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Utilization of Mango Peels as a Source of Polyphenolic Antioxidants

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ABSTRACT

Mango peels (MP) is a rich source of phenolic compounds with antioxidant activity. In order to contribute to future applications of this material, this work optimized the extraction process of phenolic compounds present in MP, and the following parameters were evaluated: total phenolic content, antioxidant activity, the quantification of individual phenolic compounds by HPLC for methanolic extracts and antimicrobial activity. A maximum extraction yield of 12.14% and total phenolic content of 515.62 mg/100g (dry weight) were obtained with methanol. The antioxidant activity of mango peel extracts on the stability of biscuit lipid oxidation was also determined. It is noted that increasing the concentration of extract improves the capacity to inhibit lipid per-oxidation. Thus, the above extract could be used as food ingredients.

Key words: Mango peels, antioxidant activity, antimicrobial activity, phenolic compounds, lipid oxidation, biscuit.

Introduction

Mango (*Mangifera indica* L.) is one of the most important tropical fruits consumed in fresh or processed form globally. Due to mango is a seasonal fruit, about 20% of the production is processed to elaborate puree, nectar, leather, pickles and canned slices, among others, which have worldwide popularity (Kim *et al*., 2010). Peels are the most important by-products of mango processing, approximately 7-24% of the fruit (Wu *et al*., 1993). Since these wastes are a disposal problem, attempts have been made to efficiently utilize this by-product (Sogi *et al.,* 2013).

Mango waste contains significant amounts of phyto-chemicals, being rich in pectin, cellulose, hemi-cellulose, lipids, protein, polyphenols and carotenoids with excellent antioxidant and functional properties, which makes it suitable to be used for value-added applications in functional foods and nutraceuticals (Ajila *et al.,* 2007 and Manthey and Perkins-Veazie, 2009). Moreover, dried mango peels products can improve the sensory properties and oxidative stability of fat rich products (Abdalla *et al*., 2007).

Artificial antioxidant compounds such as butylated hydroxyl-toluene (BHT) and butylated hydroxyl-anisole (BHA) are commonly used in processed foods. It has been reported that these compounds have some side effects and are carcinogenic (Ajila *et al.,* 2010). Natural antioxidant present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic value. The increased interest in natural antioxidants has led to the antioxidant evaluation of many species of fruits, vegetables, herbs, spices and cereals (Liyana-Pathirana and Shahidi, 2005).

Throughout processing of mango, peels are the major by-product. As mango peels are not currently utilized for any commercial purpose, it is discarded as a waste and becoming a source of pollution. Therefore, the objective of the present study was to evaluate the antioxidant and antimicrobial activities of mango peels extract and estimate the major antioxidants such as polyphenols contents. Moreover, their impact on oxidation of fatty food product such as fat-biscuit was assessed.

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Materials and Methods

Materials:

1- Samples:

Mango peels (*Mangifera indica* L.) was obtained from El-Marwa Food industry (October 6th, 2014, Egypt). Commercial soft wheat flour (72% extraction), bakery fat, sugar powder, eggs and skimmed milk powder were purchased from the North Cairo Flour Mills Company, Egypt. Food grade dextrose, sodium chloride, sodium bicarbonate and ammonium bicarbonate were used in biscuit processing.

2- Chemicals and Reagent:

Standard of phenols: gallic acid; caffeic acid; sinabic acid; chlorogenic acid; syriginc acid; ferulic acid; isoferulic acid; protocatecluic acid; vanillic acid; isovanilic acid; tyrsol; qurecetin; apignin and catechin; butylated hydroxyl-anisole (BHA); DPPH (2,2-Diphenyl-1-picrylhydrazyl)and Thiobarbituric acid (TBA) were obtained from Sigma Chemical Co., Germany. Folin-Ciocalteu reagent and TPTZ [2,4,6-Tris (2- pyridyl)-s-triazine] were purchased from Fluka Chemical Co. All other chemicals and solvents used were analytical grade.

Methods:

1- Analytical Methods:

1.1. Chemical composition:

Moisture; protein; fat; ash; and carbohydrate content of mango peels waste were determined according to the methods described by AOAC (2000). All determinations were performed in triplicates and the mean values were reported.

1.2. Mineral determination:

The mango peels samples were subjected to mineral analysis following the method of AOAC (2000). The mineral elements K, Cu, Fe, Mn, Mg, Ca, and Zn were measured after wet digestion of the material (HNO_3) and subsequent determination using an atomic absorption spectrometer Varian Spectr AA 220 Fast Sequential (Varian Inc., Palo Alto, CA, USA). Cr and Pb metals were determined by ICP-MS using Thermo Elemental X 7 equipment.

1.3. Preparation of dried extract of samples:

Extracts of sample were prepared according to the method of Zia-ur-Rehman *et al*. (2004). Fresh mango peels (MP) were washed, drained and dried in a hot air oven at 50ºC. The dried samples were ground into a fine powder in a mill (crushed in a laboratory-size mill). The materials that passed through a sieve were retained for use. Two grams of dried ground sample were extracted with 20 ml of organic solvents (ethanol, methanol, and acetone) overnight in a shaker at room temperature. The extracts were filtered through filter paper (Whatman No.1) and the residues were re-extracted under the same conditions. The combined filtrate was evaporated in a rotary evaporator (Buchi Rotavapor Switzerland) below 45 ºC. The extracts obtained after evaporation of organic solvent were used for calculating the percent yield of the natural antioxidant under investigation.

1.4. Determination of Total Phenolic Content (TPC):

The total phenolic content was determined according to the method reported by El-Hamzy *et al*. (2013) in all the extracts. Aliquots of 50 µl of each diluted extract were mixed with 1950 µl water in a

10 ml test tube. One ml of Folin-Ciocalteu reagent was added and the test tube was vigorously shaken. Immediately, 5 ml of 20% sodium carbonate solution was added, the volume of the mixture was brought up to 10 ml and shaken thoroughly again. After 20 min, the absorbance of the mixture was read at 735 nm. The spectrophotometer was zeroed with a blank cuvette. Phenolic contents of the extracts were calculated on the basis of the standard curve for gallic acid. The results were expressed as mg gallic acid equivalents per 100 g of the dry weight of the plant materials.

