increase in the interest for bioactive compounds from plant products. Polyphenols and chemical substances found in plants, have been used in the human diet in the largest quantity [1-2]. This group of compounds is recognized for the protection against the development of chronic diseases like diabetes, cancers, osteoporosis, cardiovascular and neurodegenerative diseases [2-3].

Spices, herbs, fruits and vegetables have been extensively studied in different countries because of the high antioxidant activity and their beneficial effects on human health. Antioxidants from spices, herbs, fruits and vegetables are a large group of bioactive compounds which consist of flavonoids, phenolic compounds, sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes and vitamins. These compounds demonstrate different antioxidant activities. For example, flavonoids have the ability to scavenge free radicals and can form complexes with catalytic metal ions rendering them inactive. Studies have shown that caper seeds excellent sources of antioxidants with their high content of phenolic compounds. Antioxidants can protect lipids and oils in food against oxidative degradation. When added to food, antioxidants control rancidity development, retard the formation of toxic oxidation products, maintain nutritional quality and extend the shelf-life of products.

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Because of safety concerns, synthetic antioxidants are limited to be used as food preservatives. Natural antioxidants obtained from edible materials such as spices, herbs, fruits and vegetables have been of increasing interest [2-4].

Plant seed and kernels are important sources of oils of nutritional, industrial and pharmaceutical importance [5]. *Capparis spinosa* seeds are rich in lipids, containing mainly unsaturated fatty acids [6-10]. The bioactive phytochemical analysis of *Capparis spinosa* showed that this species represented a very rich source of bioactive and nutraceutical compounds, the oil from the plant seeds oil was rich in unsaturated and rare lipids such as cis-vaccenic acid; the main glucosinolate was glucocapperin [2]. Previous chemical studies on *Capparis spinosa* have reported the richness with phenolic compounds, tocopherols, carotenoids, flavonoids and glucosinolates in different parts of this plant [2, 11, 12]. These phenolics, along with vitamin C, tocopherols and carotenoids, are responsible for the antioxidant activity of plant materials [13,14]. Antioxidant activity of various parts of *Capparis spinosa* was studied by many researchers from different countries around the world [2, 9, 10, 13, 15-18].

Caper is a plant with medicinal and aromatic properties. It is a long-lasting shrubby plant of the Mediterranean Basin that belongs to the *Capparaceae* family (North Africa, Europe, West Asia, France, Italy, Spain, Malta, Turkey, Greece, Algeria, Libya, Tunisia and Egypt [19, 20]. Moubasher *et al.* [21] mentioned that three varieties of *Capparis spinosa*. var. are grows in Egypt as independent species. Capers occur in various types (more than 350) and grow naturally in all the continents in many different regions of the world. Young shoots, flower buds and fruit are used for human nutrition. Capers have very important roles in the food industry; the flower buds are stored in brine and have become a costly product during recent years. Capers have been an important economic plant in Spain and Italy for the last 3 decades. There is limited information on physical and chemical properties of the seeds of caper plants used as food and as a condiment [2, 22].

Caper seed oil (CSO) contains high levels of tocopherols, which contribute to oil stability and oxidation resistance. Reactive oxygen species such as superoxide and hydroxyl radicals occur in the respiratory chain in the human body. Hydroxyl radicals are the most reactive and are responsible for free radical damage in the body. Living organisms do not have a specific molecule or enzyme to scavenge hydroxyl radicals. However, hydroxyl radicals can be scavenged by dietary antioxidants. Therefore, measurement of the hydroxyl radical-scavenging activity of antioxidants is important. Phenolic compounds are secondary metabolites found widely in plants. Phenolic compounds prevent oxidation through their antioxidant properties, stop the reactions caused by free radicals and hinder the development of many diseases. Sterols are an important class of organic molecules [23].

Synthetic antioxidants, although it's effectively used in the food industry and have been reported to have some side effects. Therefore, there is a need to replace synthetic antioxidants by plant-based safer natural antioxidants. The antioxidants present in the medicinal plants capture and neutralize reactive oxygen species and free radicals and thus protect the body from cancer, cardiovascular and degenerative disorders [24]. The addition of synthetic antioxidants for improving oxidative stability of edible oils is discouraged because of their toxicity and carcinogenicity [25]. Recently, there has been increased interest in identifying potential sources in order to obtain natural antioxidants [26, 27].

It is very important to know the nutritional and bioactive characteristics of products that can be consumed as such or used for food products or for food supplement production. The main purpose of this research was to use the total content of nutritional and bioactive compounds from caper seed oil (CSO) as vegetal product. The aim of the present study was carried out to investigate the effect of adding Caper seed oil (CSO) as natural antioxidants at different levels (200, 400 and 600 ppm) to sunflower and soybean oils compared to TBHQ (200 ppm) on chemical, physical characteristics of oils during frying period of potato finger at 180±5 °C.

MATERIALS AND METHODS

Materials

Oil Samples: Refined, bleached and deodorized (RBD) sunflower and soybean oils were obtained from Arma Food Industries 10th of Ramadan City, Egypt. The freshly refined oil samples packed in dark brown glass bottles were stored under frozen storage conditions (at -18°C) till further analysis and its using.

