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Research Article

Nano-encapsulation Efficiency of Lemon and Orange Peels Extracts on Cake Shelf Life

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Abstract

Bioactive compounds of citrus fruit (*Citrus unshiu*) by-product such as orange and lemon peels extract were investigated. Antioxidant activity of orange and lemon peels extract were compared with BHT and α-tocopherol at different concentrations levels. The highest antioxidant activity of OPWE and LPWE reached to 63.866 and 52.741% at extract concentration 120 mg mL^{-1}. Furthermore, antioxidant activity of OPEE and LPEE were maximized to reach to 81.347 and 61.534% at extract concentration 120 mg mL^{-1}, respectively. After nanoencapsulation, the DSC showed that the melting point was increased from 73.6-231.8°C for (OPEE) before and after encapsulation, respectively. The TEM showed that the particle size of orange extract was 38 nm before encapsulation and raised a minimum of 4.2 nm after encapsulation and re-dispersion. The encapsulation efficiency of all orange peel extract formulas (10% maltodextrin+2% arabic gum per peels extract), (8% maltodextrin+2% arabic gum per peels extract), (10% arabic gum per peels extract) and (10% maltodextrin per peels extract) were increased to 87.65, 97.62, 63.81 and 72.32%, respectively. Orange peels extract nanoencapsulated (OPNE) characterized with its highest antioxidant potential in all storage period, while the nanoencapsulated of lemon peels (LPNE) gave the lowest antioxidant 52.44% after 60 days storage at 4°C. The OPNE was applied in cake. The obtained cake samples were sensory evaluated. Cake samples showed no significant difference in color, odor, taste, texture and overall acceptability. Finally, the extracts of dried orange or lemon peels could be considered as potential antioxidant ingredients in food products.

Key words: Orange peels, lemon peels, antioxidant activity, phenolics, flavonoids, nanoencapsulation, transmission electron microscopy

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lemon and orange represent about 60% of the total citrus world production. In Egypt, about 3.23 million tons of citrus fruit was produced in 2012, contained 2014 million tons of orange. During production of citrus juice huge amounts of by-products including peel and segment membranes were represent. Peels represent the main by-product, which ranged between 50-65% of the total weight (Mohamed *et al*., 2010). Citrus juice by product could be becomes waste and produce odor, soil pollution, harborage for insects and can give rise to serious environmental pollution (Mandalari *et al*., 2006). In Egypt and Mediterranean countries, major quantities of the peels are not further processed. While, some attempts were made to use these residues as livestock feed, although their low nutritional value (Bampidis and Robinson, 2006). Accumulated large quantities of the orange peels waste along with environmental considerations to avoid health hazards derived from unsatisfactory disposal methods addressed the indispensable need for finding alternative biotechnological solutions for waste valorization (Martin *et al*., 2013; Rivas *et al*., 2008; Embaby *et al*., 2014).

Presence of many active phytochemicals in plant such as vitamins, flavonoids, terpenoids, carotenoids, cumarins, curcumins, lignin, saponin, plant sterol, etc., were play as antioxidant property. Citrus fruits and juices are an important source of bioactive compounds including antioxidants, such as ascorbic acid, flavonoids, phenolic compounds and pectins that are important to human nutrition (Fernandez-Lopez *et al*., 2005; Jayaprakasha *et al*., 2008).

The main three flavonoids found in citrus fruit are flavones, flavanones and flavonols (Calabro *et al*., 2004). While, studies proved that the main flavonoids found in citrus species are narirutin, hesperidine, eriocitrin and naringin (Schieber *et al*., 2001). Epidemiological studies on dietary citrus flavonoids reduce the risk of coronary heart disease (Hertog *et al*., 1993; Di Majo *et al*., 2005). Also, it is attracting more attention as anti-carcinogenic and anti-inflammatory agents for their lipid anti-peroxidation effects (Martyn *et al*., 2002; Tripoli *et al*., 2007). Also, in these classes of compounds is due to their pharmacological activity as radical scavengers (Cotelle *et al*., 1996).

