Evaluation of Mango Seed kernel Extract as natural occurring phenolic rich antioxidant compound

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ABSTRACT

Sunflower oil (SFO) seeds of Helianthus annuus L., is a rich source of linoleic acid, which is more susceptible to oxidation of edible oils. Usually antioxidant materials are added to improve the oxidative stability of oils .Mango seeds kernels (MSK) are a sources rich with natural antioxidant compounds. In 2012, the bio-waste was produced by processing industries of mango fruits around 3,932 mT in Egypt. Ethnolic extract of mango seed kernel (MSKE) has been used for stabilization of SFO at three concentrations (mg/kg oil) i.e., 200(MSKE-200), 500(MSKE-500) and1000 ppm (MSKE -1000), while BHT was used at a level of 200ppm (BHT-200) for comparison. Progression oxidation of SFO was followed during accelerated storage at 70°C for 72hr (at intervals of 4hr)by measuring oxidative stability tests: peroxide value(PV), p-anisidene value (P-anv), total oxidation value (TOTOX), acid value (AV) and thiobarbituric acid reactive substances (TBARS) . Results of HPLC analysis of MSKE showed that contained 20 compounds of total phenolic content. MSKE at 100ppm had the highest radical-scavenging activity (96.86%) followed by BHT (94.9%) and ascorbic acid (91.5%). The higher levels of MSKE (1000, 500 ppm) provide the best protection against primary and secondary oxidation of SFO samples. The different parameters of oxidative stability were in agreement with each other, suggesting higher efficiency of 1000ppmMSKE> 500ppm MSKE > 200ppm BHT> 200ppm MSKE > control.

Key words: *mango seed, sunflower oil, oxidative stability.*

INTRODUCTION

Sunflower oil (seeds of *Helianthus annuus* L.) is widely used in nutrition as a source of essential linoleic (9-*cis*, 12-*cis*-octadecadienoic) acid. Edible oils with higher contents of unsaturated fatty acids, especially polyunsaturated fatty acids, are more susceptible to oxidation **(Mariana-AtenaPoiana., 2012)**. Lipid oxidation is the main deterioration process that occurs during thermal processing of vegetable oils containing lipid molecules with poly unsaturation (**El Anany *et al*., 2007).**

The addition of antioxidants is considered as one of the methods of increasing shelf life of lipids and lipid-containing foods. Synthetic antioxidant, such as butylated hydroxyl anisole (BHA) and butylated hydroxyl - toluene (BHT), have restricted use in foods as these synthetic antioxidants are suspected to be carcinogenic **(Jayaprakasha *et al.*, 2001)**. Due to these safety concerns, there is an increasing trend among food scientists to replace these synthetic antioxidants with natural ones, which, in general, are supposed to be safer (**Yanishlieva and Marinova, 2001**). Antioxidants (natural and synthetic) play a significant role in retarding lipid oxidation reactions in food products. The detrimental effects of excessive lipid oxidation, such as formation of off-flavors and undesirable oxidized chemical compounds (aldehydes, ketones and organic acids) are well known **(Saad *et al*., 2007**). A number of natural antioxidants have been added during food processing and have elongated the shelf life and oxidative stability of stored products **(*Jang et al*., 2012).** The main classes of natural antioxidant compounds in nature are flavonoids and phenolic acids in free or complexes forms. These compounds have been identified and quantified in several fruits and vegetables, and show a high correlation with antioxidant activity **(Einbond *et al.*, 2004)**. According to FAO estimates **(FAOSTAD, 2015)**, world production of mango was 42,139,837 mT. Approximately, 0.5% of world mango production is used to obtain derived products; therefore the amount of bio-waste produced by processing industries is estimated to be around 210,000

mT worldwide. In 2012 the amount of bio -waste produced by processing industries in Egypt is estimated to be around 3,932 mT. Environmental, hygienic, and public health problems result in unorganized management of this waste. The objective of this study was to evaluate the antioxidant activity of mango kernel seeds extract on SFO stability.

MATERIALS & METHODS

Materials

Ripe mango fruit (*Mangifera indica*), was purchased from Egyptian local market, Cairo Egypt. Ten liters of refined bleached and deodorized (RBD) sunflower oil (SFO) free of additives was obtained from Company for Oil and Soap, Tanta, Egypt.

All chemicals used in the study, such as Gallic acid, Folin–Ciocalteu reagent and Wijs' solution and butylated hydroxyl - toluene (BHT) were purchased from Merck (Darmstadt, Germany). Potassium Ferricyanide, Potassium Phosphate, Sodium Carbonate, Sodium Hydroxide Ethanol, Methanol, Chloroform, Diethyl Ether, Petroleum Ether, Acetic acid, Sodium Thiosulfate, Boric acid, Sulfuric acid, Potassium Iodide, Aluminum Chloride, Trichloroacetic acid, Ferric Chloride and Hydrochloric acid were purchased from Fisher Scientific (Leicestershire,UK).

2,2-diphenyl- 1-picrylhydrazyl (DPPH); 1, 1, 3, 3-tetraethoxypropane ,Ascorbic acid, Acetonitrile and Standard (phenolic and flavonoid) compounds were purchased from Sigma–Aldrich (St. Louis, MO).

Methods

Sample preparation

Mango kernel stones were removed manually and separated from the pulp, then washed with excess water to remove adhering materials. The stones were opened to get kernels. Mango kernels were cut cross-section wise into thin slices before extraction. Mango seed kernels pieces were spread in single layer for drying in an electric oven at 40°C for 24 hours. The dried mango kernels were milled with grinder, then mango seed kernels were heated in an electric oven at 160ºC for 20 min to improve the yield of phenolic compound (**Soong and Barlow 2004).** According to the

extract method of **El Anany, (2015)** ten grams of fruit seeds powder were extracted overnight with 1000 ml of 80 % ethanol solution in a shaking incubator (100 rpm) at room temperature. Then the extracts were centrifuged at 3500 rpm for 15 min. The supernatants were filtered through a Whatman No.1 filter paper, then extract solutions were concentrated to dryness in a rotary evaporator at 40°C and stored at -20°C for further use.

