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Egyptian mango by-product 2: Antioxidant and antimicrobial activities of extract and oil from mango seed kernel

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Abstract

Egyptian mango seeds were collected as wastes from local fruit processing units, the kernels were separated and dried. The antioxidant and antimicrobial activities of mango seed kernel extract and oil were investigated. The results indicated that combination of both mango seed kernel extract and oil had optimum antioxidant potency higher than each one alone. Addition of 400 ppm methanol extract and 5% mango seed kernel oil increased the oxidative stability of sunflower oil incubated at ambient temperature as well as sunflower oil during frying. Moreover, both extract and oil improved the stability and quality characteristics of fresh and stored potato chips. On the other hand, mango seed kernel extract reduced total bacterial count, inhibited coliforms growth, showed remarkable antimicrobial activity against *Escherichia coli* strain and extended the shelf-life of pasteurized cow milk. These results provide useful information on the utilization of mango seed kernel as natural antioxidant and antimicrobial in foods.

Keywords: Mango seed kernel extract and oil; Sunflower oil; Potato chips; Cow milk; Antioxidant activity; Antimicrobial effect

1. Introduction

Lipid oxidation is one of major factors resulting in losses in fatty food quality by formation of products having negative effect on taste, aroma and nutritional value of the food, which are health hazard and associated with many types of biological damage in living tissues and increase risk cardiovascular disease (Addis & Park, 1989; Chow, 1992).

Antioxidants are major ingredients that protect the quality of oils and fats by retarding oxidation (Wanasundara, Amarowicz, & Shahidi, 1994). Synthetic antioxidants are used at legal limits to reduce deterioration, rancidity and oxidative discoloration (Dziezak, 1986). Butylated hydroxyl anisol (BHA) and butylated hydroxyl toluene (BHT) are quit volatile and decompose easily at high temperatures (Branen, 1975). There are some serious problems concerning the safety and toxicity of such synthetic antioxidants related to their metabolism and possible absorption and accumulation in body organ and tissues (Linderschmidt, Trylka, Goad, & Witschi, 1986; Tappel, 1995). Therefore, the search for preparation of useful natural antioxidants is highly desirable. Natural antioxidative compounds are found in numerous plant materials such as oilseeds, cereal crops, vegetables, fruits, leaves, barks and roots, spices and herbs (Ramarathnam, Osawa, Ochi, & Kawakishi, 1995). Many studies showed that natural antioxidants, as flavonoids and other phenolic phytochemicals, present in plants are associated with reduced chronic disease risk (Singh & Marimuthu, 2005; Cieslik, Greda, & Adamus, 2006). Moreover, the type of phenolic compounds has been demonstrated to inhibit lipid peroxidation of human low-density lipoprotein *in vitro* (Goncalves et al., 2004).

Various studies indicated that mango seed kernels contain different phenolic compounds and stable fat rich

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in saturated fatty acids so that it can be a good source of natural antioxidants (Parmar & Sharma, 1984; Puravankara, Bohgra, & Sharma, 2000; Nunez-Selles, 2005). Mango seed kernel extracts enhanced oxidative stability of fresh-type cheese and ghee and extended their shelf life (Parmar & Sharma, 1986; Parmar & Sharma, 1990; Puravankara et al., 2000; Dinesh, Boghra, & Sharma, 2000). This could be attributed to the phospholipids and phenolic compounds in mango seed extract that transferred to significant quantities to ghee, suggesting a synergistic action of the two types of compounds. Besides these two major classes of compounds, other factors such as tocopherols and carotenoids may also be involved in the effectiveness of mango seed kernel powder in extending the shelf-life of buffalo ghee. Youssef (1999) indicated that adding 1% of crude oil extracted from mango seed kernel exhibited antioxidant potency similar to that of 200 ppm of BHT against oxidation of sunflower oil. Zein (2000) recommended that phenolic compounds and mango seed kernel crude oil both are as good natural antioxidants due to retarding oxidation in different edible oils.

Recently, Soong, Barlow, and Perera (2004) indicated that mango seed kernel has potent antioxidant activity with relatively high phenolic contents. They referred that mango seed kernel was also shown to be a good source of phytosterols as campesterol, β-sitosterol, stigmasterol and also contain tocopherols. They suggested that mango seed kernel could be used as a potential source for functional food ingredients and, in addition, it could be further processed into therapeutic functional food products. Schiber, Berardini, and Carle (2003) and Nunez-Selles (2005) referred that the antioxidant effect of the mango seed kernel and bark was due the high content of polyphenols, sesquiterpenoids, phytosterols, and microelements like selenium, copper and zinc.

The use of natural antimicrobial compounds is important not only in the preservation of food but also safe for human consumption (Conner, 1993). Bacterial and fungal infections pose a greater threat to health, most notably in immune compromised subjects, hence the need to find natural, cheap and effective antimicrobial agents. The antimicrobial properties of mango seed kernel extract were investigated by Kabuki et al. (2000). The ethanol extract had a broad antimicrobial spectrum, and was more active against gram-positive than gram-negative bacteria with a few exceptions. The results indicated that the active component was a type of polyphenol. The antimicrobial activity of extract was stable against heat (121 °C, 15 min), freezing (−20 °C, 16 h) and pH treatment (pH 3–9) normally used in food processing. Therefore, the mango seed kernel extract could be used, together with other antimicrobial components, effective against gram-negative bacteria, such as organic acids. Although more studies are needed, the mango seed kernel extract and oil seem promising as a natural food additive for extending the shelf-life of a variety of foods products. Accordingly, the aim of the present work was to study the antioxidative effect of Egyptian mango seed kernel extract and oil on sunflower oil and potato chips as well as to estimate the antimicrobial effect of mango seed kernel extract on raw cow milk.

2. Materials and methods

2.1. Materials

Mango seeds as by-product (waste) were collected from local fruit processing units at Alexandria, Egypt. Imported refined sunflower oil containing 410 ppm natural α-tochopherol, and without addition of any synthetic antioxidant was obtained from Arma Food Industries, 10th of Ramadan, Egypt. Initial quality was checked by determining peroxide value (0.5 meq.O_2/kg oil). Fatty acid composition was 5.5% C_{16}, 4% C_{18}, 22.8% $C_{18:1}$ and 67.7% $C_{18:2}$.