Rapisarda *et al*. (1999) and Farag *et al*. (1989) reported that antioxidant and antibacterial effect of juice and edible parts of oranges as far as the peel is concerned, have a good total radical antioxidative potential (Gorinstein *et al*., 2001; Bermudez-Soto and Tomas-Barberan, 2004). There is paucity of information regarding the inhibitory effects of orange peel extract on lipid oxidation. Hegazy and Ibrahium (2012) evaluated the effect of some different solvents (such as methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate) on the extraction efficiency of effective compounds (such as polyphenolic and flavonoid compounds) from the orange peel. They determined the yield percentage, chelating activity, antioxidant/radical scavenging capacity and reducing power ability of the obtained extracts.

The shelf life of food is very limited and could be subjected to spoilage. Therefore, it has been the goal of many foods manufactures to prevent or retard oxidation in order to improve the shelf life of food products. Antioxidants have become an indispensable group of food additives. They include synthetic and natural antioxidants.

Antioxidant varieties of chemical compounds are widespread in nature, which owns a diverse mechanics, including its interaction with the free radicals and the formation of fat in products stable and non-effective (Pokorny and Korczak, 2001). Recently, synthetic antioxidant became their use controversial, where they could be cause carcinogens or toxic effects (Namiki, 1990). Therefore, attention was focused on the natural resources inherent in plants especially the good ones to eat. Especially scales that represent by-products to many factories juices, which are not possess toxic effects, i.e., phenolic compounds of the leading natural antioxidants, which include flavonoids, tannins, carotenoids, acids, phenolic and phenols compounds (Namiki, 1990; Samarth and Krishna, 2007).

Encapsulation enables to entrap essential ingredients into a carrier for protection, transport and release of the active components in a controlled manner (Chiu *et al*., 2007). Also, encapsulation could be used to extend the shelf life of materials for controlled delivery of functional substances when ingested in the intestine over a range of physiological conditions (Gharsallaoui *et al*., 2007).

Wang *et al*. (2009) and Gilles *et al*. (2012) reported that nano or micro-encapsulation involves coating of the emulsion droplets in fluidic dispersions in the microsize or nanosize regime, which having one dimension below 100 nm, take advantage for their dramatically increased surface area to volume ratio. So, when brought into a bulk system, nanomaterials can strongly influence the mechanical and textural properties such as stiffness and elasticity.

The objective of this study was to evaluate the potential of maltodextrin with gum arabic combination to building four types of materials for nanoencapsulation of orange or lemon peels extracts by ultrasonication. Also, special attention has given to the use and evaluates the effect of obtained antioxidants on stability and sensory properties of cake.

MATERIALS AND METHODS

Materials

Plant material: Orange and lemon were obtained from the local market of Giza governorate, Egypt. They were classified in department of Horticulture at the Institute of Agricultural Research. Orange and lemon were dried in an oven at a 35°C for 48 h, then peeled and grinded using mill electrical blender. The obtained peels were placed in polyethylene bags and stored in the refrigerator at of 4°C until use.

Chemicals: Chemical reagents of 2,2-diphenyl-1-picryl-hydrazyl (DPPH), Folin-Ciocalteu, Butylated hydroxyanisole (BHA), gallic acid, linoleic fatty acid and thiobarbituric acid (TBA) were obtained from Sigma-Aldrich, Chime, Steinheim, Germany. All other chemicals and solvents were analytical grade.

Methods

Preparation of orange and lemon peels extracts: Orange and lemon fruits were washed by tap water then peeled and their edible portions were carefully separated. The peels were air dried in a ventilated oven at 35°C for 48 h and ground to a fine powder and passed through a 24-mesh sieve according to the method described by Van Acker *et al.* (1996).

Preparation of Ethanolic Extract (EE): Fifty grams dried powder of orange or lemon peel was added to 500 mM ethanol 98%, then blended well using carburetor magnetic and left for 24 h at 25°C. The obtained extract was nominated using filter paper (Whatman No. 1). The residue was re-extracted (3-4 times) with ethanol. The filtrate was concentrated using rotary vacuum evaporated at 40°C to get rid of the solvent. The filtrate left at room temperature even gets a sticky substance (Mordi and Akanji, 2012) and kept in the dark at 4°C until use.