2. HPLC Analysis:

Phenolic compounds in mango peels extract were performed by HPLC analysis using the method described by Dragovic-Uzelac *et. al*. (2005).

3. **Determination of Antioxidant Activity:**

3.1. Radical scavenging activity (RSA %) assay:

Free radical scavenging activity (RSA) of the sample was measured using the method described by El-Hamzy and Yaseen (2014). An aliquot of the sample solution (40 µl) was mixed with 2.9 ml of 0.1 mM DPPH in methanol solution, incubated for 30 min at 25°C in dark; the decrease in the absorbance at 517 nm was measured. Methanol was used as blank. Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation:

Scavenging activity (%) = $1 - (A_s/A_O) \times 100$.

Where*:* A_s is the Absorbance of sample and A_O is the Absorbance of control.

Control: 40 µl of methanol mixed with 2.9 ml of DPPH methanol solution.

3.2. The ferric reducing antioxidant power (FRAP) measurement:

Antioxidant activity was measured using the ferric reducing antioxidant power reported by Benzie and Strain, (1996). The FRAP assay reduces ferric ion (Fe^{+3}) to ferrous ion (Fe^{+2}). (Fe^{+2}/TPTZ) forms a blue complex color, which increases the absorption at 593 nm. The FRAP reagent contained 2.5 ml of a 10 mmol/l TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mmol/L HCL plus 2.5 ml of 20 mmol/L $FeCl_3.6H_2O$ and 25 ml of 0.3 mol/L acetate buffer (pH 3.6) and was freshly prepared and warmed at 37°C prior to use. Aliquots of 0.1 ml sample solution were mixed with 3 ml FRAP reagent and the absorbance of reaction mixture at 593 nm was measured spectrophotometrically at 593 nm after incubation at 37 °C for 10 min. A standard calibration curve was prepared using freshly prepared ammonium ferrous sulphate (25–1600 µM Fe^{+3}).

4. Determination of Antimicrobial Activity:

The antimicrobial effect of mango peels extract on growth of gram-negative bacteria (*Escherichia coli O157:H7, Salmonella spp*.), gram-positive bacteria (*Staphylococcus aureus, Streptomyces spp., Lactobacillus Plantarum*) and fungi (*Saccharomyces cerevisiae, Aspergillu sniger*) was studied.

The antimicrobial activity of the tested strains was detected using the agar diffusion test according to the method reported by Rauha *et al*. (2000). 10ml sterile assay agar was added to each of two Petri dishes with slow shaking. Warmed agar was inoculated with 0.5ml active bacterial culture (1x10 CFU/ml) and allowed to harden in a refrigerator at 4°C for 1 h. After wards, three equidistant holes were made in the agar using sterile cork borers and 100µl MP extract was added on the top of inoculated agar layer then dried at 25°C for 30 min. Plates were kept at 4°C for 1 h then incubated at 37°C for 24-48 h. At the end of this period, inhibition zones formed on medium were accurately measured in mm.

5. Technological Application:

5.1. Preparation of biscuits:

Biscuits were prepared by addition of different levels of natural antioxidant extracts (0, 0.5%, 1%, 2% and 3%) of mango peels according to the procedure No (10–50D) described in AACC (2000). With comparative purposes, the use of synthetic antioxidant (BHA, 200 ppm) was also evaluated. Finally, a control was prepared with biscuits without addition of natural or synthetic antioxidants.

5.1.1.Formula for elaboration:

The formula used to elaborate biscuits was as follows: wheat flour (100 g) 72% Extraction, 30 g sugar (powdered sucrose), 15 g butter milk, 24 g eggs, 0.93 g salt (sodium chloride), 1.11 g of sodium bicarbonate, 3 g ammonium bicarbonate, 18 g skimmed milk powder, 3 g baking powder, 0.5 g vanilla and the required volume of water.

5.1.2. Processing of biscuits:

The natural antioxidant extract (MP) was blended with the fat and the emulsion was mixed with sugar and creamed for 3-4 min in a Hobart mixer. Sodium bicarbonate, sodium chloride and ammonium bicarbonate were dissolved in water and added to the cream. Skimmed milk powder was made into suspension with water and transferred to the cream. The contents were mixed for 6 min at 125 rpm (speed 2) to obtain a homogeneous cream. Sieved flour with eggs, baking powder and vanilla were added to the above cream and mixed for 2-3 min at 61 rpm (speed 1). Biscuit dough was sheeted to a thickness of 3.5 mm, cut using a circular mold (51 mm diameter) and baked at 205 $^{\circ}$C for 8-10 min. After baking, biscuits were cooled to room temperature and were packed in polyethylene pouches, sealed and stored until further analysis and testing.

5.2. Evaluation of Biscuits Quality:

5.2.1. Physical characteristics of biscuits:

Diameter (W) was measured by Boclase (HL 474938, STECO, Germany). Also, volume (V) and thickness (T) of biscuits were determined according to standard methods AACC No. 10-50D (2000). The spread ratio was calculated by dividing value of diameter (W) by value of thickness (T) of biscuits.

5.2.2.Color measurement of biscuits:

Objective evaluation of surface color of biscuits was measured. Hunter a^* (redness), b^* (yellowness) and L^* (brightness) parameters were measured with a color difference meter using a spectro-colorimeter (Tristimulus Color Machine) with the CIE Lab color scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode. The instrument was standardized each time with white tile of Hunter Lab Color Standard (LX No.16379): X= 72.26, Y= 81.94 and Z= 88.14 (L^* = 92.46; a^* = -0.86; b^* = -0.16) (Sapers and Douglas, 1987). Average of three values was taken for each set of samples

5.2.3. Sensory evaluation of biscuits:

Sensory characteristics of biscuits were evaluated according to the method described by Reddy *et al*. (2005). Sensory evaluation of biscuits (freshly prepared and stored) was conducted to determine the acceptability of the product prepared by addition of natural antioxidant extracts. Fifteen panelists were selected from the Staff of the Food Technology Department, National Research Center in Egypt. Sensory scores for different attributes like color, texture, taste, odor and overall quality were obtained (Evaluation of samples on a scale from zero to twenty).