Fresh potatoes were obtained from a local market at Alexandria, Egypt. The approximate composition of raw potatoes was: moisture 78%, protein (% fresh matter) 2.6%, and fat (% fresh matter) 0.2%. Raw cow milk was obtained from local market.

All chemicals used in this study were purchased from El-Gamhouria Co. for chemicals and medical requisites (Alexandria, Egypt) while *p*-anisidine and tertiary butylhydroxyquinone (TBHQ) from Sigma (St. Louis, MO, USA).The *Escherichia coli* strain was obtained from the culture collection of NIZO, Food Research, Ede, The Netherlands.

2.2. Methods

2.2.1. Preparation of mango seed kernel extract and oil

The seeds were washed and air dried and the kernels were removed manually from seeds. The kernels were chopped, and dried at 50 °C (Augustin & Ling, 1987). The dried material was ground in a hammer mill into a powdery form.

Absolute methanol was added to the mango seed kernel (MSK) powder at ratio of 2:1 (v/w) and kept overnight in a dark place with gentle shaking at 20 °C. After removing insoluble materials by filtration, the solvent was evaporated in a rotary evaporator at 30 °C under nitrogen and mango seed kernel methanol extract (MSKE) was used during experiments.

Mango seed kernel oil (MSKO) was extracted as described by Christie (1982). Total lipids from 500 g ground MSK were extracted by chloroform-methanol (2:1 by volume).

Both MSKE and MSKO were kept in a closed dark glass bottle and stored at 4 °C until utilization.

2.2.2. Antioxidant activity of MSKE or/and MSKO in sunflower oil

The antioxidant activity of MSKE, MSKO and in combination of both was tested. According to some preliminary experiments on the antioxidative effect of different levels of extract and/or oil, studies were carried out to determine the effect of MSKE (200, 400, and 800 ppm),

MSKO (1%, 3%, 5%) and their combination (200 ppm MSKE + 3% MSKO, 200 ppm MSKE + 5% MSKO, 400 ppm MSKE + 3% MSKO and 400 ppm MSKE + 5% MSKO) on the stability of sunflower oil during storage in the dark and under light exposure at ambient temperature. Samples with and without additives were transferred separately into screw-capped 300 ml glass bottles, covered externally with aluminum foil. The samples were incubated for 1 h at 60 °C in the dark to evaporate the solvent and subjected to accelerated oxidation in the dark as well as under light exposure at ambient temperature (20 ± 4 °C). The latter samples were surrounded with uniform light intensity (800 lux at the surface of the lids) generated by fluorescent lamps (Lumlux Interna L8w/41, Germany). Oxidative stability was evaluated by measuring peroxide value and anisidine value (Egan, Kirk, & Sawyer, 1981) in oil samples during storage period. Peroxide value was determined by titration with standard sodium thiosulphate and calculated as milliequivalent active oxygen per kilogram oil (meq.O_2/kg oil) according to AOCS (1989). Anisidine value was determined by colorimetric method using a Shimadzu 160 PC-UV spectrophotometer as described by Egan et al. (1981).

2.2.3. Antioxidant activity of combined MSKE and MSKO in potato chips

This study was carried out to determine the effect of combined MSKE and MSKO (400 ppm MSKE + 3% MSKO and 400 ppm MSKE + 5% MSKO) on the stability and sensory of potato chips before and after storage.

Eight kilograms of sunflower oil (with or without additives) were placed in a stainless-steel electric deep fat fryer with thermostatic control (Ontario, Canada) and the temperature of oil was raised to 180 °C. Potatoes were peeled and sliced into chips (2 mm thickness) with a mechanical slicer (Moulinex, France) and submerged in water. A batch of 300 g of potato chips was fried for 7 min. Eight batches were fried at 1 h intervals for 8 h/day. Following frying, chips were drained for 1 min with paper towels to remove excess oil, cooled at room temperature for 5 min. At the end of each day, the fryer was turned off and allowed to cool to room temperature in the dark. The frying processes for control and each treatment were conducted for 7 days. The volume of the oil was not replenished during the frying process. To monitor chips quality and stability during storage, two batches (300 g each) of mixed chips from the 1st, 4th, and 8th frying (in equal amount of 100 g) each day during continuous seven days were packed in double layer plastic and stored in the dark at ambient temperature (22 ± 2 °C) for three months. Oxidative stability was evaluated by measuring peroxide value and anisidine value in extracted oil from chips before and after three months storage. Moreover, iodine value and free fatty acids were determined (AOCS, 1989). At the same time TBHQ as a synthetic antioxidant (300 ppm) was added to sunflower oil, as a control sample. The oxidation effect of sunflower oil without additives was measured for reference purposes.

The sensory analysis of potato chips was carried out by ten trained panelists for colour, crispness, flavour, and overall acceptability as described by Warner (1989). Fresh or stored chips were presented at room temperature. Twenty quality attributes, including five for colour (1, dark; 2, brown; 3, pale; 4, bright yellow and 5, light yellow), five for crispness (1, not crisp; 2, weak; 3, slight; 4, moderate and 5, crisp), five for flavour (1, off; 2, rancid; 3, painty; 4, moderate and 5, fresh) and five for overall acceptability (1, not acceptable; 2, rather acceptable; 3, acceptable; 4, good and 5, excellent) were investigated.

2.2.4. Antimicrobial activity of mango seed kernel

2.2.4.1. Preparation of mango seed kernel extract (MSKE).

MSKE was prepared as described before and suspended in sterilized water. The neutralized and filter sterilized MSKE was used for microbiological experiments.

2.2.4.2. pH determination. Different concentrations of neutralized and filter sterilized MSKE, 3000, 4500 and 6000 ppm (pH 6.6) were added to raw cow milk (pH 6.6) and pH value of the milk was followed. The extract was added to 10 ml milk, the pH value was measured by using pH meter (Schott Gerale, CG 710) at room temperature (25 ± 2 °C) for 8 h. The electrode was immersed directly into samples.

2.2.4.3. Microbiological analysis of milk.

2.2.4.3.1. Total microbial count. Total microbial count of raw cow milk treated with MSKE (3000 ppm) was determined in comparison with control milk (untreated). The total microbial count was followed at room temperature (25 ± 2 °C) for 6 h. The conventional diluting pouring plate technique was followed for enumerating microbes in samples. Plate count agar medium (PCA, Oxoid, Hampshire, UK) was used (Oxoid Manual, 1982) and the plates were incubated for 48 h at 30 °C. All plates contain 30–300 colonies were counted and the average of two replicates from the same dilution was calculated directly by colony forming unit (CFU ml^{-1}).