Preparation of Water Extract (WE): Twenty five grams of dried powder orange or lemon peels was extracted by maceration in boiled distilled water (500 mL) for 30 min on the magnetic stirrer; the mixture was filtrated using Buchner funnel on filter paper Whatman No. 1 as described by Gulcin *et al.* (2003) and then focus using rotary evaporator at 40°C to get rid of the water, the filtrate was left and then placed in opaque bottles and kept in the refrigerator at 4°C until use.

Determination of Total Phenolic Content (TPC): The total phenolics compound of peels that extracted by water (WE) or ethanol (EE) were determined colorimetrically using Folin-Ciocalteu reagent according to the method described by Ebrahimzadeh *et al.* (2008). The extracted samples (0.5 mL different dilutions) were mixed with Folin-Ciocalteu reagent (5 mL with distilled water at rate 1:10) for 3 min and 3 mL of 2% sodium carbonate Na_2CO_3 (1 M) were added. The mixture was stand for 15 min and the polyphenols were determined by an automated UV-VIS spectrophotometer at 765 nm. The TPC was determined as gallic acid equivalents using the following linear equation based on the calibration curve according to the Eq. 1:

$$A = 0.1786\ C-0.1739,\ R^2 = 0.999 \quad (1)$$

where, A is the absorbance and C is gallic acid equivalents (mg).

Determination of total flavonoids content: Colorimetric aluminum chloride method was used for flavonoids determination according to the method described by Huang *et al.* (2004) and Ebrahimzadeh *et al.* (2008). One gram solution of each extract was mixed separately with 1.5 mL ethanol, 0.1 mL 2% aluminum chloride $AlCl_3.6H_2O$, 0.1 mL 1 M potassium acetate and 2.8 mL distilled water and then left at room temperature for 10 min. The absorbance of the mixture was measured at 367 nm on UV/visible spectrophotometer. The quercetin (μg g^{-1}) was used as a standard for the calibration curve. The total flavonoid content of the samples was calculated by using the following linear equation based on calibration curve according to the Eq. 2:

$$Y = 0.0205X-1494,\ r = 0.9992 \quad (2)$$

where, Y is the absorbance and X is the flavonoid content in μg g^{-1}.

Determination the antioxidant activity in linoleic acid system: Antioxidant activity was determined in water and ethanol extracted by using linoleic acid system (Osawa and Namiki, 1981) where, 5-120 mg mL^{-1} of each essential oils extract were added to a solution of linoleic acid (0.13 mL), 99.8% ethanol (10 mL) and 0.2 M phosphate buffer (pH 7.0, 10 mL) and α-tocopherol (5-120 mg mL^{-1}) dissolved in ethanol 99.8%. Total volume was adjusted to 25 mL by distilled water. The reaction mixture was incubated at 40°C

and the degree of oxidation was measured by using the thiocyanate. By sequentially, add the following solutions to the mixture: Ethanol (10 mL 75%), ammonium thiocyanate (0.2 mL, 30%), sample solution (0.2 mL) and ferrous chloride solution (0.2 mL) (20 mM in 3.5% HCl). After stirring the mixture for 3 min, the peroxide value was determined by reading the absorbance at 500 nm and the antioxidant activity percentage can be calculated according to the following Eq. 3:

$$\text{Antioxidant activity (\%)} = \left[100-\frac{\text{Absorbance increase of sample}}{\text{Absorbance increase of standard}}\right]\times 100 \quad (3)$$

Determination of reducing power: The reducing power of orange (OPE) and lemon (LPE) peel extracts was quantified by the method described previously with minor modification compared to BHT and α-tocopherol concentrations from (5-120 mg mM^{-1}) in 98% ethanol with 2.5 mL of sodium phosphate buffer 200 mM, pH 6.6. Orange or lemon peel extract (5, 10, 30, 60, 100 or 120 mg) in 1 mL ethanol (98%) were mixed with 5 ml phosphate buffer (2 M, pH 6.6) and 5 mL potassium ferricyanide (1%). These mixtures incubated at 50°C for 20 min. About 5 mL trichloroacetic acid (1%) was added and the mixture was centrifuged at 2000 rpm for 10 min. The upper layer of the solution (5 mL) was mixed with 5 mL distilled water and 1 mL ferric chloride (0.1%). The absorbance of the pink color mixture was measured spectrophotometrically at 700 nm according to the method described by Oyaizu (1986).