Caper Seed Oil: Fresh caper fruits (*Capparis spinose* L) were obtained from the Saint Catherine, South Sinai Governorate, Egypt.

All chemicals and reagents used in the analytical methods (Analytical grade) were obtained from El-Gamhouria Trading Chemicals and Drugs Co., Egypt.

Methods

Preparation of Coper Seed Oil: The fruits were divided, then seeds discarded and dried at 45 °C in vacuum air oven. The dried seeds were grounded in a mortar and about 5 g of the seeds doughs extracted with petroleum ether (40-60°C) in a Soxhlet apparatus for 8 hrs. The solvent was concentrated using a rotary evaporator, under reduced pressure at 45°C. The oil obtained was stored at -18°C until use, according to ISO [28].

The Addition of Caper Seed Oil to Frying Oils: Caper seed oil (CSO) was added separately to sunflower and soybean oils as antioxidants with the following ratios: (200, 400 and 600 ppm) as well as 200 ppm tertiary butyl hydroquinone (TBHQ) as control sample [29].

Potatoes Sample Preparation: Potato tubers (*Solanum tuberosum*) were peeled and were cut using knives to identical fingers in shape and size. The potato fingers were rinsed with distilled water to eliminate starch material on the surface and dried with paper towels before each experiment, then it was immediately ýfried in oil.

Frying Process: Deep-frying process was carried out according to the methods of Alizadeh *et al.* [30] using a deep-fat fryer (SASHO Deep Fryer- SH 308). Two kg of each oil sample was placed into the fryer and heated to 180±5°C. Six batches of potato fingers, (300 g per batch), were consecutively introduced into hot oil and fried for 6 min, total frying time was 180 min. for the treatment. 100 g samples of frying oils were taken after each 30 min and cooled to room temperature and frozen at -18 °C for further analyses.

Physical and Chemical Properties of Oil Samples Used: Refractive index of oil samples was measured using Carl Zeiss Refractometer at 25 °C, Free fatty acid (FFA%) (as % Oleic acid); Peroxide value (meq. active O_2/kg) and Iodine value (g I_2/100g) were determined according to AOAC [31]. The insoluble polymers % were determined according to the method described by Wu and Nawer [32], while TBA (mg malonaldehyde/kg oil) was determined as described by Sidwell *et al.* [33].

Fatty Acids Profile: Fatty acid composition of oil samples was determined using gas liquid chromatography technique. Methylation process was carried out using BF3 in methanol (20%) [30]. The methyl esters of the authentic fatty acids were analyzed with a GCV Hewlett Packard gas chromatography model 5890 equipped with dual flame ionization detector and dual channel recorder. The fractionation of fatty acid methyl esters was conducted using a coiled glass column (1.5m x 4mm) packed with diameter C (100 –120 mesh) and coated with 10% polyethylene glycol adipate (PEGA). The column oven temperature was programmed at 8°C/min from 70°C to 190°C, then isothermally at 190°C for 25 min with nitrogen at 30 ml/min. Detector, injector temperatures and hydrogen, air flow rates were generally 300°C, 280°C and 33ml, 330 ml / min; respectively. Fatty acid methyl esters were identified by comparing their relative and absolute retention times to those of the authentic pure standards of fatty acid methyl esters. All of the quantification was done by a built-in data-handling program. The peak areas were measured by triangulation and the relative percentage of each individual fatty acid was estimated as the ratio of its % partial area to the total area.

Trans Fatty Acid: Trans fatty acid in the oils were detected using a Schimadzu FTIR 8101 PC Infrared spectrophotometer according to Nicolova [34].

Determination of Oxidative Stability: The oxidative stability (induction period, IP of tasted oil samples was evaluated by the Rancimat method (Mod 679 Metrohm Ud. CH-9100, Herisau, Switzerland) [31]. The assays were carried out using 5 g of oil sample at 110±0.5 °C with an air flow of 20 L/h. Oil stability was expressed in terms of IP in hrs according to the method described by Tsaknis *et al.* [35].

Determination of Natural Antioxidants:

- Total tocopherols as vitamin E were determined according to the Ferric Chloride-Dipyridyl method of Philip *et al.* [36].
- The total carotenoid content was determined by spectrophotometry according to the method described by Rodriguez-Amaya [37].
- Total phenolic content was conducted according to the Folin–Ciocalteu colorimetric method Singleton *et al.* [38]. All values were expressed as mean (mg of Gallic Acid Equivalents/ 100g of dry weight)
- Phenolic compounds were determined by HPLC according to Goupy *et al.* [39]. HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was an Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A)

and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8 ml/min for a total run time of 60 min and the gradient program was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 µl and peaks were monitored simultaneously at 280 nm, 320 nm and 360 nm for the benzoic acid and cinnamic acid derivatives, respectively.

- The total flavonoid content was measured with aluminum chloride colorimetric assay according to Bahorun *et al.* [40]. The total flavonoid content was expressed as mg of catechin equivalents per 100g dry weight.
- Total glucosinolates content was analyzed as allyl isothyocianate (mg/100g dry weight) according to the method described by Mukhopadhyay and Bhattacharyya [41].