2.2.4.3.2. Count of coliforms bacteria. Coliforms bacteria were enumerated in raw cow milk untreated and treated with MSKE (3000 ppm) at room temperature (25 ± 2 °C) for 6 h. The coliform count was followed in control and treated milk samples by measuring CFU ml^{-1} using violet red bile agar (Oxoid). The plates were incubated for 48 h at 37 °C.

2.2.4.3.3. Antimicrobial assay. Antimicrobial activity was determined in agar well-diffusion assay against target organism as described by Ayad, Verheul, Wouters, and Smit (2000). Plates were prepared by adding 2 ml from an overnight culture of Enterophathogenic *E. coli* obtained from the culture collection of NIZO Food Research, Ede, The Netherlands, as indicator to 200 ml of plate count agar medium (oxoid) held at 45 °C. Approximately 10^5 CFU ml^{-1} were added. The agar was then immediately dispensed

into round sterile 8.5 cm diameter Petri dishes and after solidification, wells (diameter 3 mm) were made by removing the agar by a sterile metal borer. Subsequently, 30 μl of the neutralized and filter sterilized of MSKE in different concentration (3000, 4500 and 6000 ppm) were dispensed in individual wells. The plates were incubated for 2 h at 4 °C and subsequently overnight at 37 °C after which the diameter of the inhibition zones was measured.

2.2.4.3.4. Effect of antimicrobial activity of MSKE on the keeping quality of pasteurized cow milk. The neutralized and filter sterilized MSKE (3000 ppm) was added to 50 ml of pasteurized milk (72 °C, 5 min). The treated and untreated milk (control) were stored at 4 °C and at room temperature (25 ± 2 °C) for 15 days. The antimicrobial activity of MSKE was tested by following the curdling time of milk. Curdling time was determined by observing beed formation on a microscope slide surface.

2.2.4.3.5. Sensory evaluation of pasteurized milk treated with different concentrations of MSKE. The MSKE was added in different concentrations, 3000, 4500 and 6000 ppm to 50 ml of pasteurized milk (72 °C for 5 min). Sensory evaluation of treated and untreated milk was carried out by 12 grades from the stuff members and assistant at Food Science Department, Faculty of Agriculture – Saba Basha, Alexandria University.

The following scale was used to evaluate samples: 1, bad; 2, sufficient; 3, good; 4, very good and the overall grade out of (100). The intensity of flavour attributes (taste and smell) and colour were scored on a scale from 1, slightly; 2, moderate; 3, strong; 4, very strong.

3. Results and discussion

3.1. Antioxidant activity of MSKE on sunflower oil

Antioxidative activity of MSKE was assayed during storage of sunflower oil at ambient temperature for 12 months in dark and for 12 weeks under light exposure. Addition of mango seed kernel extract at various concentrations of 200, 400 and 800 ppm did not affect the colour of appearance of sunflower oil. Measurements of peroxide value for monitoring primary oxidation products and anisidine value for evaluating secondary oxidation products were suitable potent method to characterize oxidative changes in the stored sunflower oil samples. The development of oxidative rancidity was estimated every month for stored samples in the dark and every week for stored samples under light exposure and compared with those of samples mixed with 300 ppm TBHQ and untreated sunflower oil samples as control (Fig. 1). It can be noticed that peroxide and anisidine values of sunflower oil without additives (control) was increased rapidly after 12 months in the dark and after 12 weeks under light exposure and slightly increased in samples with 300 ppm TBHQ. Data showed that increasing the levels of MSKE added to sunflower oil (200, 400 and 800 ppm) led to decreasing peroxide and anisidine values formed and retarding oxidatives rancidity in sunflower oil. 800 ppm of MSKE added to sunflower oil was the best natural antioxidant due to decreasing peroxide and anisidine values of oil, followed by 400 ppm of extracts. Accordingly the shelf life of sunflower oil, which is very rich in linoleic triglycerides and extremely susceptible to oxidative deterioration, was more than 12 months in the dark and up to 12 weeks under light exposure. These results were in agreement with those cited by Zein (2000) and Mohamed and Girgis (2005) who referred to the antioxidative effects of mango seed kernel extract. Mango seed kernel extracts were reported to contain phenolic compounds which can act as antioxidant (Parmar & Sharma, 1990; Puravankara et al., 2000). Abdalla, Darwish, Ayad, and Elhamahmy (2006) identified eight phenolic compounds from mango seed kernel extract. They found that tannin and vanillin were the most abundant compounds.

3.2. Antioxidant activity of MSKO on sunflower oil

Mango seed kernel oil MSKO was added at various concentrations (1%, 3% and 5%) to sunflower oil, and then samples were stored in the dark for 12 months and under light exposure for 12 weeks. The addition of oil (total lipids) extracted from mango seed kernels did not affected on the colour or the appearance of sunflower oil samples. Results in Fig. 2 showed that the oxidative stability of sunflower oil with 5% mango seed kernel oil similar to sunflower oil with 300 ppm TBHQ during storage for 12 months in the dark. Peroxide and anisidine values of sunflower oil samples with 5% MSKO were in the lowest levels during 12 months storage in the dark. During storage sunflower oil samples under light exposure, deterioration of samples were faster and both peroxide and anisidine values sharply increased. Youssef (1999) reported that addition of 1% of crude mango seed kernel oil to refined sunflower oil had antioxidant potency similar as 200 ppm BHT after 36 hours of incubation the oil at 90 °C. From data it can be concluded that MSKO can be used as a natural antioxidants due to their fatty acid pattern rich with saturated fatty acids and with monounsaturated oleic acid besides tocopherols and different sterol fractions. Gordon and Magos (1983) found that Δ-avenasterol and fucosterol were effective as antioxidants whilst other sterols including sitosterol and stigmasterol in olive oil were ineffective. Concerning the antioxidative effect of squalene, Gowind Rao and Achaya (1968), Sims, Fioriti, and Kanuk (1972), Malecka (1991) and Malecka (1994) found that squalene markedly retarted the degradation of unsaturated fatty acids in safflower and rapeseed oils and limited the extent of polymerization. Abdalla (1999) published that the addition of 1% of unsaponifiable matter (from olive oil deodorizer distillate) to sunflower oil showed the highest effect in retarding the oxidative deterioration of oil during frying of potato chips. This protective effect was attributed to high levels of squalene, Δ-avenosterol, and tocopherols as reported in previous study (Abdalla et al., 2006).