Sample preparation for accelerated oxidative storage

A known weight of the dry extract was dissolved in ethanol and addition to SFO as a source of antioxidant. The extract of mango seed kernel (MSKE) was applied to RBD edible SFO (free of any synthetic antioxidants) at different concentrations (200, 500 and 1000 ppm, based on extract weight), in a series of glass bottles having a volume of 100 ml each, to examine their antioxidative activity. BHT at a level of 200 ppm was also applied for comparison. The bottles were completely filled with oil and sealed. A control sample was prepared by using the same amount of ethanol used to dissolve the antioxidant and the extracts **(Mohdaly *et al*., 2010).** The antioxidant enriched oil samples were evaporated in a rotary evaporator below 40 ºC to evaporate the solvent and subjected to accelerated oxidation in dark oven at 70ºC for 72 hr **(Fennema, 1976).** One hundred ml of each oil sample was taken for oxidative analysis of (PV, Pan, AV and TBARS), at 4 h intervals for analysis. Immediately after storage period, oil samples were withdrawn for triplicate analyses. Oil sampled were taken for each measurement from separate bottle.

Chemical Composition of seeds

Moisture, protein, ash, crude fiber and ether extract in the mango seed kernel were determined according to **AOAC (2005)** methods. The nitrogen free extract was calculated by difference on dry weight basis.

Determination of phenolic content

Total phenolic content of the extract was measured using the Folin–Ciocalteu assay developed by **Singleton and Rossi (1965).** This is a colorimetric assay, involving production of a blue

molybdenum tungsten complex in the presence of phenolics which can be measured relative to gallic acid as the standard. Aqueous Folin –Ciocalteu reagent (1:10) was added to the kernel extract or the standard, incubated for 5 min before addition of 0.115 mg/ml of Na_2CO_3. After 2 h incubation period, absorbance was read at 765 nm using a spectrophotometer (Unicam, Helios Alpha, UK). Gallic acid was used as standard and calibration curve was plotted in concentration range 50-200 mg/l. Results were expressed as mg of Gallic acid equivalents/g dried seeds.

Determination of flavonoid content

Flavonoid content was determined according to the procedure described by **Zhishen *et al.*, (1999).** Solutions of the extracts in Methanol 80% were prepared to give a final concentration of 6 mg/ml. Each solution (5ml) was mixed with 0.3 ml of 5% aqueous $NaNO_2$ (w/v) and allowed to stand at room temperature for 5 min; 0.6 ml of 10% $AlCl_3$ solution (w/v) was added to the mixture. After 6 min, 2 ml of 1M NaOH and 2.1 ml of water were added to the mixture. The absorbance was measured at 510 nm using a spectrophotometer (Unicam, Helios Alpha, UK). The results were expressed as mg quercetin (Q) equivalents per 100 g of dry mater.

Free radical scavenging activity

The free radical scavenging activity of the extract was measured by using 2,2-diphenyl-2-picryl hydrazyl (DPPH) method as described by **Yang *et al.*, (2009)** with some modifications. Briefly, 0.1 ml of samples (100µg/ml in 80% methanol) was added to 3.9 ml of 0.2 mM DPPH methanolic solution. The reaction mixture was agitated and allowed to stand at room temperature in dark for 30 min. The absorbance at 515 nm was used to measure the concentration of the remaining DPPH using a spectrophotometer (Unicam, Helios Alpha,UK). Ascorbic acid and BHT (100 µg /ml in each methanol) were used as positive control, the percentage inhibition of the DPPH radical was calculated according to the following formula:

% Inhibition = ((A control −A sample) / A control) X 100

Where: A is absorbance

Determination of reducing power

The reducing power of the extracts was measured as described by **Oyaizu (1986).** The reaction mixture contained 1.0 ml of extract, 1.0 ml of 0.2 M phosphate buffer (pH 6.6) and1.5 ml of potassium ferricyanide (1%, w/v, in water). The mixture was incubated at 50^{o}C for 30 min and the reaction was stopped by addition of 1.5ml of tri chloroacetic acid (10%, w/v, in water), followed by centrifugation at 980 g for 10 min. Aliquots of 2 ml of the supernatant were mixed with 2ml of distilled water and 0.5 ml of ferric chloride (0.1%, w/v, in water) and the absorbance was measured at 700 nm, using spectrophotometer (Unicam, Helios Alpha,UK), against blanks that contained all reagents except the sample extracts.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay of the MSKE was carried out according to the procedure of–**Benzie and Szeto, (1999**). One hundred micro liters of the diluted extract in methanol (conc.) was added to 3ml of the FRAP reagent. After 4 min, the absorbance of the mixture was measured at 593 nm using UV/VIS spectrophotometer (Unicam, Helios Alpha, UK). A calibration curve was prepared using BHT as a standard at 200, 400, 600, 800 and 1000 µm. FRAP values were expressed on a fresh weight basis as micromoles of BHT equivalent per gram of sample (µm BHT equivalent/g dry weight).

Identification of phenolic acids using HPLC

Phenolic acids of the dried extract were identified according to the method described by **Janas *et al.*, (2009).** Shimadzu HPLC system (Shizadzu Corp., Kyoto, Japan) comprising an LC-10AD pump, SCTL 10A system controller and SPD 10A photodiode array detector. Each sample was first filtered through a 0.45 µm nylon membrane and then injected onto a prepacked LiChrospher 100RP-18 column (4 x 250 mm, 5 µm; Merck, Darmstad Germany). The mobile phase consisted of water,

acetonitrile, acetic acid (88:10:2; v/v/v) the flow rate, was 1 ml / min, and detection of phenolic acid was monitored at 320 nm.

Oxidative stability tests

Peroxide value (PV)

PV was determined according to the standard method of **IUPAC (1987).**

Measurement of P-Anisidin value (P-anV)

P-anV was determined according to **AOCS method (1995).**

Total oxidation value (TOTOX)

TOTOX values of the oil samples were determined based on the obtained PV and p-an values **(Nyam *et al.*, 2013)**. Values were calculated using the following Equation: TOTOX = 2PV + P-anV

Acid value (AV)

AV was determined according to **AOCS method (1995).**

Thiobarbituric Acid Reactive Substances (TBARS) assay

The Thiobarbituric Acid test was based on the color reaction of TBA with malon-dialdehyde (MDA) mg/kg in the sample **(David, 1976).** The absorbance was measured at 531 nm against the blank solution (distilled water). A standard curve of MDA was prepared using 1, 1, 3, 3-tetraethoxypropane. TBARS values were expressed as mg of MDA per kg of sample.