Determination of Antioxidant Activity: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to determine the antioxidant activity of the CSO which contain natural compounds. The free radical scavenging activity of the CSO was measured using the method described by Kalantzakis *et al*. [42].

Statistical Analysis: The obtained data were statistically analyzed by one-way analysis of variance using SPSS 16.0 for windows performed on all experimental datasets. Post-hoc multiple comparisons were carried out by Duncan analysis to determine significant differences between sample means at the 5% level as described by SPSS [43].

RESULTS AND DISCUSSION

Fatty Acid Composition of Caper Seeds, Sunflower and Soybean Oils: The present work was undertaken to analyze the fatty acid composition of the caper seed oil (CSO). The seeds of caper *(Capparis spinosa*) may serve as a new potential source of oil. Contents of seed oil and the fatty acid composition were measured in *Capparis spinosa* populations from the Saint Catherine, South Sinai Governorate, Egypt. The total oil content of Egyptian *Capparis spinosa* seeds was 30.47% on a dry weight basis. These results are in agreement with those reported by Matthäus and Özcan [6], who reported that oil content ranged between 27.3 to 37.6%. These results encourage the use of this plant species as a new source of vegetable oil for both food and industrial applications. Table (1) shows a typical fatty acid profile of CSO oil extracted from caper seeds. Total unsaturated fatty acids represented (79.29%), the main fatty acid was oleic acid (45.02%), followed by linoleic acid (28.13%), palmitic acid (15.23%), palmitoleic acid (5.05%) and stearic acid (4.22%). These results are in agreement with those of Matthaus and Özcan [6] Tlili, *et al.* [8], Badr and El-Waseif [44] and Zhang and Ma [2]. Also, the fatty acid composition of fresh sunflower and soybean oils is shown in Table (1). Oleic acid and linoleic acid were presented as major constituents of sunflower and soybean oils. Iodine value of sunflower, soybean oils and the CSO were 124.62, 130.87 and 99.38 I_2/100g, respectively, besides trans fatty acids not detected in all tested oil.

Table 1: Fatty acid profile (%) of sunflower, soybean oils and caper seed oil

	Oil samples		
Fatty acids (%)	Sunflower oil	Soybean oil	CSO
Luaric acid $C_{12:0}$	0.10	ND	0.30
Myristic acid $C_{14:0}$	0.34	0.08	0.52
Palmitic acid $C_{16:0}$	7.46	11.27	15.23
Palmitoleic acid $_{16:1}$	0.33	0.30	5.05
Stearic acid $C_{18:0}$	4.98	4.81	4.22
Oleic acid $C_{18:1}$	29.24	22.27	45.02
Linoleic acid $C_{18:2}$	56.80	53.9	28.13
Linolenic acid $C_{18:3}$	0.30	6.91	1.09
Arachidic acid C $_{20:0}$	0.45	0.46	0.44
Total Saturated Fatty Acids	13.33	16.62	20.71
Total Unsaturated Fatty Acids	86.67	83.38	79.29
Trans fatty acid	ND	ND	ND
Iodine Value (g I_2/100g)	124.62	130.87	99.38

ND: Not Detected.

Natural Antioxidant Contents of CSO: Antioxidants are indispensable for the food preservation from oxidative deterioration, as well as for the protection of unsaturated lipids in animal and human tissues. Therefore, the usage of essential oil in foods, which may act as natural antioxidant preservatives, may prolong the shelf life of relevant food products, as well as an influence on the health of consumers. Essential oils from caper represent an alternative for synthetic antioxidants and potential of functional food [45]. The concentration of the natural antioxidant contents of CSO including total tocopherols, total carotenoids, total phenolic compounds (phenolic acids and flavonoids), glucosinolates and DPPH % assay was used to determine the antioxidant activity of the CSO were recorded and listed in Table (2).

From the obtained data (Table 2), showed that the total phenolic compounds content (phenolic acids and flavonoids) were highest concentration of the natural antioxidant contents of CSO (858.54 mg/100g on a dry weight basis, followed by total tocopherol content

Table 2: Natural antioxidant content (mg/100g) and DPPH activity % of caper seed oil (on dry weight basis)

Caper seeds oil (CSO)	
Natural Antioxidant	mg/100g
Total Tocopherols (as vitamin E)	758.12±2.59
Total Carotenoids	675.8±2.31
Total Phenolic Compounds	858.54±2.03
- Phenolic acids	674.03±1.47
- Flavonoids	184.31±1.76
Total Glucosinolates	506.77±3.19
DPPH activity assay %	152.40±0.76

M±SE: Means±standard error for chemical composition

(758.12 mg/100 g), total carotenoids (675.8 mg/100 g) and total glucosinolates (506.77 mg/100 g). From the same Table (2), it could be observed that the DPPH activity test percentage of CSO was recorded (152.40 %). The results were relatively comparable with the data given by Özcan and Aydin [46], Tlili *et al*. [8, 9], Al-Snafi [12], Badr and El-Waseif [44] and Zhang and Ma [2].