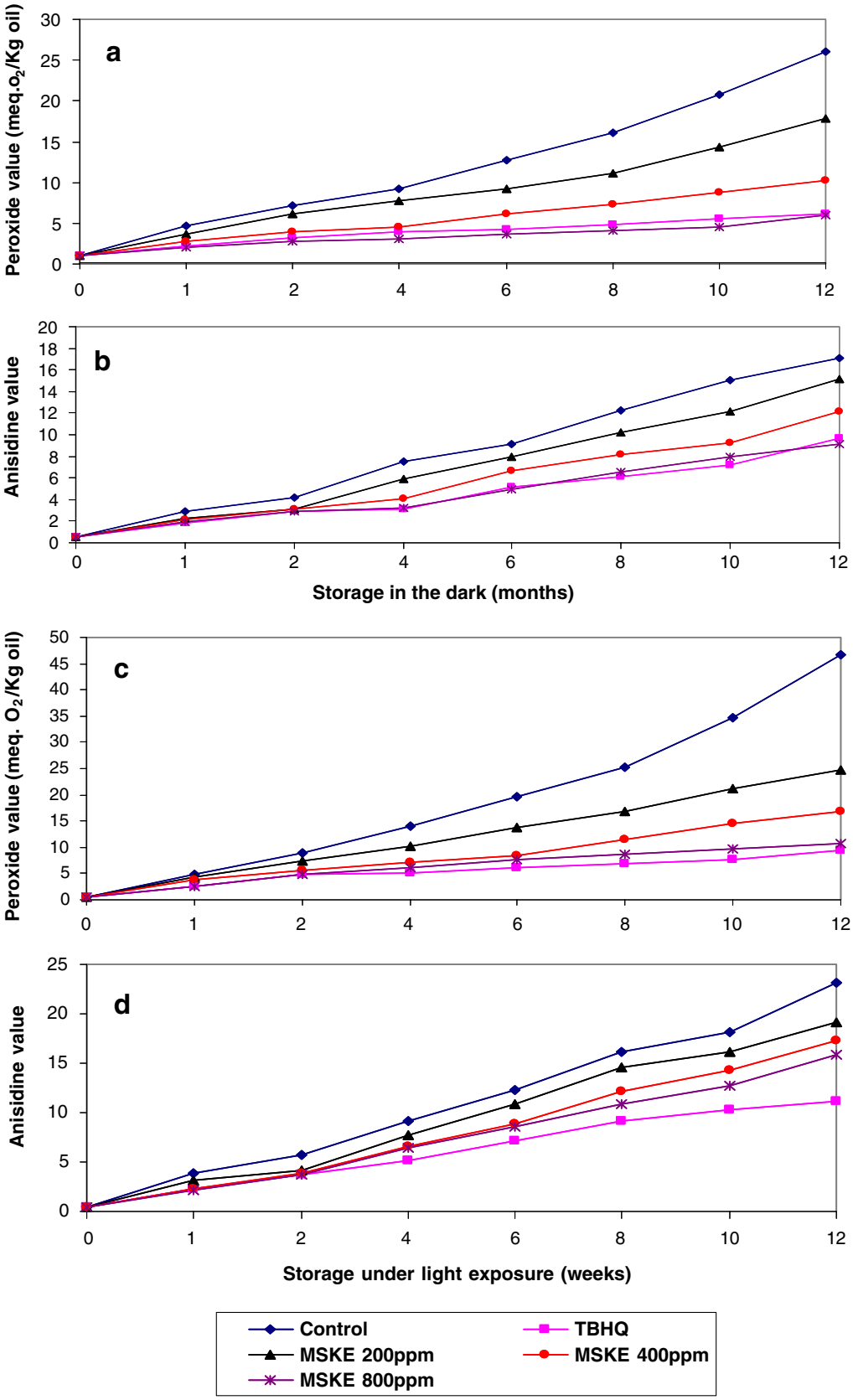

Fig. 1. Effect of mango seed kernel extract MSKE on the determination of peroxide and anisidine values in sunflower oil during storage in the dark (a, b) for 12 months and under light exposure (c, d) for 12 weeks at ambient temperature 20 ± 4 °C (mean of three determinations). Sunflower oil without synthetic antioxidants containing 410 ppm α-tocopherol. TBHQ is tertiary butylated hydroxy quinon.