Hydrogen peroxide-scavenging activity: The hydrogen peroxide-scavenging activity of OPE and LPE were determined according to Ruch *et al.* (1989). The oil extract was dissolved in 3.4 mL of 1 M sodium phosphate buffer (pH 7.4) and mixed with 600 µL of 43 mM solution of hydrogen peroxide. The absorbance value (at 230 nm) of the reaction mixture was recorded at 10 min intervals between zero and 40 min for each concentration, a separate blank was used 1 mL sodium phosphate buffer solution without the addition of plant extracts and used ascorbic acid and rutin. The following Eq. 4 has calculate the scavenging of hydrogen peroxide:

$$\text{Scavenging (\%)} = \left[\frac{A_{Blank}-A_{Sample}}{A_{Blank}}\right]\times 100 \quad (4)$$

where, A_{Sample} is absorbance of sample and A_{Blank} is absorbance of blank (distilled water instead extract).

Chelating activity: Chelating activity (Fe^{+2}) was measured by 2,2-bipyridyl competition assay according to the method described by Re *et al.* (1999). The reaction mixture containing 0.25 $FeSO_4$ solution (1 M), 0.25 mL antioxidant solution. About 1 mL tris-HCl buffer (pH 7.4), 1 mL 2,2-bipyridyl solution (0.1% in 0.2 M HCl) and 2.5 mL ethanol. The final volume made up 6.0 mL with distilled water. The absorbance was measured at 522 nm and used to evaluate chelating activity using disodium ethylene diamine tetracetate (NaEDTA) and citric acid in the same manner for the purpose of comparison as a standard. The sample control carried out in the same way as above without essential oil extracted.

Preparation of suspensions and capsules: Maltodextrin (MD) was dissolved in distilled water for one day before the preparation of the suspension, then kept overnight in a shaking water bath. Arabic Gum (AG) was dissolved in distilled water and mixed at 50°C using centrifuged at 10,000 rpm angular velocity for 2 min and filtered through a 0.45 µm filter paper. Additionally, nano-suspension were prepared by homogenization AG and MD solution using a homogenizer (Homogenizer PRO 400PC) at 15.000 rpm for 15 min to encapsulation. Total concentration of dissolved solid was 10% (w/w). Two different coating solutions were prepared. The first solution was composed of 10% MD prepared and 90% distilled water and the second one was composed of 8% MD prepared, 2% AG prepared and 90% distilled water. All suspensions were prepared in two stages: (a) Pre-suspensions were obtained by weighing coating material solutions and homogenize for 15 min at 15.000 rpm and (b) The obtained presuspensions were further homogenized using the ultrasonic probe (Probe Sonicator Sonics, VCX-750 Vibra-Cell Sonicator, USA) with a diameter of 3.8 mm and was applied at 160 W with 50% pulse for 20 min. During the homogenization process, samples were placed in water bath with cold water at 4°C to prevent the overheating of the suspensions. Each experiment was duplicated. Suspensions were freeze dried at -52°C and at 0.075 mbar for 48 h. Dried samples were manually crushed and kept at -20°C while, excluding light for further analysis.

Finally, four formulas were prepared in this study as follows: 10% maltodextrin+2% arabic gum with orange or lemon peels ethanol extract (MD+AG/OPE_1 or LPE_1), 8% maltodextrin+2% arabic gum with orange or lemon peels ethanol extract (MD+AG/OPE_2 or LPE_2), 10% arabic gum with orange or lemon peels ethanol extract (AG/OPE or LPE) and 10% maltodextrin with orange or lemon peels ethanol extract (MD/OPE or LPE).