Generally, it can be announced that the addition of CSO to food products such as frying oils led to increasing amounts of natural antioxidants, so this addition causes the prolongation of its shelf-life as well as the maintenance or enhancement its original quality properties of foods containing the CSO, beside healthy beneficial functions to food consumption.

Identification of Phenolic Compounds of CSO by HPLC: The phenolic compounds are divided into different classes based on their chemical structures. Phenolic acids include hydroxybenzoic acids (gallic acid, syringic acid) and hydroxycinnamic acids (caffeic acid, cinnamic acid, chlorogenic acid, ferulic acid). Flavonoids include flavonols (quercetin, rutin), flavanones, flavanols (catechin), flavonones, anthocyanins and isoflavones. Plants rich in these active principles have antioxidant effects and are widely used nowadays for their health beneficial properties [47].

Identification of the phenolic compounds (mg/100g) of CSO by using HPLC analysis was listed in Table (3). From the obtained data in Table (3), it can be noticed that Caper seed oil (CSO) include two parts of phenolic compounds: (A) Phenolic acids included: gallic, ellagic, pyrogallol, caffeic, vanillic, sinapic, p-oh-benzoic, p-coumaric, syringic, iso-ferulic, 3,4,5-methoxy-cinnamic, ferulic, benzoic and salycilic. (B) Flavonoids which included: rutin, kaempferol, quercetin, catechein and epicatachin. From the same data in Table (3), showed that the highest content of percentage total phenolic acids it was recorded to gallic acid (14.62 %), pyrogallol (13.56%) and p-coumaric (11.53%), while the other phenolic

Table 3: Phenolic compounds (mg/100g) of caper seed oil

Phenolic compounds	Concentration level (mg/100g) on Dry basis	Phenolic compounds (%)
----------A-Phenolic acids----------		
Gallic acid	98.47	14.62
Ellagic	47.65	7.07
Pyrogallol	91.38	13.56
Caffeic	50.04	7.43
Vanillic	31.34	4.65
Sinapic acid	36.61	5.43
P-OH-benzoic	24.30	3.60
P-coumaric	77. 76	11.53
syringic acid	32.49	4.82
Iso-ferulic	27.71	4.12
3,4,5-methoxy-cinnamic	52.03	7.72
Ferulic	45.85	6.81
Benzoic	56.16	8.33
Salycilic	2.06	0.31
Total Phenolic acids	673.85	100%
----------B-Flavonoids----------		
Rutin	99.80	54.23
kaempferol	17.78	9.66
Quercetin	15.10	8.20
Catechein	30.84	16.76
Epicatachin	20.52	11.15
Total Flavonoids	184.04	100%
Total Phenolic compounds		857.89

compounds content in Caper seed oil were recorded amount ranged between (3.60% - 7.72%). On the other hand, the highest content of total flavonoids was rutin (54.23%), while the other flavonoids (catechein, epicatachin, kaempferol and quercetin it was recorded (16.76%, 11.15%, 9.66% and 8.20%, respectively). The mentioned data are in accordance with those given by Lafka *et al.* [48], Jayaprakasha *et al.* [49], Tlili *et al.* [7,8], Al-Snafi [12] and Zhang and Ma [2].

For instance, large amounts of phenolic compounds which are responsible for their antioxidant actions, which have many favorable effects on human health, such as inhibiting low-density protein oxidation, decreasing the heart disease risks and possessing anticarcinogenic properties [48, 49]. They also have been proven to be food lipid antioxidants [50]. The mechanism of antioxidative activity of these compounds consists in their capability of radical scavenging, metal chelating and synergism with other antioxidants [51, 52].

Oxidative Stability of Oil Samples Treated with CSO Compared to Samples Treated with TBHQ: The induction period (IP) in hrs was taken as an index for the oxidative stability of the oil samples investigated. Different concentration of the CSO was added to sunflower and soybean oils at levels of 200, 400 and 600 ppm, while

Table 4: Oxidative stability of sunflower and soybean oil samples treated with different levels of CSO compared to oil samples treated with TBHQ

	Sunflower oil				Soybean oil			
Oxidative stability	TBHQ	CSO200	CSO400	CSO600	TBHQ	CSO200	CSO400	CSO600
IP (hrs)	6.67[b]	5.13[a]	7.21[c]	9.89[d]	5.85[b]	4.93[a]	6.30[c]	8.11[d]
IP (months) (Validity period)	8.13[b]	6.26[a]	8.79[c]	12.06[d]	7.13[b]	6.01[a]	7.68[c]	9.89[d]

*TBHQ: 200 ppm tertiary butyl hydroquinone; CSO200: 200 ppm; CSO400: 400 ppm and CSO600: 600 ppm.