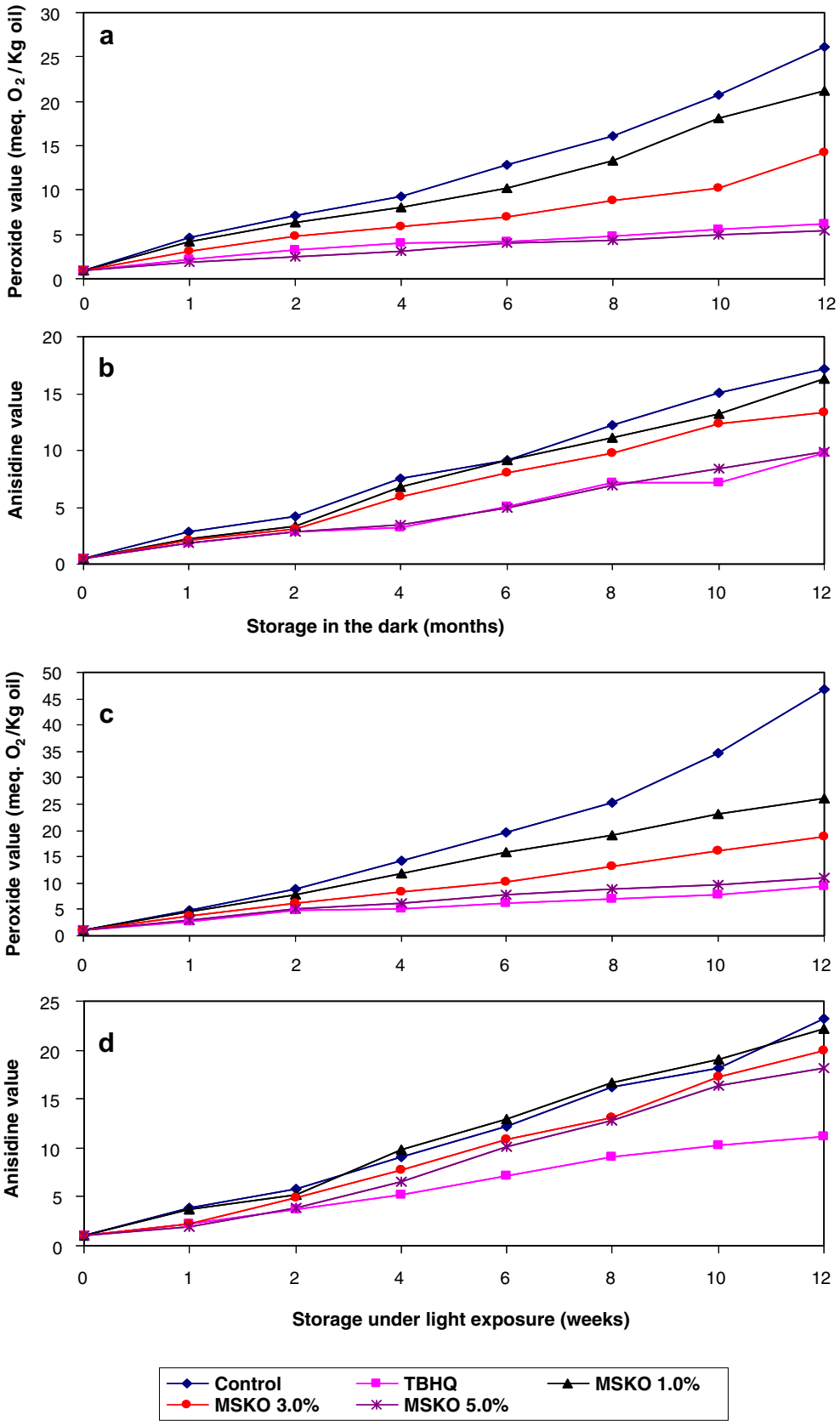

Fig. 2. Effect of mango seed kernel oil MSKO on the determination of peroxide and anisidine values in sunflower oil during storage in the dark (a, b) for 12 months and under light exposure (c, d) for 12 weeks at ambient temperature 20 ± 4 °C (mean of three determinations). Sunflower oil without synthetic antioxidants containing 410 ppm α-tocopherol. TBHQ is tertiary butylated hydroxy quinon.

3.3. Antioxidant activity of combined MSKE and MSKO on sunflower oil

Different concentrations of MSKE combined with MSKO were added to sunflower oil to study their antioxidative activities during storage of oil samples in the dark or under light exposure at ambient temperature. Combination of both MSKE and MSKO had an optimum antioxidant potency higher than each one alone (Fig. 3). The oxidative activity of oil 400 ppm MSKE and 5% MSKO was higher than 300 ppm TBHQ after 12 months of incubation the oil at room temperature, while 400 ppm extracts and 3% was similar to 300 ppm TBHQ. In general, the obtained data showed that the peroxide values of sunflower oil samples with different concentrations of mixed MSKE and MSKO were lower than that recommended by Egyptian standard (10 meq.O_2/kg oil) (Anonymous, 1993) during storage in the dark for 12 months.

From the above results, it can be concluded that adding both extract and mango seed kernel oil increased shelf-life of edible oils rich with polyunsaturated fatty acids as sunflower oil. These increments in oxidative stability may be due to methanolic extracts of mango seed kernel which contained the phenolic compounds and to mango seed kernel oils which contained high saturated fatty acids and mono-unsaturated oleic acid as well as high levels of tocopherols, squalene and phytosterols in unsaponifiable matter of oil as described in previous study. Zein (2000) found that oxidation of some edible oils was inhibited more by pure catechin and mango seed kernel oil at concentrations 1–3% than that of 300 ppm BHT.

3.4. Antioxidant activity of combined MSKE and MSKO on the compositional quality and stability of oil extracted from potato chips

The compositional quality and stability of oil extracted from chips before and after storage are presented in Table 1. The amount of oil absorbed by potato chips ranged from 37.5% to 39.5%. The addition of both extract and mango kernel oil has no effect on oil uptake.

Adding 400 ppm of MSKE and 5% of MSKO together to sunflower oil showed a protective effect on the rate of oxidation of the oil extracted from potato chips followed by 400 ppm extract and 3% oil. The changes in the characteristics of the extracted oil indicated that the extent of oxidation change occurred during intermittent seven frying days dependent mainly on the frying numbers, whereas oxidation values increased with subsequent frying. Potato chips samples collected from the first frying day (1st, 4th and 8th frying number in equal amount) were more stable against oxidation than other samples. The extent of oxidation in chips fried in sunflower oil with 400 ppm extract and 5% mango kernel oil was generally lower in fresh chips as well as in stored chips for three months than those fried in 300 ppm TBHQ and in 400 ppm extract combined with 3% mango kernel oil or without additives. The effectiveness of these natural antioxidants during storage of chips was directly proportional to antioxidant concentration at the levels incorporated in chips, so that chips with highest amount of phenolic compounds, tcopherols and sterols showed the highest oxidative stability during storage (Abdalla, 1999). Zein (2000) reported that increasing the level added from 200 to 400 ppm phenolic compounds and from 1% to 3% mango kernel oil lead to decreasing peroxide values formed and retarding oxidative rancidity in sunflower oil at 90 °C.