5.3. Chemical Analysis of Biscuits:

5.3.1. Determination of peroxide value (PV):

Peroxide value of fat was determined according to AOCS Official Method Cd 3d-63 (1996).

5.3.2. Free Fatty Acids (FFA):

Titrimetry was classically used to determine the acid value (free fatty acid content) of oils and fats. Acid value was determined according to the method described in AOCS Official Method Cd 8-53 (1996).

5.3.3.Thiobarbituric Acid Test (TBA):

Malonaldehyde compound as an index of secondary lipid peroxidation was determined using the method described by Norhidayah *et al*. (2011).

6. Statistical analysis:

All data are presented as the mean ± SD. One-way analysis of variance (ANOVA), unpaired and paired t-tests were performed using SAS version 8.1. $P<0.05$ was the level of significance (SAS, 1996).

Results and Discussion

1. Composition of Mango Peels Waste:

The chemical composition of the mango peels waste is recorded in Table 1. Composition analysis of mango peels showed 12.07% moisture, 5.34% of crude protein, 2.42% of fat, 3.06% of ash, 16.62 of crude fiber and 72.56% of carbohydrates by difference. These results are in agreement with Ajila *et al*. (2010) and Martinez *et al*. (2012). These results indicate that mango peel waste could be used as a source of protein, fiber and carbohydrate.

Table 1: Chemical composition of Mango peels waste

Parameters	Dry weight (%)
Moisture*	12.07±0.06
Crude protein	5.34 ±0.05
Fat	2.42 ±0.13
Ash	3.06 ±0.01
Crude fiber	16.62±0.14
Carbohydrates**	72.56±0.67

*The results are the mean ± SD of three replicates. * On fresh weight basis. **By difference.*

2. Minerals Content:

The results of the mineral analysis shown in Table 2, K, Ca, and Mg were presented in higher concentrations. The high levels of potassium can lead to a mineral balance that favors hypertension control. A diet rich in potassium lowers blood pressure and consequently the risk of morbidity and mortality due to cardiovascular diseases; in addition, potassium intake can decrease urinary calcium excretion and consequently reduce the risk of developing osteoporosis (National Academy of Sciences, 2011).

Comparing with Dietary Reference Intakes (DRI) (Cetin *et al*., 2011), the amount of iron found in this study (388 mg/kg) supplies the adult daily requirements for iron (8 mg/day for men and 8 to 18 mg/day for women). For zinc, the recommended daily intake is 11 mg for men and 8 mg for women. The amount of zinc found was 18.65 mg/kg. These minerals are considered essential for the human

body. Iron, among other functions, is associated with the production of blood cells, and zinc is essential for the immune system.

Table 2: Content of minerals in mango peels waste

Minerals	Results
K (g/kg)	13.05±0.85
Ca (g/kg)	3.13±0.01
Mg (g/kg)	1.23±0.05
Fe (mg/kg)	43.50±2.05
Zn (mg/kg)	11.95±0.23
Cu (mg/kg)	9.25±0.52
Mn (mg/Kg)	8.45±0.11
Cr (µg/g)	<5.0
Pb (µg/g)	<5.0

The results are the mean ± SD of three replicates.

3. Extraction yield:

As shown in Table 3, the extraction yields of mango peels extract by three different solvents varied from 6.33 to 12.14% on dry weight (DW). The highest yield was observed with methanolic extraction followed by ethanolic and acetone. It should be noted that variation in the yields of various extracts is attributed to differences in polarity of compounds present in plants, and such differences have been reported in the literature on fruit seeds (Jayaprakasha *et al*., 2001).

4. Total phenolic compounds:

Polyphenols one of the major groups of compounds acting as primary antioxidants or free radical terminators. Hence the total amount of phenolic compounds, as well as the content of individual phenolic acids in mango peels extracts was determined. The content of total phenolics quantified in different solvent extracts of mango peels is illustrated in Table 3.

Table 3: Extraction yield and total phenolic contents of mango peels extract of some organic solvents

Sample	Solvent	Extraction yield (%)	Total phenolic contents mg GAE 100 /g (DW)
Mango peels	Methanol	12.14±0.81	515.62 ± 2.00
	Ethanol	10.79±0.62	382.61 ± 1.02
	Acetone	6.33±0.15	286.51 ± 0.97

The results are the mean ± SD of three replicates.

Generally, the total phenolic content of MP was found to be 515.62, 382.6 and 286.51 mg/100g (DW) for methanol, ethanol and acetone extracts, respectively. The results revealed that methanol and ethanol were better ($P < 0.05$) than the acetone at extracting phenolic compounds owing to their higher polarity and good solubility for phenolic components from plant materials (Wieland *et al*., 2006). The values of total phenolic contents of MP extracts in this study were similar to the amounts reported by Ribeiro *et al*. (2008); Ajila *et al*. (2010) and Kim *et al.* (2010).

5. HPLC Analysis of Mango Peels Extract:

The phenolic compounds (mg/100g) of methanolic extracts from MP were analyzed by high performance liquid chromatography (HPLC) against standard compounds. Data in Table 4 showed that gallic acid was the highest amount of total polyphenol compounds found in MP. On the other hand, chlorogenic acid was the lowest amount of total polyphenols found in peels. Based on the HPLC analysis, and by comparison with standards, 9 phenolic compounds could be identified in MP extract. From the above- mentioned data it could be concluded that, mango peels proved that it is a good source of phenolics. This result is comparable with another research (Ajila *et al*., 2010).