**In the same row values with different superscript are significantly different (P<0.05).

Table 5: Changes in refractive index of oil samples during frying periods

	Sunflower oil				Soybean oil			
Time of frying (min.)	TBHQ	CSO200	CSO400	CSO600	TBHQ	CSO200	CSO400	CSO600
Zero time	1.4746[a]	1.4746[a]	1.4746[a]	1.4746[a]	1.4735[a]	1.4735[a]	1.4735[a]	1.4735[a]
After 30	1.4751[b]	1.4754[b]	1.4749[a]	1.4748[a]	1.4748[b]	1.4755[c]	1.4747[b]	1.4744[a]
After 60	1.4758[b]	1.4760[b]	1.4755[a]	1.4754[a]	1.4765[c]	1.4769[b]	1.4766[b]	1.4759[a]
After 90	1.4766[b]	1.4768[b]	1.4763[a]	1.4760[a]	1.4787[b]	1.4792[c]	1.4785[b]	1.4777[a]
After 120	1.4770[c]	1.4775[b]	1.4771[a]	1.4768[a]	1.4796[b]	1.4830[c]	1.4798[b]	1.4788[a]
After 150	1.4781[b]	1.4784[b]	1.4778[a]	1.4775[a]	1.4819[c]	1.4865[d]	1.4821[b]	1.4796[a]
After 180	1.4789[b]	1.4801[c]	1.4787[a]	1.4784[a]	1.4868[c]	1.4887[d]	1.4852[b]	1.4834[a]

**TBHQ: 200 ppm tertiary butyl hydroquinone; CSO200: 200 ppm; CSO400: 400 ppm and CSO600: 600 ppm.*

***In the same row values with different superscript are significantly different (P<0.05).*

TBHQ at 200 ppm were added. The IP for oil samples were tested using Rancimate at 110°C and the results presented in Table (4). Data revealed that, the use CSO as natural antioxidant at levels 400 and 600 ppm caused an increase in IP of sunflower and soybean oils compared to TBHQ.

The results in Table (4) show that the IP of sunflower and soybean oil samples contain 600 ppm of CSO (CSO600) exhibited (9.89 and 8.11 hrs, respectively), followed by the samples containing 400 ppm of CSO (CSO400) (7.21 and 6.30 hrs, respectively) and 200 ppm TBHQ (6.67 and 5.85 hrs, respectively), while the oil samples contain 200 ppm of CSO (CSO200) was exhibited the lowest values of IP (5.13 and 4.93 hrs, respectively).

From the same Table (4), it can be observed that addition of CSO to oils at different levels led to increase its stability against oxidation and the extent of this phenomenon was basically depending on the high natural antioxidant activity in CSO. The phenolic compounds and tocopherols are important for the oxidative stability of oils and the higher concentrations of it in CSO led to longer induction periods of lipid peroxidation [53, 54].

Effect of Deep Frying at (180±5 °C) on the Refractive Index for Oil Samples Investigation: The refractive index (RI) of the edible oils and fats is an important quality assurance characteristic because it is useful for identification, purity, processing purposes and following the reactions which occurred in lipids and fatty acids such as isomerization, hydrolysis, polymerization and oxidation throughout thermal processing. RI is affected by oil saturation degree, free fatty acid content and its oxidative state [55, 56].

Table (5) demonstrates the RI of testing oil samples during frying process at 180±5°C. Table (5) shows that there is gradual increase in RI of all investigated frying oil samples used in frying potato process with increasing frying time. RI was influenced by frying time, with significant increases in the values during the frying process [57]. Meanwhile, Table (5) shows that the addition of the CSO as antioxidants with levels 400 and 600 ppm to sunflower and soybean oils caused a reduction in the RI of frying oil during frying periods when compared to an oil sample containing TBHQ. The highest inhibitory effect on oxidation was observed for the oil samples treated by CSO600 which exhibit highest ability to retard oil degradation during the frying process, followed by oil samples treated by CSO400 and TBHQ. However, the increment of RI might be due to formation new compounds with a high molecular weight during frying periods, but addition the CSO (400 and 600 ppm) protect the oil from degradation, since it has a higher content of antioxidants, which reduce oxidation and polymerization [30]. These results indicate that the RI increased as oil degradation, increased, but the rate of degradation clearly depends on content of antioxidants and the nature of food fried in the oil [58].

Table 6: Changes in Free fatty acids (%) of oil samples during frying periods

	Sunflower oil				Soybean oil			
Time of frying (min.)	TBHQ	CSO200	CSO400	CSO600	TBHQ	CSO200	CSO400	CSO600
Zero time	0.06[a]	0.06[a]	0.06[a]	0.06[a]	0.09[a]	0.09[a]	0.09[a]	0.09[a]
After 30	0.09[b]	0.10[c]	0.10[c]	0.08[a]	0.15[c]	0.17[d]	0.14[b]	0.12[a]
After 60	0.16[b]	0.17[c]	0.15[b]	0.11[a]	0.19[c]	0.21[d]	0.18[b]	0.17[a]
After 90	0.23[d]	0.24[c]	0.22[b]	0.16[a]	0.26[c]	0.28[d]	0.24[b]	0.23[a]
After 120	0.27[b]	0.29[c]	0.27[b]	0.22[a]	0.33[c]	0.35[d]	0.32[b]	0.29[a]
After 150	0.31[b]	0.34[c]	0.30[b]	0.27[a]	0.38[c]	0.41[d]	0.36[b]	0.34[a]
After 180	0.35[c]	0.39[d]	0.34[b]	0.33[a]	0.44[c]	0.46[d]	0.42[b]	0.38[a]