Sensory data including colour, crispness, flavour and overall acceptability of fresh and stored chips fried in sunflower oil with or without both MSKE and MSKO are shown in Table 2. The colour of the chips was affected by the frying number and during storage. Chips obtained from the first and third frying days showed bright yellow colour, while chips obtained from the seventh frying day showed pale or brown colour. These colours changes by storage to dark. Crispness was slightly changed in fresh and stored chips, it was between moderately and slightly crispy. Flavour quality and overall acceptability were gradually decreased following the frying number and after the storage period. Potato chips fried in sunflower oil treated with 400 ppm MSKE and 5% MSKO showed bright yellow colour, moderate crispress high score flavour, and were well accepted much more than chips fried in sunflower oil with 300 ppm TBHQ or oil with 400 ppm extract and 3% oil as indicated by the mean score values of the panelists. In contrast, chips fried in sunflower oil without additives obtained from third to seventh frying days showed pale or dark colour, sign of rancidity and just or not accepted scores after three months of storage. These results indicated that oxidation had taken place during frying and during storage of these samples. Chemical analyses of oil extracted from fresh and stored chips tended to support data of trained panelists, which indicated that chips fried in sunflower oil obtained from the third to the seventh frying days had highest levels of peroxide and anisidine values as well as free fatty acids and were deteriorated most rapidly. These results are in agreement with Hawrysh, Erin, Kim, and Hardin (1995) and Abdalla (1999). From these recent results above, it could be concluded that mango seed kernel extract and oil are suitable as natural antioxidant for frying oils and are not decompose easily at high temperatures as synthetic antioxidants.

3.5. Antimicrobial activity of mango seed kernel

3.5.1. Screening of antimicrobial effect of MSKE

Effect of adding MSKE on the acid development of raw cow milk showed that the acid production in milk was affected by extract concentration. The concentration of MSKE added into the milk samples were choosen according to the results of preliminary microbiological study assayed in our lab. Addition of different concentrations of MSKE (3000, 4500 and 6000 ppm) caused a considerable

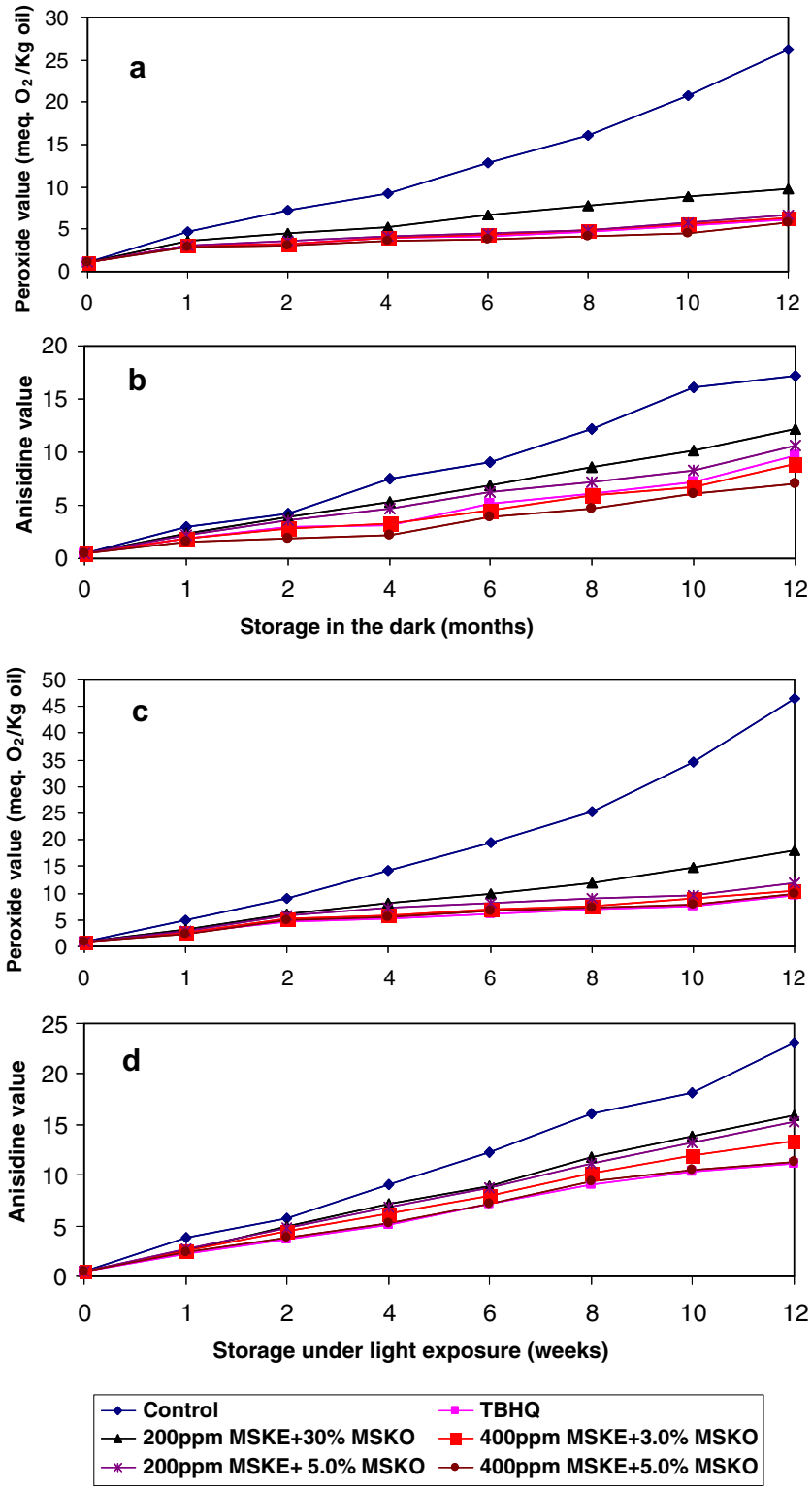

Fig. 3. Effect of combined MSKE and MSKO on the determination of peroxide and anisidine values in sunflower oil during storage in the dark (a, b) for 12 months and under light exposure (c, d) for 12 weeks at ambient temperature 20 ± 4 °C (mean of three determinations). Sunflower oil without synthetic antioxidants containing 410 ppm α-tocopherol. TBHQ is tertiary butylated hydroxy quinon.

decrease in acidity development, the pH of the treated milk samples being always higher (6.6–6.1) than that of control sample (6.6–4.7) after incubation time up to 8 h at 25 °C. These results revealed that MSKE has an antimicrobial effect against the microorganisms (flora) of raw milk which are responsible for the acidifying of control sample (e.g., lactic acid bacteria and other gram-positive and gram-negative bacteria).