RESULTS & DISCUSSION

Proximate composition: The results in Table1, showed the chemical composition of MSK powder showed its contents of crude protein, total lipid, crude fiber and ash were found to be 6.9%, 12.5%, 2.9% and 2.3%, on a dry weight basis, respectively, moisture content was 8.1%. These results are close to those reported by **(Abdalla *et al.*, 2007**), who reported that the moisture content of dried MSK powder samples was 8.1%. Crude protein, total lipid, crude fiber and ash contents of MSK were 6.7%, 12.3%, 2.7% and 2.5% on dry weight basis, respectively. The total lipid content in this study was higher than that showed by others as **Youssef,(1999).** This may be due to different mango varieties.

The yield of extract from mango seed kernel powder showed in Table 2 was 9.43 g/100g powder. **Jung *et al*.,(2006)** compared the influence of different solvents on total phenolic and total flavonoid extracted from ginseng leaves and they found that, the ethanol extracts contained higher amounts of total phenolic and Flavonoids (2333 and 1199 mg/100g resp.) than water (1932 and 503 mg/100g resp.)and methanol extracts (2286 and 720 mg/100g resp.). The ethanolic extracts of heated MSK at 160ºC for 20min were showed increase in the total phenolic content from 50.3 to 160 mg GAE/g (**Soong and Barlow, 2004).** This increase could be due to the release of more bound phenolics from the breakdown of cellular constituents due to the thermal treatment (**Gallegos-Infante *et al*., 2010**) and to the degradation of polymerized polyphenols, specifically hydrolysable tannins, and the hydrolysis of other glycosylated flavonoids (**Monagas *et al*. 2009**). Roasting process caused significant decreases in the nutritional factors (Tannins and phytic acid) content (**El Anany, 201**5). Likewise, in a study on acorn nut Gallic acid increased after thermal treatment to almost 2-fold its content (0.142 to 0.270 %) in a dry powder extract (**Rakic *et al*., 2006)**. Pyrroles and furans which are the major compounds formed by the Maillard reaction may contribute to the increased in total phenolic compounds of roasted samples (**Yanagimoto *et al*., 2002**). They were reported an increased in the antioxidative function due to the generation and accumulation of Maillard-type antioxidants (MRP) during the heating process. This suggestion was confirmed by **(Antonio *et al*., 2003)** who showed an increase in the optical density of at 280 nm, in plums dried at 60 or 85ºC.

Total phenolic content

Polyphenols are phytochemicals from plants and are being used for prevention of various diseases that are mainly caused by free radicals. The higher polyphenol content would then exhibit stronger inhibition and also higher antioxidant activity (**Jayaprakasha *et al*., 2003**). Results in table 2 showed that, total phenolic content of the extract from mango seeds was

21.29 mg/g on dry basis. The result of total phenolic content of mango seed kernels are in agreement with those of Thai mango which contained 11-28.62 mg gallic /g (**Pitchaon, 2011)** and 28.33- 44.76mg gallic /g (**Eva Dorta *et al.*, 2014**). The technique of phenolic isolation from plant material, including the methods and type of extracting solvent, depends generally on the type of phenolic compound and the solvents (**Goli *et al.*, 2004**).

Total flavonoid content

Flavonoids are the most common and widely distributed group of plant phenolic compounds (**Guo *et al.*, 2012**) and are generally categorized as phenolics depending on their chemical structure (**Sung and Lee, 2010**). Results in table 2 showed that, mango seeds contained 22.20 mg total flavonoids/g dw. Total flavonoids contents different conditions depend on extracting methods and sources of seeds.

Antioxidant activity

The most common methods to determine the antioxidant activity in a practical, rapid and sensitive manner are those that involve a radical chromophore, simulating the reactive oxygen species, and the free radical DPPH, of purple coloration that absorbs at 515 nm, is one of the most widely used for *in vitro* evaluation of plant extracts and fractions **(El Gengaihi *et al.*, 2014)**. The results in Table 3 showed that, MSKE at a concentration of 100ppm, ethanol extract of mango seeds had the highest radical - scavenging activity (96.86) at a concentration of 100ppm, followed by BHT(94.9%) and ascorbic (91.53%). These results are higher than that obtained by (**Mansour and Khalil 2000**). This difference might be due to the interspecies variation.

The ferric reducing antioxidant power (FRAP) assay was used to evaluate the antioxidant capacities of the seeds extract. The FRAP assay is based on the capacity of antioxidants to reduce ferric (III) ions to ferrous (II) ions **(Benzie and Szeto, 1999)**, The reducing power of the seeds extracts was measured by direct electron donation in the reduction of $[Fe(CN)6]^{3-}$ to $[Fe(CN)6]^{4-}$. The product was visualized by addition of free Fe^{3+} ions after the

reduction reaction, by forming the intense Prussian blue color complex, $(Fe^{3+})_4[Fe2+(CN^-)_6]_3$, and quantified by absorbance measurement at 700 nm **(Oyaizu, 1986)**. The FRAP value of the MSKE are shown in Table 3. The highest FRAP value was 4710µ mol Fe (II)/g. The values of absorbance at 700 nm for the extracts of mango seeds kernel in Table 3 revealed that, MSKE had a high capacity to reduce iron (III). The reducing power (RP) of the MSKE was 0.85. The extracts from the mango seed had RP values higher than BHA at100 ppm. **Wojdylo *et al.*, (2007)** reported that herbs can be classified into their antioxidant capacity as: very low FRAP (<10 µM/100 g), low FRAP (10–50 µM/100 g), good FRAP (50–100 µM/100 g), high FRAP (100–500 µM/100 g) and very high FRAP (>500 µM/100 g). According to this classification MSKE could be considered as a high antioxidant capacity.