**TBHQ: 200 ppm tertiary butyl hydroquinone; CSO200: 200 ppm; CSO400: 400 ppm and CSO600: 600 ppm.*

***In the same row values with different superscript are significantly different (P<0.05).*

Table 7: Changes in Peroxide value (meq O_2/kg oil) of oil samples during frying periods

	Sunflower oil				Soybean oil			
Time of frying (min.)	TBHQ	CSO200	CSO400	CSO600	TBHQ	CSO200	CSO400	CSO600
Zero time	0.17[a]	0.17[a]	0.17[a]	0.17[a]	0.28[a]	0.28[a]	0.28[a]	0.28[a]
After 30	2.51[b]	3.60[d]	2.78[c]	1.86[a]	3.01[c]	3.56[d]	2.95[b]	2.63[a]
After 60	4.83[c]	5.22[d]	4.65[b]	3.79[a]	6.16[b]	7.31[d]	6.29[c]	5.71[a]
After 90	7.05[c]	8.10[d]	6.77[b]	6.08[a]	9.23[c]	11.64[d]	8.80[b]	8.58[a]
After 120	9.45[b]	11.56[d]	9.93[c]	8.15[a]	12.41[d]	9.15[a]	11.53[c]	10.30[b]
After 150	11.64[c]	9.91[a]	12.08[d]	10.71[b]	8.26[b]	7.66[a]	10.17[c]	12.42[d]
After 180	10.13[b]	7.28[a]	11.73[c]	12.64[d]	5.72[a]	6.79[b]	7.26[c]	9.75[d]

**TBHQ: 200 ppm tertiary butyl hydroquinone; CSO200: 200 ppm; CSO400: 400 ppm and CSO600: 600 ppm*

***In the same row values with different superscript are significantly different (P<0.05).*

Effect of Deep Frying on the Free Fatty Acids of Oil Samples Investigation: Free fatty acids (FFA %) are used to asses frying oil degradation and is related to fried food quality [59]. The changes in the FFA (%) of oils during frying process at 180±5°C for 180 min. are given in Table (6). The initial FFA values of fresh sunflower and soybean oils under this investigation were seen in 0.06 and 0.09%, respectively. The addition of CSO with 600 and 400 ppm for sunflower and soybean oils led to decrease in FFA (%) during frying periods and the end of frying periods after 180 min., which recorded was (0.33 and 34%) and (0.38 and 0.42%), respectively, compared to the other oil samples treated with TBHQ and CSO200 which recorded was (0.35 and 39%) and (0.44 and 0.46%) at the end of the frying process. The FFA (%) increased with increase the time of the frying process. The highest change in the FFA (%) was shown for soybean oil, whereas the lowest changes in FFA (%) were observed for sunflower oil samples at the end of frying period. These increments are attributed to the high content of unsaturated fatty acids in oils investigated, especially soybean oil, which containing linolenic acid (C18:3) 6.91% (Table 1) [60, 61].

Generally, the addition of natural or synthetic antioxidants to sunflower and soybean oils caused a reduction in FFA (%) during frying periods [62]. FFA showed a little changed during frying process for all oil samples and it does not reach to the limits of 2.5% (in the limits of discarded frying the oil). A significant level of FFA may be present in unrefined oils before use and increase acidity due to hydrolysis and to some extent of formation of acidic products. However, poor correlation of FFA with total polar materials and polymer has been reported for frying oil [63].

Effect of Deep Frying on the Peroxide Values of Oil Samples Investigation: Peroxide value (PV) is one of the most widely used methods of monitoring the initial stage of lipid oxidation and reflects the concentration of peroxides and hydroperoxides. The PVs for the fresh oil were very low which indicate the high quality of the oils Girgis *et al.,* (2015). Frying process at 180±5°C for 180 min. was used in this work. Table (7) illustrates that all treatments showed a gradually increment in PV as frying time increased, then began to decrease, which in agreement with the findings of PV reduction can be explained by the decomposition of unstable hydroperoxides to secondary oxidation products such as: hydrocarbons, alcohols, ketones and aldehydes [62].