Table 1
Effect of combined MSKE and MSKO on the compositional quality and stability of oil extracted from potato chips before and after three months storage at ambient temperature 22 ± 2 °C

Treatment	Frying day	Iodine value (wijs)	Peroxide value (meq.O_2/kg oil)	Anisidine value	FFA (as oleic%)
Sunflower oil (control)					
Before storage	1	122.5 ± 3.2	15.6 ± 1.	8.7 ± 1.1	0.40 ± 0.10
	3	110.8 ± 2.1	28.5 ± 2.1	19.3 ± 1.8	0.80 ± 0.20
	7	98.4 ± 2.3	38.7 ± 2.3	32.4 ± 1.9	2.10 ± 0.40
After storage	1	118.6 ± 3.7	19.2 ± 1.1	10.1 ± 0.5	0.62 ± 0.10
	3	116.6 ± 2.8	35.4 ± 2.2	25.4 ± 1.3	0.98 ± 0.35
	7	86.5 ± 1.6	44.2 ± 2.8	36.8 ± 1.5	2.80 ± 0.48
+300 ppm TBHQ					
Before storage	1	125.2 ± 3.8	5.4 ± 0.8	3.6 ± 0.5	0.22 ± 0.10
	3	122.4 ± 3.3	7.9 ± 0.6	4.8 ± 0.4	0.32 ± 0.12
	7	119.1 ± 2.5	9.8 ± 0.8	8.1 ± 0.7	0.85 ± 0.28
After storage	1	123.6 ± 3.8	5.8 ± 0.8	4.4 ± 0.3	0.25 ± 0.10
	3	120.3 ± 2.6	8.3 ± 0.9	6.7 ± 0.6	0.40 ± 0.20
	7	117.1 ± 2.4	11.7 ± 0.8	9.8 ± 0.7	1.25 ± 0.18
+400 ppm Extract and 3% oil					
Before storage	1	111.6 ± 2.9	5.2 ± 0.7	3.8 ± 0.4	0.25 ± 0.10
	3	106.4 ± 2.2	7.9 ± 0.8	4.9 ± 0.4	0.33 ± 0.15
	7	99.3 ± 1.1	10.3 ± 1.1	9.2 ± 1.1	0.95 ± 0.30
After storage	1	108.5 ± 2.2	6.1 ± 0.5	4.5 ± 0.5	0.35 ± 0.25
	3	101.3 ± 2.4	8.9 ± 0.9	7.5 ± 0.7	0.46 ± 0.26
	7	94.7 ± 1.1	12.4 ± 1.3	10.9 ± 0.8	1.35 ± 0.48
+400 ppm Extract and 5% oil					
Before storage	1	105.8 ± 2.7	5.0 ± 0.5	3.2 ± 0.3	0.20 ± 0.10
	3	102.8 ± 2.2	6.9 ± 0.6	3.9 ± 0.3	0.30 ± 0.15
	7	98.9 ± 1.3	9.1 ± 0.6	6.8 ± 0.5	0.75 ± 0.30
After storage	1	103.9 ± 2.1	5.3 ± 0.6	3.6 ± 0.3	0.22 ± 0.10
	3	99.4 ± 1.1	7.8 ± 0.5	4.8 ± 0.4	0.40 ± 0.14
	7	97.6 ± 1.2	10.4 ± 1.1	8.9 ± 0.6	1.10 ± 0.30

Values are mean of three determinations ± SD.

3.5.2. Effect of MSKE on the total bacterial and coliforms count of raw cow milk

Fig. 4 illustrates the total bacteria counts and coliforms in raw cow milk before and after addition of MSKE. The results showed that total bacterial and coliforms counts in untreated raw milk were 3.5×10^6 and 1.1×10^4 CFU ml^{-1}, increased to 9.9×10^8 and 2.1×10^6, respectively after 6 h of incubation at 25 °C. Addition of 3000 ppm MSKE to raw cow milk resulted in reduction of total bacterial count to 5.0×10^3, whereas coliforms growth was completely inhibited. The results in this study indicated that MSKE reduced aerobic plate and coliform counts of raw milk therefore it can be used to facilitate the collection of raw uncooled milk by increasing its keeping quality. These results agreed with those reported by Kabuki et al. (2000) who published that the MKE had a broad antimicrobial spectrum against gram-positive and gram-negative bacteria. They showed that the active antimicrobial component of the MSE was a type of polyphenol. However, numerous studied have been carried out to study the mode of action of antimicrobial agents containing antibiotics (Franklin & Snow, 1989). To argue the difference of susceptibility of MKE against gram-positive and gram-negative bacteria, the mode of action must be studied in detail.

3.5.3. Antimicrobial assay

Antimicrobial activity of neutralized and filter sterilized MSKE (3000, 4500 and 6000 ppm) was investigated using agar well-diffusion assay against target Enterophathogenic *E. coli*. The results indicated that the extract showed remarkable antimicrobial activity against *E. coli* strain. A positive relationship was found between antimicrobial activity and the concentration of extract. Diameter of inhibition zones were 21, 19 and 18 mm for 6000, 4500 and 3000 ppm, respectively, compared with no inhibition zone for control sample without extract. These results agreed with Kabuki et al. (2000) who reported that the MKE showed an antimicrobial activity against gram-negative bacteria, e.g., *E. coli* and *Salmonella* spp. Different phenolic compounds were found to inhibit the growth of several gram-negative and gram-positive bacteria (Branen & Davidson, 1983).

3.5.4. Effect of antimicrobial activity of MSKE on the keeping quality of pasteurized cow milk

Neutralized and filter sterilized MSKE (3000 ppm) was added to 50 mL of pasteurized cow milk (72 °C, 5 min). Milk samples were stored at 4 °C and at room temperature (25 ± 2 °C) for 15 days. Beed formation was observed on a microscope slide surface daily. The results indicated that

Table 2
Sensory evaluation of fresh and stored potato chips for three months at ambient temperature (22 ± 2 °C)

Treatment	Frying day	Properties			
		Colour	Crispness	Flavour	Overall acceptability
Sunflower oil (control)					
Fresh	1	4.5	4.0	4.0	4.0
	3	3.0	3.5	3.0	3.0
	7	2.5	3.5	2.0	2.5
Stored	1	4.2	4.0	3.5	3.8
	3	3.0	3.5	2.6	2.5
	7	2.2	3.0	1.0	1.5
+300 ppm TBHQ					
Fresh	1	4.8	4.5	4.6	4.8
	3	4.6	4.5	4.5	4.5
	7	4.0	4.0	3.5	4.0
Stored	1	4.5	4.0	4.2	4.1
	3	4.0	3.6	4.0	3.6
	7	3.5	3.4	3.0	3.2
+400 ppm extract and 3% oil					
Fresh	1	4.6	4.3	4.5	4.6
	3	4.0	4.6	4.0	4.0
	7	3.8	3.6	3.0	4.0
Stored	1	4.4	4.0	4.0	4.0
	3	4.0	3.2	4.0	3.4
	7	3.0	3.0	2.5	3.0
+400 ppm extract and 5% oil					
Fresh	1	4.9	4.7	4.8	4.9
	3	4.8	4.5	4.6	4.8
	7	4.3	4.2	4.5	4.5
Stored	1	4.7	4.2	4.4	4.4
	3	4.5	4.0	4.2	4.0
	7	4.0	3.5	3.4	3.4

Values are mean of ten panelists.