Identified content of the phenolic compounds

HPLC is the preferred technique for both separation and quantification of phenolic compounds **(Naczk and shahidi 2004**). The HPLC analysis of the phenolic compounds in mango seeds extracts were compiled in Table 4. Results revealed that the alcoholic extract (80%) of MSkE contained 20 identified phenolic compounds. The highest amount of phenolic compounds were Ellagic, Pyrogallol, Chlorogenic, Catachin, Mangiferin, Protocatechuic, Gallic, Cinnamic, Catechol and Myricetin (2613, 1337, 1182, 757, 516, 433, 349, 217, 202 and 106 mg/100g) on a dry wt basis, respectively. **Puravankara *et al.*, (2000)**, identified six phenolic compounds in mango seed extracts, mainly gallic acid, ellagic and gallates. The chromatogram corresponding for the ethanolic extract of mango seed showed that three compounds were found, of which one was identified, corresponding to gallic acid as the main component of the extract, in a concentration of 586.68 mg/g dw **(Vega-Vega *et al.*, 2013). Yilmaz and Toledo (2004)** concluded that aqueous solutions of ethanol, methanol mixture was better solvent than pure either of them as solvent system for the extraction of phenolics compound from

Muscadine seed. **Rodtjer *et al.*, (2006)** showed that the extraction yield of phenolic compounds is greatly dependent on the solvent polarity. The differences in composition presented between the extracts reported in the literature and in the present study may be due variety, degree of ripeness of the fruit used and the method of extraction. An important factor is the solvent used for the extraction of phenolic compounds, because solvents with different polarity extracted different compounds in varying quantities **(Chen *et al.*, 2011)**.

Peroxide value (PV)

Hydroperoxide is the primary oxidation product produced as result of lipid oxidation. It may break down into nonvolatile and volatile secondary products, which decrease the quality of the oil. This is an indicator of the initial stage of oxidative changes (**Erwin *et al.*, 2004**). The presence of hydroperoxide in the oil can be determined based on the oxidation of iodine ion with hydroperoxide. A saturated iodine solution added to the oil sample reacts with the produced hydroperoxide from the lipid oxidation and releases free iodine as the end product. The liberated iodine is then titrated against sodium thiosulphate. The titration value can be calculated and reported as peroxide value, mill equivalents of oxygen per kilogram oil sample (meq O_2/kg) (**Shahidi and Wanasundara 1996**). In this study, oxidation degree of sunflower oil samples were determined by measuring PV during accelerated oxidative storage of sunflower oil samples (with or without synthetic antioxidant or natural mango seed extracts) at70^o C for 72 hr, as shown in Table 5. The results showed a continuous increase in PV with the increasing of storage times for all the samples. Initial rate increasw in PV was very slow, but it started to increase after 40 hr of storage and went on increasing further with the increase in storage times (72hr). Peroxide values were in the range of 0.81- 16.14 meq O_2/kg SFO for MSKE. SFO samples without antioxidant control (-ve) reached a maximum PV of 21.3 meq O_2/kg SFO after 72 h of storage. The results in Table 5 showed significant differences in PV were observed between the control and

SFO samples containing seeds extracts and synthetic antioxidants, which decreased the rate of peroxide formation. The PV of SFO sample containing 200 ppm of BHT was 17.62 meq O_2/kg, while SFO samples containing (200, 500, 1000 ppm) MSKE were16.14, 14.95, 13.25 meq O_2 /kg SFO, respectively after72 hr at 70^o C. This data suggests the superiority of the antioxidant activity of MSKE at 200ppm over synthetic antioxidants. These results were in agreement with **Abdalla *et al.,*(2007)** who showed that ,increasing the levels of MSKE added to sunflower oil (200,400 and 800 pmm) led to a decrease in PV formed and retarding oxidatives rancidity during storage of sunflower oil at ambient temperature for 12 months. One can say that, the total antioxidant activity of by-product fruit seed extracts was linearity proportional to the concentration of total phenolic compounds. However, all oil samples with seed extract at 500 and 1000ppm were better than that of the synthetic antioxidants.

P-Anisidin value (P-anV)

During lipid oxidation, hydroperoxides, the primary reaction products, decompose to produce secondary oxidation products (aliphatic aldehydes, ketones, alcohols, acids and hydrocarbons) which are more stable during the storage time and an responsible for the off-flavors and off-odors of oxidized edible oils. In order to ensure a better monitoring of lipid oxidation process in the storage time, the simultaneous detection of primary and secondary lipid oxidation products is necessary. p-anV is a reliable measurement of the amount of secondary oxidation products **(Zhang *et al.*,2010; De Abreu *et al.*,2010)**. The added anisidine reacts with the aldehyde and produces a yellow colored solution. The yellow color is measured at the absorbance value of 350 nm. A lower P-anV indicates a better quality of oil **(Shahidi and Zhong 2005)**. Results in Table 6 presents the changes recorded in p-anV during accelerated storage of SFO at 70ºC for72 hr, as effected by supplementation with BHT and different constitutions of MSKE. It can be observed that accelerated storage promoted rapid transformation to secondary products which contributes to the

off-flavors of sunflower oil. Addition of BHT and various levels of MSKE resulted in significant decrease in p-anV relative to the control sample. A gradual continuous increase in p-anV with increasing storage times for all the samples was noticed. Initially the rate in p-anV was very slow, but it started to increase after 44 hr of storage and went on increasing further with increasing the storage times (72hr). All levels of MSKE provide good protection against secondary oxidation of SFO samples. The active compounds present in the fruit seeds extract might decompose with storage time. However, an increase in the extracts concentration could extend the period, which was evidenced in the results of this study. The p-anV of the treated samples showed a similar trend with the reported PVs, as shown in Table 6. At the end of storage, there were no significant differences between supplemented SFO samples with BHT or MSKE (200 mg/kg). This means that MSKE at a level of 200 ppm provide a protection effects equivalent to 200 ppm of BHT against secondary lipid oxidation. MSKE can be suggested as a potential source of antioxidants in the prevention of the production of secondary products in edible oils during storage. These data are in agreement with those reported by **Iqbal and Bhanger, (2007); Yim *et al.*, (2013)** which highlight that the natural extracts showed a significant inhibitory effect against oxidation of refined oils.