PV of sunflower oil sample contain TBHQ used in frying potato reached to 11.64 meq O_2/kg oil after 150 min of the initial of frying process, while the oil samples

Table 8: Changes in TBA (mg malonaldehyde/kg oil) of oil samples during frying periods

	Sunflower oil				Soybean oil			
Time of frying (min.)	TBHQ	CSO200	CSO400	CSO600	TBHQ	CSO200	CSO400	CSO600
Zero time	0.096[a]	0.096[a]	0.096[a]	0.096[a]	0.121[a]	0.121[a]	0.121[a]	0.121[a]
After 30	0.114[b]	0.526[d]	0.224[c]	0.110[a]	0.633[c]	0.760[d]	0.557[b]	0.464[a]
After 60	0.763[b]	1.400[d]	0.879[c]	0.657[a]	1.307[c]	1.584[d]	0.948[b]	0.782[a]
After 90	1.501[b]	1.772[d]	1.684[c]	1.315[a]	1.811[c]	2.115[d]	1.672[b]	1.334[a]
After 120	1.824[b]	1.954[d]	1.891[c]	1.726[a]	2.500[c]	3.074[d]	2.182[b]	1.865[a]
After 150	2.165[b]	2.625[d]	2.270[c]	1.913[a]	3.274[c]	4.005[d]	2.811[b]	2.342[a]
After 180	2.436[c]	3.121[d]	2.420[b]	2.224[a]	4.018[c]	4.876[d]	3.967[b]	3.256[a]

**TBHQ: 200 ppm tertiary butyl hydroquinone; CSO200: 200 ppm; CSO400: 400 ppm and CSO600: 600 ppm*

***In the same row values with different superscript are significantly different (P<0.05).*

CSO400 and CSO600 reached at 12.08 and 12.64 meq O_2/kg oil after 150 and 180 min. respectively, which is lower than the PV limit of the Egyptian Standard Specifications [64, 65] and reflect the high oxidative stability of CSO. In this concern PV of soybean oil samples CSO400 and CSO600 reached at 11.53 and 12.42 meq O_2/kg oil after 120 and 150 min., respectively, of initial frying process, while the oil sample contain TBHQ reached to 12.41 meq O_2/kg oil after 120 min of the initial of the frying process.

These decrements in PV may be attributed to the high content of natural antioxidants to CSO which was added in these samples (as shown Table 2 and 3). The PV is a useful measure of fresh oil quality. The heating during of frying processes, the PV increases, but the PV is rapidly breakdown at high temperature, so PV is not relying in correlation during the oil deterioration [66]. The anti-oxidative effect may have contributed to the oxidative stability of oils with the addition of natural antioxidants. When added into the oils, antioxidants prevent the lipid peroxides formed during frying and delayed oxidation. This could be due to that antioxidant components decrease the permeation rate the oxygen into unsaturated fatty acids in the oils (as shown Table 4)

Generally, the PV increased with increase the time of frying process. The highest change in the PV at the end of frying period was shown for soybean oil [60, 61]. These results showed that the addition of CSO to sunflower and soybean oils used as frying media of potato significantly retarded the formation of hydroperoxides and kept peroxide value under the Egyptian standard specifications limit after 180 min. of frying process as reported in Codex Stand. [67] and ESS [64,65]. These results are in agreement with those obtained by Jaswir *et al.* [68], Chen *et al*. [61] and Alizadeh *et al.* [30].

Effect of Deep Frying on the Thiobarbituric acid (TBA) of Oil Samples Investigation: Thiobarbituric acid (TBA) test is a condensation reaction between TBA and malonaldehyde, the most predominate product of the secondary oxidation of oil and measure aldehyde contents in oil, principally 2, 4-dienals and 2-alkenals, produced from decomposition of hydroperoxides, which are formed during food lipids oxidation. Therefore, the TBA value considered a good chemical quality criterion to identify the oxidative state of edible oils and fats, since it reflects the extent of occurred oxidation [56]. From Table (8), observed that the changes occurred in the TBA value of the oil samples treated by TBHQ and the oil samples treated by CSO during frying periods at 180±5°C for 180 min. Data indicated that the TBA values of all samples increased with increasing the frying time.

TBA values of sunflower and soybean oils samples contain 600 ppm of CSO (CSO600) used in frying potato exhibited the lowest TBA value (2.224 and 3.256 mg malonaldehyde/kg oil, respectively), followed by the samples containing 400 ppm of CSO (CSO400) (2.420 and 3.967 mg malonaldehyde/kg oil, respectively) and 200 ppm TBHQ (2.436 and 4.018 mg malonaldehyde/kg oil, respectively), while the oil samples contain 200 ppm of CSO (CSO200) was exhibited the highest increment in the TBA values (3.121 and 4.876 mg malonaldehyde/kg oil, respectively) at the end of frying periods.

The TBA values of oil samples treated by CSO (natural antioxidant) at levels of 400 and 600 ppm were kept lower than that of the samples treated by TBHQ which exhibited higher TBA values during frying periods. This means that the CSO at the highest level (400 and 600 ppm) protect the oil of oxidation and produce the lowest level of TBA reacting substance. This observation could be due to the presence of a high concentration of natural antioxidants in CSO compared to the oil sample treated TBHQ.