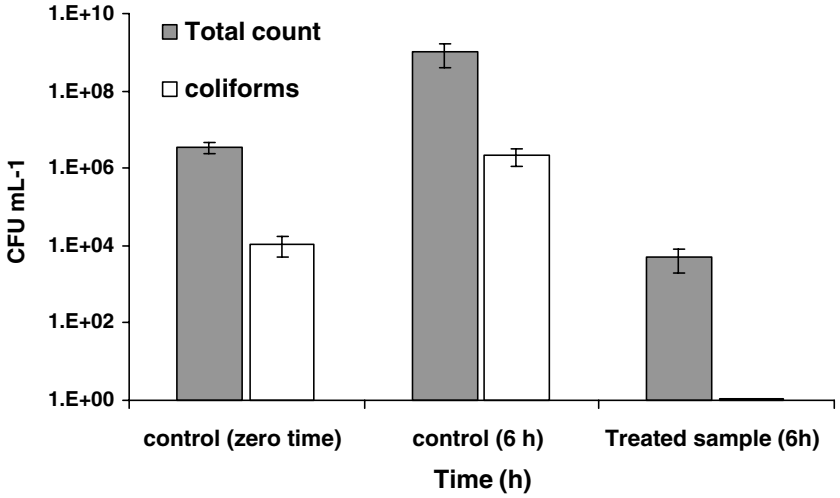

Fig. 4. Effect of MSKE on the total microbial and the coliforms count of raw cow milk at 25 °C.

untreated pasteurized cow milk samples were deteriorated after 3 and 6 days of storage at 25 ± 2 °C and 4 °C respectively. Beeds were not formed in treated milk samples after 15 days of storage at both temperatures. Keeping quality of pasteurized milk produced in Egypt is low. This is due to unsanitary conditions of production and handling of raw milk and the lack of chilling facilities and the long time interval between the production of milk and subsequent delivery to dairy plant. Thus, MSKE could be added to pasteurized milk to provide a subsequent shelf life extension. A few papers reported the safety of mango seed kernels as food additives for extending the shelf-life of a variety of food products (Rukmini & Vijayaraghavan, 1984; Arogba, 1997).

Table 3
Sensory evaluation of pasteurized milk treated with different concentrations of MSKE

Sample	Taste		Smell		Colour		Overall grade (100)
	Grade[a]	Description[b]	Grade	Description	Grade	Description	
Control	4	Normal, swetty (1), flat	4	No smell, normal pasteurized milk	4	Normal	100
+3000 ppm	4	No specific flavour, swetty (1), normal	4	No smell, normal pasteurized milk	3	White, tress yellow (1)	85
+4500 ppm	3	Normal, less swetty	4	No smell, normal pasteurized milk	2	Yellowish (1)	80
+6000 ppm	3	Mineral taste (1)	4	No smell, normal pasteurized milk	2	Yellowish (2)	75

Values are mean of 10 panelists.
[a] Grade (1–4): 1:bad, 2: sufficient, 3: good, 4: very good.
[b] Intensity (1–4): 1: slightly, 2: moderate, 3: strong, 4: very strong.

3.5.5. Sensory evaluation of pasteurized milk treated with MSKE

Pasteurized milk was treated with MSKE (3000, 4500 and 6000 ppm). The treated and untreated (control) milk were assessed sensorically (Table 3). The treated milk (3000 ppm) sample received the highest overall grade (out of 100) 85 and also the highest flavour and colour score. The colour was changed to tress yellow (3000 ppm), slightly yellow (4500 ppm) and moderate yellowish (6000 ppm).

The results showed that the concentration of 3000 ppm extract was the best one which was comparable to the control and no effect was found on the flavour, smell and colour of the pasteurized milk.

4. Conclusion

According to the determination and identification of phenolic compounds in mango seed kernel extract and to the evaluation of mango seed kernel oil characteristics and composition in previous study, the antioxidant and antimicrobial activities of mango seed kernel extracts and oil were investigated in this study. The results showed that adding 800 ppm of MSKE or 5% of MSKO to sunflower oil were the best levels as natural antioxidant due to decreasing peroxide and anisidine values of oil. Sunflower oil is characterized by the presence of high amount of linoleic acid and extremely susceptible to oxidative deterioration during storage. The shelf life of sunflower oil with MSKE or/and MSKO increased and reached up to more than 12 months in the dark and up to 12 weeks under light exposure. The results indicated that combination of both mango seed kernel extract and oil had optimum antioxidant potency higher than each one alone. Addition of 400 ppm extract and 5% mango seed kernel oil increased the oxidative stability of sunflower oil incubated in the dark and under light exposure at room temperature as well as sunflower oil during frying. Moreover, both extract and oil improved the stability and quality characteristics of fresh and stored potato chips. On the other hand, mango seed kernel extract reduced total bacterial count, inhibited coliforms growth, showed remarkable antimicrobial activity against *E. coli* strain and extended the shelf-life of pasteurized cow milk.

It can be concluded that mango seed kernel extract and oil can be use as natural antioxidant and antimicrobial in different kind of foods, due to high content of different phenolic compounds, their fatty acid pattern rich with saturated fatty acids and with mono-unsaturated oleic acid besides tocopherols, squalene, and different sterol fractions.

More studies are needed to focus on isolation of the most important natural antioxidative compounds from MSK by different procedures and recent equipments and study its mode of action as natural antioxidants and antimicrobials in different fatty foods and emulsion.

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