TOTOX Value

This parameter gives a measurement value of the total oxidation, including primary and secondary oxidation products **Hashemi *et al*., (2014).** The total oxidation of the oil sample can be calculated based on the determined PV and p-anV values **(Nyam *et al.*, 2013)** are calculate TOTOX value according to the following equation: {TOTOX value = 2PV + p-anV}. These values reflect the initial and later stages of the oil oxidation. It measures the primary product, hydroperoxide, and its breakdown product, aldehyde. Therefore, it provides a better estimation of the progressive oxidative deterioration of the oil. The lower TOTOX value indicates a higher

quality of the oil (**O'Keefe *and Pike* 2010**).

The TOTOX values of the supplemented sunflower oil samples are shown in Figur1. All the supplemented samples showed positive effects in inhibiting oxidative rancidity. As compared to the control, all the supplemented samples were observed to have lower TOTOX values. The TOTOX values of all the samples including synthetic antioxidants were as follow: control > 200ppm MSKE > 200ppm BHT > 500ppm MSKE> 1000ppm MSKE. The highest level of MSKE had the best inhibitory effect on oil oxidation in the storage time.

Acid value (AV)

The acid value of an oil or fat is defined as the number of milligrams of potassium hydroxide required to neutralize the free acidity in one gram of sample. The results are often expressed as the percentage of free fatty acids (FFA), especially in UK. The acid value is a measure of the extent to which the glycerides in the oil have been decomposed by lipase or other action. The decomposition is accelerated by heat and light **(David, 1976)**. Free fatty acid and acid value of any lipid are measure of hydrolytic rancidity **(Rehab, 2010)**. The results in Table 7 represent the effect of MSKE on acid values of SFO during accelerated storage at 70°C for 72hr. The results showed that, a continuous increase in AV with increasing storage time (72hr) for all the samples, MSKE had pronounced antioxidant activity against hydrolytic oxidative rancidity of SFO samples during storage. The antioxidant activity of 1000 ppm MSKE was higher than 200, 500 ppm or 200 ppm of BHT. The acid value in the samples with MSKE or BHT were lower than that in the control sample, indicating that these extract or BHT can retard SFO rancidity. The high amount of total phenolic compounds in MSKE may be responsible for decreased AVs in SFO samples during accelerated storage at 70°C for 72hr. These results were in agreement with **Jayaprakash *et al.*, (2001)** noted that phenolic compounds donate hydrogen atoms to scavenge and stabilize lipid radicals. Also, the results in agreement with **Sikwese and**

Doudo (2007) who reported that the contribution of plant extracts to inhibit lipid oxidation due to their phenolic content. These results indicate that the MSKE have significant potential to be used as natural antioxidants to retard oil oxidation

Thiobarbituric Acid Reactive Substances value (TBARS)

TBARS values have been widely used as the marker for oxidative stress **(Yim *et al*., 2013).** The basic principle of the method is the reaction of one molecule of Malondialdehyde (MDA) and two molecules of thiobarbituric acid (TBA) to form a red MDA-TBA complex, which can be quantified spectro photometrically at 530 nm. In many cases, the TBA test is recommended for comparing samples of a single material at different states of oxidation. Currently, it is known that TBARS assay is a general method for the detection of lipid peroxidation **(Lee *and Yoon* 2013).** MDA is a component of the fatty acids with three or more double bonds. It is produced following the degradation of polyunsaturated fatty acids during lipid oxidation. The primary product, hydroperoxide reacts with oxygen to form MDA, which contributes to the off-flavor of the oil (**Zhang *et al*., 2010**). The TBA values are expressed as milligrams of MDA equivalents per kilogram of sample **(Zhang *et al*., 2010).** Results in Table 8 presents the changes recorded in TBARS values of the supplemented sunflower oil samples during accelerated storage at 70ºC for 72 hr, as effected by supplementation with BHT, MSKE. The results show that MSKE were able to inhibit the formation of TBARS at all concentrations. The TBARS values of all the supplemented samples and the control increased gradually from zero time to 72 hours. As the concentration of seeds extracts increased, the amounts of secondary products detected were lower. This result was in accordance with the obtained P-anV. The secondary products were determined to be lower at higher concentrations of seeds extracts. Results in Table 8 showed that, significant differences in TBARS values were observed between the control and sunflower oil samples containing seeds extracts or synthetic antioxidants, which slowed the

rate of lipid peroxidation. The TBARS value of SFO sample without antioxidants was 3.62 mg/kg, while the TBARS value of SFO sample containing 200 ppm of BHT was1.49 mg/kg. TBARS values of SFO samples containing 200,500, 1000 ppm MSKE were 1.79, 1.56 and1.42 mg /kg respectively, at 70^{o}C for72 hr. The inhibitory effect of the SFO samples at high concentration was stronger than that of BHT at 200ppm (1.49 mg/kg).

CONCOLUSION:

The highest level of MSKE (500, 1000 ppm) provides the best protection against primary and secondary oxidation of SFO samples during storage than synthetic antioxidant BHT. Showing that a high potential of mango seed kernel extract for use as sources of natural antioxidants.

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Table 1: Proximate composition of Mango seeds powder (g\100g) *

Nutrients	Moisture Mean ± SD	Protein Mean ± SD	Fat Mean ± SD	Fiber Mean ± SD	Carbohydrate Mean ± SD	Ash Mean ± SD
g / 100 g	8.1 ± 0.3	6.9 ± 0.25	12.5 ± 0.45	2.9 ± 0.2	67.3 ± 2	2.3 ± 0.2

* *Data obtained from at least three replicates.*

Table 2: Yield, total phenolic compounds and total flavonoids content of mango seeds kernel *

Samples	Mango seed
Yield % (g/100 g)	9.43±0.64
Total phenolics (mg gallic/ g dw)	21.29±0.85
Total flavanols (mg catechin / g dw)	22.20±0.66

** Data obtained from at least three replicates.*

Table 3: Antioxidant activity of MSKE compared with BHT*

parameters	Mango seed kernel	BHT	Ascorbic acid
DPPH Inhibition % (100µl/ml) 100ppm	96.86±3.2[a]	94.9±2.7[a]	91.53±3.3[b]
FRAP value(µmol BHT equivalent g^{-1})	4710.33±142[a]	-	-
Reducing power(mg/ml) (absorbance at 700 nm)	0.85±0.02[a]	0.36±0.03[b]	-