From Tables (7) and (8) it could be noticed that CSO has a clear inhibitory effect on the propagation stage of oxidation in oils used in the deep frying process. So, CSO exhibited high inhibitory effects towards both primary and secondary oxidation changes, which is higher than that of TBHQ. This result agrees with that of Zhang *et al.* [69]

Table 9: Changes in polymer content (%) of oil samples during frying periods

	Sunflower oil				Soybean oil			
Time of frying (min.)	TBHQ	CSO200	CSO400	CSO600	TBHQ	CSO200	CSO400	CSO600
Zero time	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
After 30	0.55^{c}	0.74^{d}	0.41^{b}	0.38^{a}	0.76^{c}	0.86^{d}	0.62^{b}	0.51^{a}
After 60	0.81^{c}	1.13^{d}	0.63^{b}	0.57^{a}	1.11^{c}	1.44^{d}	0.98^{b}	0.82^{a}
After 90	1.56^{c}	1.95^{d}	1.45^{b}	1.39^{a}	1.72^{c}	2.06^{d}	1.65^{b}	1.35^{a}
After 120	2.05^{c}	2.64^{d}	1.87^{b}	1.70^{a}	2.43^{c}	2.77^{d}	2.20^{b}	1.89^{a}
After 150	2.70^{c}	3.16^{d}	2.46^{b}	2.25^{a}	3.13^{c}	3.61^{d}	3.06^{b}	2.56^{a}
After 180	3.29^{c}	4.02^{d}	3.11^{b}	2.87^{a}	4.50^{c}	5.05^{d}	4.39^{b}	3.87^{a}

**TBHQ: 200 ppm tertiary butyl hydroquinone; CSO200: 200 ppm; CSO400: 400 ppm and CSO600: 600 ppm*

***In the same row values with different superscript are significantly different (P<0.05).*

and Alizadeh *et al.* [30]. This fact might be contributed to the presence of donate hydrogen donating compounds in CSO (natural antioxidants, Table 2 and 3), which change peroxides to stable hydroperoxides resulting in a slower decomposition rate of hydroperoxides and formation of fewer secondary products [70].

Effect of Deep Frying on the Polymer Content % for Oil Samples Investigation: Polymerization of frying oil cause formation of compounds with high molecular weight and polarity, which can have formed from free radicals or triglycerides by the Diels-Alder reaction. Oxidized polymer compounds accelerate oxidation, further degradation, increase oil viscosity [71], reduce heat transfer, produce foam during deep fat frying, develop undesirable color in fried food and cause high oil absorption of foods [72]. Oil quality control regulations IUPAC [73] indicate that polymer levels must not exceed 1.5%.

The formation of polymeric material (polymer content %) one of the important changes in fat during frying period. The polymers are responsible for the increase of viscosity and refractive index in oils [59,74]. Oils generally have polymeric content during frying because of their high polyunsaturation [75].

Data presented in Table (9) demonstrates the changes in polymer content % sunflower and soybean oils during the frying process at 180±5°C. Frying process caused and gradual increase in the polymer compounds content with frying time. Results in Table (9) showed that polymer content (%) of sunflower and soybean oil samples contain 600 ppm of CSO (CSO600) used in frying potato exhibited the lowest polymer content (2.87 and 3.87 %, respectively) at the end of frying periods (180 min), followed by the samples containing 400 ppm of CSO (CSO400) (3.11 and 4.39%, respectively) and 200 ppm TBHQ (3.29 and 4.50%, respectively), while the oil samples contain 200 ppm of CSO (CSO200) was exhibited the highest increment in the polymer content (4.02 and 5.05%, respectively). From the same Table (9), noticed that the rate of polymer formation was faster in the oil samples with TBHQ than the oil samples with natural antioxidants (CSO). Data revealed that the CSO had the strongest effect in retarding formation of polymer content during frying followed by TBHQ. The results took the same trend of the other chemical quality properties which indicate that the CSO can act as a good antioxidant in protecting sunflower and soybean oils as reported by Lumley [76].

Generally, oil samples treated by CSO (natural antioxidant) during frying periods at a level of 400 and 600 ppm were kept lower than that of the samples treated by TBHQ which exhibited higher polymer content (%). These decrements are nearly due to the high content of antioxidants in CSO in these oil samples. This means that the CSO at the highest level (400 and 600 ppm) protect the oil of oxidation and produce the lowest level of polymer content reacting substance. These results are in agreement with Tabee *et al.* [77] and Farhoosh and Tavassoli-Kafrani [78] who reported that the formation of dimers and polymers depends on the oil type, frying temperature and number of frying. As the number of frying and the frying temperature increase, the amounts of polymers increased.

CONCLUSION

The natural antioxidants in food play an important role to avoid toxic effects of synthetic antioxidants. Thus, the addition of Caper seed oil (CSO) to oils under this study at different levels led to increase its stability against oxidation and the extent of this phenomenon was basically depending on the high concentration of the natural antioxidant contents and high natural antioxidant activity in CSO. Although, soybean and sunflower oils had the highest amounts of polyunsaturated fatty acids, the protective role of natural antioxidants present in CSO may protect these frying oils from drastic oxidation as

compared to frying with soybean and sunflower oils containing TBHQ (synthetic antioxidants). However, the frying oil (soybean oil) containing higher amount of linolenic acid was shown to be less stable to oil deterioration during deep frying. Moreover, the quality of soybean and sunflower oils used in this study increased by adding CSO to it. Finally, the CSO was exhibited superior ability in protecting the oil from oxidation during frying process which suggests that the CSO may be a good natural antioxidant alternative to TBHQ.

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