On the other hand, the differences in phenolic acid composition in mango peels samples maybe attributed to variations in cultivars, growing conditions, and extraction solvents and techniques used by different scientists (Ajila *et al*., 2010 and Kim *et al*., 2010).

Among the bound flavonoids identified, quercetin and catchin were the major flavonoids. Presence of quercetin in MP as free flavonoids was reported by several studies (Ribeiro *et al*., 2008; Ajila *et al.,* 2010 and Mostafa, 2013).

Table 4: Identification of phenolic compounds of MPE using HPLC analysis.

Individual phenolic acids	Concentration (mg/100g)
Gallic acid	241.9±1.01
Caffeic acid	ND
Sinabic acid	ND
Chlorogenic acid	18.11
Syriginc acid	ND
Ferulic acid	101.06
Isoferulic acid	ND
Protocatecluic acid	47.42
Vanillic acid	25.31
Isovaniliic acid	22.25
Tyrsol	19.23
Qurecetin	65.59
Apigenin	ND
Catechin	53.57

The results are the mean ± SD of three replicates. ND= not detected.

6. Antioxidant Activity:

6.1. DPPH Radical-Scavenging Activity:

The antioxidant activity depends on the type and polarity of the extracting solvent, the isolation procedures, purity of active compounds, as well as the test system and substrate to be protected by the antioxidant (Meyer *et al*., 1998). The scavenging activity of MPE against DPPH is shown in Table 5. The mango peels extracts showed relatively high antioxidant activity (90.12 ±0.72%), which is much higher to that obtained with BHA (65.20±0.49%). The increase in the free radical scavenging maybe attributed to increase in the contents of polyphenols as shown in Table 5. This may be attributed to its hydrogen donating ability. This concept is in accordance with Ribeiro *et al*. (2008) and Ajila *et al*. (2010).

6.2. Ferric-Reducing Antioxidant Power (FRAP):

The FRAP assay is commonly used to study the antioxidant capacity of plant materials. The antioxidant capacity of plant extracts is determined by the ability of the antioxidants to reduce ferric to ferrous iron using the FRAP reagent (Benzie and Strain, 1996). As shown in Table 5, the mango peels extract exhibited the highest reduce ferric to ferrous iron in FRAP reagent (57.06 mmol Fe_2SO_4 /100g) compared to the BHA (52.46 mmol Fe_2SO_4 /100g).

Table 5: Antioxidant activities of MPE and BHA

Tested material	% Radical scavenging[a]	FRAP (mmol Fe_2So_4/100g)
Mango peels	90.12 ±0.72	57.06 ±0.64
BHA	65.20±0.49	52.46±0.53

The results are the mean ± SD of three replicates. [a]*: DPPH assay.*

On the other hand, in the FRAP assay, not all reductants that are able to reduce Fe^{+3} are antioxidants and some antioxidants that can effectively scavenge free radicals may not efficiently reduce Fe^{+3}. The FRAP assay is an indirect test of total antioxidant power and not specific for radical scavenging as the DPPH assay (Ou *et al*., 2002; Wangcharoen and Morasuk, 2008).

7. Antimicrobial Effect of Mango Peels Extract on Microorganisms:

The use of natural antimicrobial compounds in foods has gained much attention by the consumers and the food industry. These compounds can degrade the cell wall and disrupt cytoplasmic membrane-integrated enzymes, which may eventually lead to cell death (Shan *et al*., 2007). The mango peels extract showed various degrees of inhibition against the growth of investigated microorganisms as shown in Table 6. Thus, with the exception of *Aspergillus niger*, which exhibited the lowest antimicrobial value, the results showed that there were significant differences ($p < 0.05$) among inhibition effects on the tested microorganisms, with inhibition zones ranging from 5.4 to 12.3 mm. Hence, the highest inhibition was obtained for *Streptomyces spp.* (inhibition zone 12.3 mm), followed by *Staphylococcus aureus* (12.0 mm), while the lowest inhibition was for *Aspergillus niger* (1.1 mm).

Consequently, these results evidence the presence of antimicrobial phenolic compounds in MP extracts, meanwhile the study provides helpful information on the utilization of mango peel as natural antimicrobials in foods. This by-product could be of great benefit from economic and environmental perspectives as sources of low cost natural antimicrobials (Taveira *et al*., 2010).

Table 6: Effect of mango peels extract on growth of the tested microorganisms

Sample	Inhibition zone (mm)						
	Gram-negative		Gram-positive			Fngi	
	Escherichia coli	*Salmonella spp*.	*Staphylococcus aureus*	*Streptomyces Spp.*	*Lactobacillus Plantarum*	*Saccharomyces cervisiae*	*Pergillusniger*
MP	9.3±0.22	11.0±0.21	12.0±0.33	12. 3±0.38	11.5±0.29	5.40±0.41	1.1±0.12

The results are the mean ± SD of three replicates.

8. Using of MPE in Preparing Biscuits:

8.1.Physical Measurements of Biscuits:

The effect of replacing 0.5, 1, 2 and 3 % of wheat flour with MPE on physical properties of biscuits was studied and the data are presented in Table 7. The results showed that all selected MPE treatments caused significant increase in biscuit diameter as compared with 6.86 cm for control. The largest diameter (7.08 cm) was found with MPE at 3% without significant difference that combined with 1 and 2% MPE. Using of BHA (200 ppm) in preparing biscuit gave less diameter value than the control (6.76 cm), without significant difference with control sample. It was also clear that using MPE at all concentrations in biscuit preparation resulted in decrease in thickness when compared with 0.89 cm for control. This observation is in agreement with the results observed by Sudha *et al*. (2007) who reported a decrease in diameter and increase in breaking strength of biscuits upon addition of cereal bran and decrease in thickness upon addition of barley bran.