**Means in a row followed by the same letter are not significantly different at <0.05. Data obtained from at least three replicates.*

Table 4: yield of identified phenolic compounds (mg /100g dr matter) in mango seeds extract as determined by HPLC

No	phenolic compounds	(mg/100g dry extract)
1	Gallic	349.66
2	Pyrogllol	1337.66
3	Chlorogenic	1182
4	Protocatechuic	433.65
5	Catechol	202
6	Caffeic	7.1
7	Vanillic	21.6
8	Catechin	757
9	Caffeine	12.56
10	Ellagic	2613
11	Coumaric	12.9
12	Mangiferin	516
13	Sinapic acid	17.63
14	Ferulic acid	32.06
15	Salicylic	78.66
16	Kaempferol	4
17	Cinnamic	217
18	Quercetin	39.3
19	Myricetin	106.3
20	tannins	21.6

Table (5): Effect of MSKE at different concentration on PV * (meq.O_2/Kg SFO) during accelerated storage at70° for 72h

Time (hr)	Control	BHT 200 ppm	MSKE		
			200ppm	**500 ppm**	**1000 ppm**
0	0.81±0.01^{a}	0.81±0.02^{a}	0.81±0.02^{a}	0.81±0.03^{a}	0.81±0.02^{a}
4	2.10±0.04^{a}	1.89±0.05^{b}	1.83±0.03^{bc}	1.81±0.03^{c}	1.40±0.04^{c}
8	3.21±0.06^{a}	2.63±0.04^{b}	2.47±0.04^{c}	2.25±0.04^{d}	2.07±0.07^{e}
12	4.44±0.05^{a}	3.59±0.05^{c}	4.24±0.04^{b}	3.55±0.05^{c}	3.54±0.05^{c}
16	5.66±0.04^{a}	4.13±0.05^{c}	4.48±0.03^{b}	3.90±0.04^{d}	3.67±0.04^{e}
20	6.12±0.05^{a}	4.47±0.04^{b}	4.50±0.05^{b}	4.37±0.04^{c}	4.16±0.05^{d}
24	7.29±0.05^{a}	5.45±0.05^{b}	4.77±0.04^{c}	4.65±0.03^{c}	4.45±0.04^{d}
28	8.12±0.04^{a}	5.66±0.04^{b}	4.92±0.05^{c}	4.72±0.04^{d}	4.69±0.04^{d}
32	8.36±0.05^{a}	6.36±0.03^{b}	5.14±0.04^{c}	4.91±0.05^{d}	4.78±0.04^{d}
36	9.11±0.06^{a}	6.52±0.04^{b}	5.89±0.05^{c}	5.39±0.08^{d}	5.55±0.05^{e}
40	9.62±0.04^{a}	7.22±0.05^{b}	6.65±0.07^{c}	5.10±0.06^{d}	5.39±0.04^{e}
44	10.43±0.05^{a}	8.31±0.06^{b}	6.63±0.05^{c}	6.43±0.06^{d}	6.23±0.08^{e}
48	12.36±0.06^{a}	9.17±0.06^{b}	8.14±0.05^{c}	7.12±0.10^{d}	6.85±0.05^{e}
52	13.62±0.05^{a}	10.44±0.07^{b}	8.94±0.07^{b}	8.26±0.08^{c}	7.22±0.03^{d}
56	14.94±0.08^{a}	11.75±0.06^{c}	10.49±0.08^{b}	9.13±0.07^{d}	8.22±0.09^{e}
60	15.35±0.06^{a}	12.98±0.11^{c}	13.41±0.08^{b}	10.24±0.05^{d}	9.07±0.08^{e}
64	16.88±0.06^{a}	13.42±0.12^{b}	14.87±0.11^{c}	12.67±0.07^{d}	10.92±0.1^{e}
68	18.09±0.05^{a}	14.83±0.13^{b}	15.64±0.09^{c}	13.76±0.10^{d}	12.11±0.1^{e}
72	21.30±0.06^{a}	16.62±0.17^{b}	16.14±0.06^{c}	14.95±0.15^{d}	13.25±0.13^{e}

Means in a row followed by the same letter are not significantly different at <0.05.
Data obtained from at least three replicates.
** According to Egyptian standard (2013) PV of SFO should be less than 10 meq .O_2/Kg SFO.*

TABLE (6): Effect of MSKE at different concentration on p-Anisidine values of SFO during accelerated storage at 70°C for 72 h

Time (hr)	Control	BHT 200 ppm	MSKE		
			200ppm	**500 ppm**	1000 ppm
0	0.55±0.02^{a}	0.55±0.02^{a}	0.55±0.03^{a}	0.53±0.04^{a}	**0.53±0.03^{a}**
4	2.15±0.05^{a}	1.21±0.04^{cd}	1.38±0.03^{b}	1.29±0.06^{bc}	**1.16±0.06^{d}**
8	3.85±0.04^{a}	2.28±0.08^{b}	2.36±0.07^{b}	2.30±0.09^{b}	**2.22±0.14^{b}**
12	4.03±0.05^{a}	2.48±0.07^{cd}	2.65±0.04^{b}	2.53±0.03^{c}	**2.35±0.04^{d}**
16	4.34±0.08^{a}	2.74±0.08^{d}	2.93±0.07^{bc}	2.81±0.06^{cd}	**2.65±0.05^{e}**
20	4.71±0.08^{a}	2.98±0.10^{de}	3.15±0.09^{bc}	3.05±0.05^{cd}	**2.89±0.07^{e}**
24	5.11±0.10^{a}	3.19±0.10^{de}	3.34±0.05^{bc}	3.26±0.09^{cd}	**3.11±0.03^{e}**
28	5.79±0.09^{a}	3.56±0.09^{d}	3.72±0.04^{bc}	3.69±0.06^{cd}	**3.46±0.05^{e}**
32	6.24±0.07^{a}	3.99±0.06^{c}	4.13±0.03^{b}	4.02±0.04^{c}	**3.88±0.04^{d}**
36	6.85±0.06^{a}	4.38±0.07^{de}	4.59±0.08^{bc}	4.46±0.06^{cd}	**4.31±0.04^{e}**
40	7.48±0.05^{a}	4.89±0.07^{d}	5.01±0.06^{b}	4.90±0.05^{cd}	**4.76±0.06^{e}**
44	8.09±0.07^{a}	5.29±0.05^{d}	5.56±0.05^{b}	5.37±0.05^{c}	**5.22±0.06^{e}**
48	9.24±0.04^{a}	5.84±0.05^{d}	6.08±0.05^{b}	5.94±0.04^{c}	**5.77±0.05^{e}**
52	10.62±0.07^{a}	6.56±0.06^{de}	6.81±0.06^{b}	6.62±0.06^{cd}	**6.49±0.07^{e}**
56	11.84±0.04^{a}	7.12±0.06^{d}	7.39±0.07^{b}	7.20±0.05^{c}	**7.07±0.06^{d}**
60	12.46±0.07^{a}	7.42±0.05^{d}	7.65±0.05^{b}	7.48±0.04^{c}	**7.31±0.05^{e}**
64	13.13±0.07^{a}	7.82±0.04^{b}	7.85±0.03^{b}	7.71±0.04^{c}	**7.53±0.05^{d}**
68	13.98±0.06^{a}	8.34±0.04^{b}	8.37±0.04^{b}	8.27±0.06^{c}	**8.19±0.06^{d}**
72	**14.88±0.05^{a}**	**8.98±0.04^{b}**	**9.02±0.04^{b}**	**8.91±0.05^{c}**	**8.86±0.05^{c}**

Means in a row followed by the same letter are not significantly different at <0.05.
Data obtained from at least three replicates.

TABLE (7): Effect of MSKE at different concentration on acid values * (AV) of SFO during accelerated storage at 70°C for 72 h

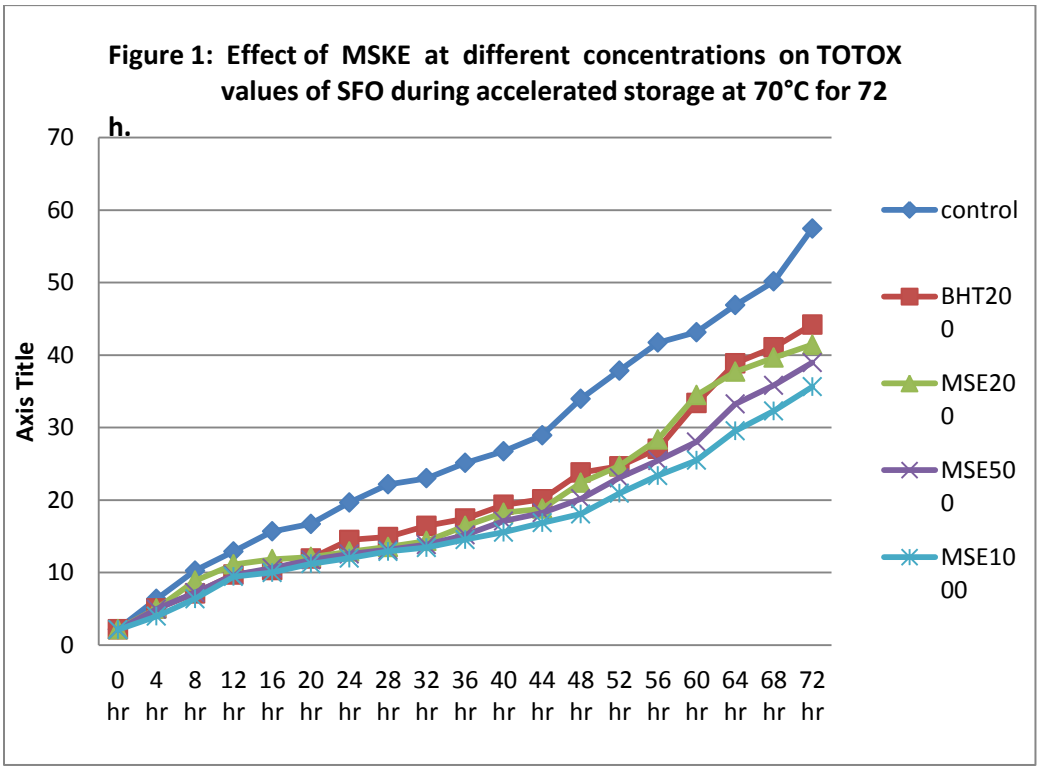
Time (hr)	Control	BHT 200 ppm	MSKE		
			200ppm	**500 ppm**	**1000 ppm**
0	0.09±0.01^{a}	0.09±0.01^{a}	0.09±0.01^{a}	0.09±0.01^{a}	0.09±0.01^{a}
4	0.15±0.02^{a}	0.11±0.01^{bc}	0.12±0.01^{b}	0.10±0.01^{c}	0.09±0.01^{c}
8	0.19±0.03^{a}	0.14±0.02^{bc}	0.15±0.01^{b}	0.13±0.01^{c}	0.10±0.01^{d}
12	0.26±0.02^{a}	0.17±0.02^{bce}	0.17±0.01^{bc}	0.16±0.02^{ce}	0.13±0.02^{e}
16	0.31±0.03^{a}	0.21±0.01^{b}	0.21±0.02^{b}	0.20±0.01^{b}	0.16±0.01^{c}
20	0.37±0.03^{a}	0.29±0.02^{b}	0.28±0.02^{bc}	0.24±0.02^{c}	0.19±0.03^{c}
24	0.41±0.02^{a}	0.34±0.01^{b}	0.33±0.02^{bc}	0.31±0.01^{c}	0.25±0.03^{d}
28	0.46±0.03^{a}	0.38±0.02^{b}	0.37±0.02^{bc}	0.34±0.02^{ce}	0.29±0.03^{e}
32	0.49±0.02^{a}	0.41±0.03^{b}	0.43±0.02^{b}	0.37±0.02^{c}	0.33±0.01d
36	0.53±0.03^{a}	0.45±0.03^{b}	0.46±0.03^{b}	0.41±0.02^{c}	0.37±0.01d
40	0.57±0.03^{a}	0.48±0.03^{b}	0.47±0.03^{bc}	0.44±0.02^{cd}	0.40±0.03^{d}
44	0.61±0.02^{a}	0.51±0.03^{b}	0.50±0.03^{b}	0.49±0.02^{bc}	0.44±0.03^{c}
48	0.68±0.02^{a}	0.54±0.03^{b}	0.53±0.03^{b}	0.51±0.02^{bc}	0.46±0.03^{c}
52	0.77±0.05^{a}	0.57±0.03^{bc}	0.59±0.02^{b}	0.54±0.03^{cd}	0.51±0.03^{d}
56	0.89±0.03^{a}	0.61±0.03^{bc}	0.64±0.03^{b}	0.57±0.02^{cd}	0.54±0.03^{d}
60	0.94±0.04^{a}	0.65±0.04^{bc}	0.69±0.02^{b}	0.63±0.03^{cd}	0.57±0.03^{d}
64	1.07±0.05^{a}	0.70±0.03^{b}	0.73±0.03^{b}	0.70±0.03^{b}	0.61±0.04^{c}
68	1.15±0.04^{a}	0.74±0.04^{b}	0.78±0.03^{b}	0.75±0.03^{b}	0.68±0.03^{c}
72	1.28±0.04^{a}	0.79±0.03^{c}	0.91±0.03^{b}	0.89±0.02^{b}	0.76±0.02^{c}