The changes in diameter and thickness of biscuits are reflected in spread ratio values, which consistently increased in all samples compared to the control. Also, it was observed that partial replacing of 3% wheat flour by MPE recorded the highest value (9.69) when compared with control (7.97) and 8.45 for BHA (200 ppm).

8.2.Color of Biscuits:

Color is one of the parameters used for quality control during baking, because brown pigments appear as browning and caramelization reactions progress (Moss and Otten, 1989).

Table (8) shows the values of the color parameters L^*, $a^*$and b^* of the biscuit samples containing the different concentration of MPE. It can be seen in the same Table that all the biscuit samples stand in the clear zone, with values of brightness (L^*) above 50. In all cases the values of a^* are positive, thus indicating the predominance of the color red over the green. Also, the coordinate b^* assumes positive and relatively high values, indicating a strong predominance of the yellow coloration, in disfavor of the blue.

In general, the L^* and a^* values decreased with the increase in the levels of MPE compared to control. The change in b^* value, which indicates the yellowness, gradually decreased with the increase

in MPE level. Therefore, due to the enzymatic browning, brightness and yellowness of the biscuits may be decreased as reported by Ajila *et al*. (2008).

8.3.Sensory Evaluation of Biscuits:

Sensory evaluation studies showed that the surface color of biscuits containing up to 1% of MPE was as acceptable as those of control biscuits (Table 9). It was noticed that incorporation of 3% MPE in biscuits caused relatively dark color, the increase in darkness was reflected on L^* values (Table 8) which may be due to the enzymatic browning. Aziah and Komathi (2009) reported that mango peels flour imparts a dark brown color to crackers; this might have given the panelists an impression of over-baked product, thus affecting their likings. Results also showed that biscuits had acceptable texture with increased levels of MPE up to 1% as compared to the BHA and control. Also, the textural profile plays an important role in justifying the overall acceptability of biscuits. The taste and odor of biscuits were changed with incorporation of MPE. However, at levels of 2% and 3% of MPE the biscuits had a slight bitter taste which may be due to high polyphenols content. These data are in agreement with Ajila *et al*. (2008).

With regard to overall quality, it could be observed that biscuits incorporated with MPE up to 1% showed good scores. The lower score values of MPE could be due to the unattractive color and the unpleasant taste. It could be concluded that biscuits with acceptable overall quality can be prepared by substituted 0.5% of wheat flour with MPE.

Table 7: Influence of mango peel extracts with different levels on the physical measurements of biscuits

Treatments	Diameter W (cm)	Thickness T (cm)	Spread ratio W/T
Control	6.86±0.04	0.89±0.01	7.97±0.06
BHA	6.76±0.03	0.8±0.09	8.45±1.11
MPE (%)			
0.5	7.06±0.08	0.8±0.03	8.82±0.71
1	7.03±0.05	0.76±0.04	9.25±0.53
2	7.06±0.08	0.76±0.04	9.28±0.71
3	7.08±0.07	0.73±06	9.69±0.68

The results are the mean ± SD of three replicates.

Table 8: Influence of mango peels extracts with different levels on the color parameters of biscuits

Treatments	L^*	a^*	b^*
Control	63.58±2.64	14.04±0.01	25.41±0.26
BHA	56.57±0.02	11.85±1.17	24.5±0.01
MPE (%)			
0.5	60.72±1.54	10.14±0.44	23.43±0.30
1	59.16±1.79	7.85±0.86	22.73±0.32
2	56.35±1.41	7.43±0.72	22.43±0.22
3	53.04±0.64	6.61±0.25	21.40±0.24

The results are the mean ± SD of three replicates. L, lightness, a*, redness, b*, yellowness*

Table 9: Influence of mango peels extracts with different levels on Sensory evaluation of biscuits

Treatments	Color	Odor	Taste	Texture	Overall quality
Control	17.8±1.37	18.13±1.40	17.2±1.82	18.13±1.76	17.93±1.22
BHA	16.46±1.84	17.00±1.92	15.73±2.52	16.66±1.75	16.26±2.25
MPE (%)					
0.5	15.33±1.33	15.4±2.41	15.66±1.36	15.73±1.43	16.53±2.60
1	13.53±1.72	14.00±2.11	14.73±1.15	14.33±1.42	14.73±2.08
2	12.93±1.17	13.6±1.93	13.00±1.36	13.06±1.24	13.6±2.29
3	12.86±1.16	12.66±2.08	12.93±1.23	13.00±1.22	13.8±1.84

The results are the mean ± SD of three replicates.

8.4. Peroxide Value (PV):

Peroxide value (PV) is a measure of the concentration of peroxides and hydro-peroxides formed in the initial stages of lipid oxidation. Peroxide value is one of the most widely-used tests for the measurement of oxidative rancidity in oils and fats (Rossel, 2005). A continuous increase in PV with the increase in storage period was observed for all different treatment groups (Fig. 1). Initially rate in PV was very slow, but it started to increase after two months of storage and tended to increase further with the increase in storage period. Biscuits samples without antioxidant (control) had the highest peroxide value 21.84 meq kg^{-1} at the end of the storage period compared to other formulation groups. Peroxide value was in the range of 1.36±0.01-11.15±0.02 meq kg^{-1} for using MPE, whilst it was 1.45±0.03 - 15.13±0.05 meq kg^{-1} for biscuits containing 200 ppm of BHA. A significant difference ($P < 0.05$) in PV was observed between the control and biscuits samples containing extracts and synthetic antioxidants, which slowed the rate of peroxide formation. The PV of biscuits containing 0.5, 1, 2, 3% of MPE and BHA (200 ppm) were found to be 11.15±0.06, 8.195±0.04, 6.74±0.03, 4.45±0.01 and 15.13±0.05 meq kg^{-1} after 6 months of storage, respectively.

From the same Fig, it could be observed that the peroxide value of biscuit samples decreased by increasing mango peels extract concentration from 0.5 to 3%. This data suggests the superiority of antioxidant activity of mango peels extracts over synthetic antioxidants. The results also indicate that the presence of mango peels extracts in biscuits was able to retard the formation of peroxides in the lipid, similar to the observations of Reddy *et al*. (2005).

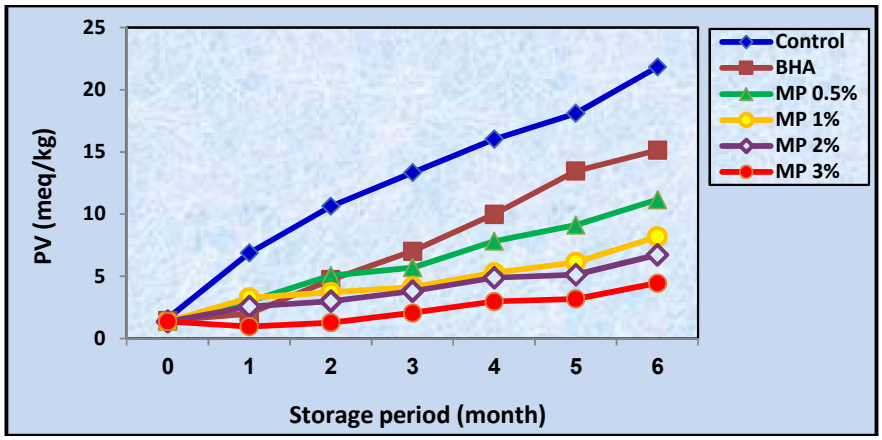

Fig. 1: Response of BHA and different MP extracts on PV of stored biscuits for 6 months.

8.5. Free Fatty Acids (FFA):

Generally, the antioxidants are mainly used in lipids to delay the accumulation of primary oxidation products and thus to improve the oxidative stability. The primary products of lipid per-oxidation are hydro-peroxides, which are generally referred to as peroxides. Therefore the results of PV estimation give a clear indication of lipid aut-oxidation (Mohdaly *et al*., 2010). For further confirmation of these results, other oxidation parameters, such as free fatty acid was also measured. The free fatty acid (FFA) of different treatment groups is shown in Fig. 2.

From the same figure, it could be observed that the mango peels extract was the most effective in reducing the FFA value (from 1.11 to 1.00%) compared with FFA of control (1.62%) and sample containing 200 ppm BHA (1.07%) at zero time. FFA values continuously increased ($P < 0.05$) in all biscuit samples during the storage for 6 months at room temperature. The increase in FFA value could be attributed to breaking of double bonds of unsaturated fatty acids of lipid during storage of biscuits at room temperature, as reported by earlier workers (Noor and Augustin, 1984). The increment rates in the values were decreased with increment of extracts concentration from 0.5 to 3%. These results confirm the findings of Ajila *et al*. (2008) and Ashoush and Gadallah (2011).

8.6.Thiobarbituric Acid Test (TBA):

TBA value measures the formation of secondary oxidation products, mainly malonaldehyde, which may contribute off-flavor to oxidize oil (Rossel, 1994). The effect of BHA and different mango peels extracts on the TBA content of biscuit samples during the storage for 6 months at room temperature is illustrated in Fig. 3.

The addition of MP extracts at different concentrations (0.5 - 3%) resulted in a slight decrease (from 0.378 to 0.375 mg malonaldehyde / kg sample) in TBA contents of biscuits when compared with TBA of control biscuit (0.385 mg/kg) and the sample containing BHA (0.383 mg/kg) at zero time as shown in Fig. 3. These results are consistent with findings of Izzreen and Noriham (2011) and Ibrahium *et al*. (2013). They found that the addition of some natural antioxidants to cakes and biscuits formula decreased TBA content in all samples at zero time and during 28 days storage compared to synthetic antioxidant effect.

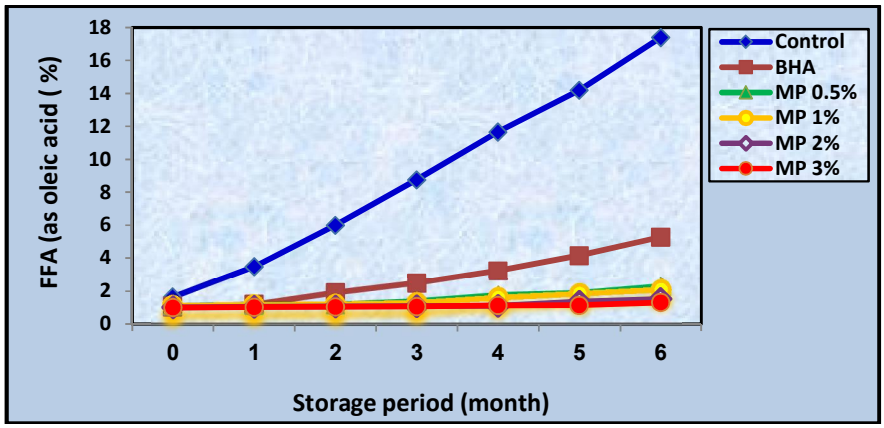

Fig. 2: Response of BHA and different MP extracts on FFA of stored biscuits for 6 months.

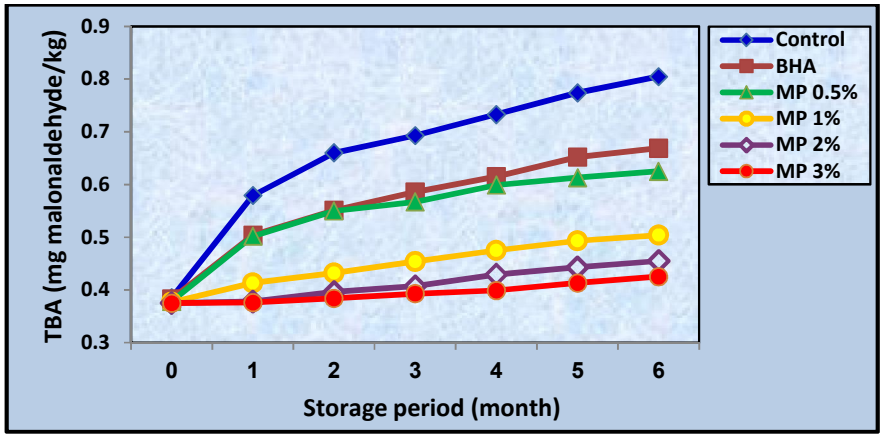

Fig. 3: Response of BHA and different MP extracts on TBA content of stored biscuits for 6 months.