Means in a row followed by the same letter are not significantly different at <0.05.
Data obtained from at least three replicates.
** According to Egyptian standard (2013) AV of SFO should be less than0.6 mg KOH/Kg SFO.*

TABLE (8): Effect of MSKE at different concentration on TBARS of SFO during accelerated storage at 70°C for 72 h

Time (hr)	Control	BHT 200 ppm	MSKE		
			200ppm	**500 ppm**	**1000 ppm**
0	0.08±0.01^{a}	0.08±0.01^{a}	0.08±0.01^{a}	0.08±0.01^{a}	0.08±0.01^{a}
12	0.41±0.02^{a}	0.17±0.01^{d}	0.29±0.02^{b}	0.21±0.02^{c}	0.13±0.02^{e}
24	0.68±0.02^{a}	0.31±0.01^{d}	0.47±0.02^{b}	0.34±0.01^{c}	0.23±0.02^{e}
36	1.42±0.03^{a}	0.42±0.02^{d}	0.62±0.02^{b}	0.47±0.02^{c}	0.39±0.03^{d}
48	2.12±0.04^{a}	0.73±0.02^{d}	0.95±0.03^{b}	0.80±0.03^{c}	0.69±0.03^{d}
60	2.73±0.03^{a}	1.17±0.03^{d}	1.36±0.04^{b}	1.24±0.03^{c}	1.10±0.03^{e}
72	3.62±0.04^{a}	1.49±0.03^{d}	1.79±0.04^{b}	1.56±0.03^{c}	1.42±0.03^{e}

Means in a row followed by the same letter are not significantly different at <0.05.
Data obtained from at least three replicates.



تقييم مستخلص أنوية بذور المانجو كمضادات أكسده طبيعيه غنيه فى الفينولات

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٢) قسم علوم الاغذية – كلية الزراعة جامعة الزقازيق

الملخص العربى

زيت بذرة عباد الشمس من المصادر الغنية بحمض اللينوليك والذى يعتبر من اكثر العوامل المسببة لأكسدة الزيوت الغذائية . عادة ما يضاف للزيوت المواد المضاده للأكسدة لتحسين الثبات الأكسيدى لها. أنوية بذور ثمرة المانجو من المصادر الغنية بمضادات الأكسدة الطبيعية . بلغت كمية المخلفات (الناتجة عن تصنيع عصائر المانجو) فى مصر سنة ٢٠١٢ نحو٣٩٣٢ طن من المخلفات الحيوية، و التخلص منها يمثل مشكلة كبيرة. فى هذا البحث تم إضافة ثلاث تركيزات من المستخلص الإيثانولى لأنوية بذور المانجو(٢٠٠،٥٠٠، ١٠٠٠مليجرام) لكل كيلو جرام من زيت عباد الشمس، مقارنة بإحدى مضادات الأكسدة الصناعية (BHT) بتركيز٢٠٠مليجرام/كيلوجرام زيت عباد الشمس، وأيضا بالمقارنة بزيت عباد الشمس بدون أى إضافات . تم تتبع تطورات عملية الأكسدة السريعة للزيت (على فترات زمنية ثابتة كل ٤ ساعات) أثناء التخزين على درجة ٧٠ مئوي لمدة ٧٢ ساعة، وذلك بإستخدام إختبارات الثبات الأكسيدى مثل: قياس قيم كل من رقم البيروكسيد، ورقم البارأنيسيدين، ورقم الأكسدة الكلية ، وقياس رقم حموضة الزيت وقياس قيم حمض الثيوباربيتيوريك. أوضحت النتائج: أنه بالتحليل الكروماتوجرافى السائل(HPLC) لمستخلص أنوية بذورثمار المانجو(MKSE) وجد أنها تحتوى على 20 من المركبات الفينولية الكلية. كما وجد أنه عند إضافة١٠٠مليجرام من المستخلص/ كيلوجرام من الزيت كان له إعلى نشاط للتخلص من الشوارد الحرة (٩٦,٨٦%) بالمقارنة بكل من حمض الأسكوربيك (٩١,٥%) ومضاد الأكسدةBHT (٩٥,٢%). كما وجد أن إستخدام النسب العالية من المستخلص MKSE (١٠٠٠،٥٠٠ ملجم/كيلوجرام زيت) أدى الى أحسن وقاية ضد الأكسدة الأولية والثانوية لزيت عباد الشمس عند تخزينه على درجة٧٠ مئويه لمدة٧٢ ساعة. كما أظهرت النتائج وجود درجة كبيره من التوافق بين إختبارات الثبات الأكسيدى والتى أوضحت أن أعلى كفائة لحماية زيت عباد الشمس أثناء التخزين كان ترتيبها تبعا للإضافة بالمليجرام كما يلى:
١٠٠٠ MKSE > MKSE500> BHT200 >MKSE200 > عينة الكنترول(بدون إضافات)

الكلمات المفتاحية: *بذور المانجو – زيت دوار الشمس – ثبات الأكسدة*