On the other hand, TBA contents continuously increased ($P< 0.05$) in all biscuit samples during the storage for 6 months at room temperature . The increment rates in the values were decreased with increasing of extracts concentration from 0.5 to 3% as shown in Fig (3).

Generally, it was apparent after 6 months storage that addition of MP extracts at various concentrations retard the development of rancidity in biscuits. Also, it could be observed that the mango peels with all its concentrations showed the best protection towards reducing the TBA contents in biscuits (from 0.625 to 0.375 mg/kg) during different storage periods in comparison with control sample (0.805 mg/kg) and BHA at 200 ppm (0.669 mg/kg). These finding are in agreement with the results obtained by Hara (2001) and Reddy *et al*. (2005).

Conclusion

The data of this research proved that the best extraction conditions is a vital step in gaining extracts rich in antioxidants from mango peels (MP). The most serious factor is the extraction solvent. The best suitable solvent to obtain extracts with high antioxidant capacity and high phyto-chemical compound content from MP was methanol. Mango peel extracts were effective inhibitor to lipid per-oxidation in biscuit during storage. There are many antioxidant compounds in mango peels and they could be used as a natural antioxidant and antimicrobial compounds in food applications and very inexpensive alternative to synthetic food additives.

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References

AACC., 2000. American Association of Cereal Chemists. Approved Method of the AACC, 10th ed., American Association of Cereal Chemists, St., Paul, Minnesota, USA.

Abdalla, A.E.M., S.M. Darwish, E.H.E. Ayad and R.M. El-Hamahmy, 2007. Egyptian mango by-product 2: Antioxidant and antimicrobial activities of extract and oil from mango seed kernel. Food Chemistry, 103: 1141-1152.

Ajila, C.M., K.A. Naidu, S.G. Bhat and R.U.J.S. Prasada, 2007. Bioactive compounds and antioxidant potential of mango peels extract. Food Chemistry, 105: 982-988.

Ajila, C.M., M. Aalami, K. Leelavathi and R.U.J.S. Prasada, 2010. Mango peels powder: A potential source of antioxidant and dietary fiber in macaroni preparations. Innovative Food Science and Emerging Technologies, 11: 219-224.

Ajila, C.M., K. Leelavathi and U.J.S. Prasada Rao, 2008. Improvement of dietary fiber content and antioxidant properties in soft dough biscuit with the incorporation of mango peels powder. Journal of Cereal Science, 48: 319-326.

AOAC., 2000. "Official Methods of Chemical Analysis" Association of Official Analytical Chemists, Arlington, Virginia, USA.

AOCS, 1996. Official Methods Recommended Practices of the AOCS, 6th ed., Champaign, IL USA. Association of Cereal Chemists, INC. st., Paul, Minnesota, USA.

Ashoush, I.S. and M.G.E. Gadallah, 2011. Utilization of Mango Peels and Seed Kernels Powders as Sources of Phytochemicals in Biscuit. World Journal Dairy and Food Sciences, 6(1): 35-42.

Aziah, N.A.A. and C.A.K omathi, 2009. Acceptability attributes of crackers made from different types of composite flour. International Food Research Journal, 16: 479-482.

Benzie, I.F.F. and J.J. Strain, 1996. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Analytical Biochemistry, 239: 70-76.

Cetin, E.S., D. Altinöz, E. Tarçan and N.G. Baydar, 2011. Chemical composition of grape canes. Industrial Crops and Products, 34(1): 994-998.

Dragović-Uzelac, V., J. Pospišil, B. Levaj and K. Delonga, 2005. The study of phenolic profiles of raw apricots and their purees by HPLC for the evaluation of apricot nectars and jams authenticity. Food Chemistry, 91 (2): 373-383.

El-Hamzy, E.M.A. and A.A. Yaseen, 2014. A study on characterization of onion (*Allium cepa* L.) wastes as natural food ingredients and their inhibitory effect on polyphenol oxidase and enzymatic browning of apricot puree. Journal of Applied Sciences Research, 10(13): 59-71.

El-Hamzy, E.M.A., M.M.S. Ashour, R.K. Moawad and G.M. Abd El Moniem, 2013. Physico-Chemical, Sensorial and Antioxidative Characteristics of Date Syrups as Affected by Pectinase/Cellulase Enzymes Treatments. J. App. Sci. Res., 9(11): 5835-5849.

Hara, Y., 2001. Green tea: Health benefits and applications, Marcel Dekker, New York, pp: 183-220.

Ibrahium, M.I., M.E. Abd El-Ghany and M.S. Ammar, 2013. Effect of Clove Essential Oil as Antioxidant and Antimicrobial Agent on Cake Shelf Life. World Journal Dairy and Food Sciences, 8(2): 140-146.

Izzreen, I. and A. Noriham, 2011. Evaluation of the antioxidant potential of some Malaysian herbal aqueous extracts as compared with synthetic antioxidants and ascorbic acid in cakes. International Food Research Journal, 18: 583-587.

Jayaprakasha, G.K., R.P. Singh and K.K. Sakariah, 2001. Antioxidant activity of grape seed (*Vitisvinifera*) extracts on peroxidation models in vitro. Food Chemistry, 73: 285-290.

Kim, H., J.Y. Moon, H. Kim, D.S. Lee, M. Cho, H.K. Choi, Y.S. Kim, A. Mosaddik and S.K. Cho, 2010. Antioxidant and antiproliferative activities of mango (*Mangifera indica* L.) flesh and peels. Food Chemistry, 121: 429-436.

Liyana-Pathirana, C.M., and F. Shahidi, 2005. Antioxidant activity of commercial soft and hard wheat (*Triticuma estivum*) as affected by gastric pH conditions. Journal of Agricultural and Food Chemistry, 53: 2433-2440.

Manthey, J.A., and P. Perkins-Veazie, 2009. Influences of harvest date and location on thelevels of β-carotene, ascorbic acid, total phenols, the in vitro antioxidant capacity, and phenolicprofiles of five commercial varieties of mango(*Mangifera indica* L.). Journal of Agricultural and Food Chemistry, 57: 10825-10830.

Martinez, R., P. Torres, M.A. Meneses, J.G. Figueroa, G.A. Pérez-Alvarez and M. Viuda-Martos, 2012. Chemical, technological and in vitro antioxidant properties of mango, guava, pineapple and passion fruit dietary fiber concentrate. Food Chemistry, 135: 1520-1526.

Meyer, A.S., M. Heinonen and E.N. Frankel, 1998. Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin, and ellagic acid on human LDL oxidation. Food Chemistry, 61: 71-75.

Mohdaly, A.A.A., M.A. Sarhan, A. Mahmoud, M.F. Ramadan and I. Smetansk, 2010. Antioxidant efficacy of potato peels and sugar beet pulp extracts in vegetable oils protection. Food Chemistry, 123: 1019-1026.

Moss, J.R. and L. Otten, 1989. A relationship between color development and moisture content during roasting of peanuts. Canadian Institute of Food Technology Journal, 22: 34-39.

Mostafa, U.E., 2013. Phenolic Compounds and antioxidant potential of mango peels and kernels (*Mangifera indica* L.) on the frying oil stability, lipid profile and activity of some antioxidant serum enzymes in rats. The Journal of American Science, 9(11): 371-378.

National Academy of Sciences, 2011. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academy Press, Washington, D.C. pp: 82-442.

Noor, N. and M. Augustin, 1984. Effectiveness of antioxidant onthe stability ofbanana chips. Journal of Science Food and Agriculture, 35: 805-812.

Norhidayah, A., A.S. Babji, M.S. Shazali, M.N. Norazmir and H. Norazlanshah, 2011. Effects of Mango (*Mangifera indica*L.) and Guava (*Psidium guajava* L.) Extract on Frozen Chicken Meat Balls' Storage Quality. Pakistan Journal of Nutrition, 10(9): 879-883.

Ou, B., D. Huang, M. Hampsch-Woodill, J.A. Flanagan and E.K. Deemer, 2002. Analysis of antioxidant activities of common vegetables employing Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) assays: a comparative study. Journal of Agricultural and Food Chemistry, 50(11): 3122-3128.

Rauha, J.P., S. Remes, M. Heinonen and A. Hopia, 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. International Journal of Food Microbiology, 56: 3-12.

Reddy, V., A. Urooj, and A. Kumar, 2005. Evaluation of antioxidant activity of some plant extracts and their application in biscuits. Food Chemistry, 90: 317-321.

Ribeiro, S.M.R., L.C.A. Barbosa, J.H. Queiroz, M. Kno¨dler and A. Schieber, 2008. Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. Food Chemistry, 110: 620-626.

Rossel, J.B., 2005. Measurements of rancidity. In: Rancidity in Foods. 3 ed. Eds., Allen, J. C. and R. J. Hamilton.Blackie Academic and Professional, Glasgow, UK.

Rossel, J.B., 1994. Measurements of rancidity. In: J.C. Allen and R.J. Hamilton, Editors, Rancidity in foods (third ed.), Blackie, UK, pp: 22-53.

Sapers, G. and F. Douglas, 1987. Measurement of enzymatic browning at cut surfaces and in juice of raw apple and pear fruits. Journal of Food Science, 52: 1258-1262.

SAS, 1996. SAS/STAT Software: Changes and Enhancements through Release 6.11. SAS Inst. Inc., Cary, NC.

Shan, B., Y. Cai, J. Brooks and H. Corke, 2007. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. International Journal of Food Microbiology, 117: 112-119.

Sogi, D.S., M. Siddiq, I. Greiby and K.D. Dolan, 2013. Total phenolics, antioxidant activity, and functional properties of 'Tommy Atkins' mango peels and kernel as affected by drying methods. Food Chemistry, 141: 2649-2655.

Sudha, M., R. Vetrimani and K. Leelavathi, 2007. Influence of fiber from different cereals on the rheological characteristics of wheat flour dough and on biscuit quality. Food Chemistry, 100: 1365-1370.

Taveira, M., L.R. Silva, L.A. Vale-Silva, E. Pinto, P. Valentaeo and F. Ferreres, 2010. Lycopersiconesculentum seeds: an industrial by product as an antimicrobial agent. J. Agric. Food Chemistry, 58(17): 9529-9536.

Wangcharoen, W. and W. Morasuk, 2008. Antioxidant capacity changes in Chili Spur Pepper (*Capsicum annuum Linn*. var. *acuminatumFing*erh.) during drying process.Asian Journal of Food and Agro-industry, 1(02): 68-77.

Wieland, P., S.F erran, D. Wilfried, P. Andreas, G. Irene, J.D iego, L. Rosa, B. Susana and C. Carles, 2006. An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chemistry, 97: 137-150.

Wu, J.S.-B., H. Chen and T. Fang, 1993. Mango Juice. In S. Nagy, C.S. Chen, & P.E. Shaw (Eds.), Fruit juice processing technology, pp: 620-655. Auburndale' Ag science, Inc.

Zia-ur-Rehman., Habib, F. and W.H. Shah, 2004. Utilization of potato peels extract as a natural antioxidant in soy bean oil. Food Chemistry, 85: 215-220.