Differential scanning calorimetry: Ten milligrams samples were placed in aluminium crucibles. The samples were analyzed under a flow of nitrogen gas (40 mL min^{-1}). A dynamic scan was performed at a heating rate of 10°C min^{-1} over a temperature range of -150-300°C. Evaporation enthalpies were calculated by peak area integration of DSC profiles and the results were compared with the estimated vaporization enthalpy of essential oils major components.

Transmission Electron Microscope (TEM) of extracted and nano-encapsulated extracts: Twenty microliters of diluted samples was placed on a film-coated 200-mesh copper specimen grid for 10 min and the fluid excess was eliminated using filter paper. The grid was then stained with one drop of 3% phosphotungstic acid and allowed to dry for 3 min. The coated grid was dried and examined under the TEM microscope. The samples were observed by operating at 160 kV (Saloko *et al*., 2013).

DPPH radical scavenging activity: The 2,2-diphenyl-2-pcrylhydrazyl was used as generating substance of free radicals in order to determine the radical scavenging activity of OPE or LPE and encapsulated MD+AG/OPE or LPE (Dima *et al*., 2014). Different quantities of OPE, LPE or encapsulated (MD+GA/OPE or LPE) were added in a hydroalcoholic solution of water:ethanol (2:1 w/w) that was homogenized through ultrasonication (Sonoplus Bandelin, Germany) for 1 min.

Solutions with concentrations between 1 and 50 μg mL^{-1} OPE or LPE were obtained. The quantity of MD+AG/OPE or LPE complex was computed in relation with the retention degree of OPE or LPE in MD+AG. A volume of 1 mL sample OPE or LPE or 1 g coriander (MD+AG/OPE or LPE) complex in different concentrations was added on 2 mL DPPH ethanolic solution (5 mg mL^{-1}) and was strongly stirred. The mixtures were kept in darkness for 60 min, after that the absorbance was measured at wavelength of 515 nm. The antiradical activity (AA%) was established according to the Eq. 5:

$$AA\ (\%) = \frac{A_{sample} - A_{empty\ sample}}{A_{control}} \times 100 \quad (5)$$

where, A_{sample} is the absorbance of the sample with DPPH, $A_{empty\ sample}$ is the absorbance of the sample without DPPH (2 mL ethanol+1 mL sample with different OPE or LPE content), $A_{control}$ is the control absorbance of a blend formed from 2 mL DPPH solution+1 mL ethanol.

Also, it was calculated the IC_{50} which represents the OPE or LPE concentration needed for the quench 50% of initial DPPH radicals under the experimental conditions. The antioxidant activity of OPE or LPE and encapsulated OPNE or LPNE was compared with those of chemical antioxidants butylated hydroxytoluene (BHT) and ascorbic acid (AAc).

Encapsulation efficiency (EE): Encapsulation Efficiency (EE) was determined according to the method described by Bae and Lee (2008). Fifteen milliliters of ethanol were added to 1.5 g of sample powder encapsulated in a glass jar with a lid, which was shaken by hand for 2 min to OPE or LPE extract. The solvent mixture was filtered through a Whatman filter paper No.1 and the collected powder from filter paper was rinsed three times with 20 mL ethanol. The non-encapsulated OPNE or LPNE was determined by mass difference between the initial clean flask and that containing the extracted oil residue (Carneiro *et al*., 2013). Total OPE or LPE was assumed to be equal to the initial extract, since preliminary tests revealed that all the initial extract was retained, which was expected, since peel extracted is not volatile. Encapsulation efficiency (EE) was calculated from Eq. 6:

$$EE\ (\%) = \frac{T_0 - S_0}{T_0} \times 100 \quad (6)$$

where, T_0 is the total OPE or LPE content and S_0 is the non-encapsulated OPNE or LPNE content.

Preparation of cakes: Cakes were prepared according to the method described by Lu *et al*. (2010) by using the following formula: 1000 g wheat flour (72% extraction) without antioxidants, 850 g sugar, 50 g shortening, 40 g baking powder, 250 g eggs, 200 mL milk and 17 g vanilla. The BHT or encapsulated OPNE or LPNE was added to the formula. The ingredients were mixed for 3-4 min and placed on aluminum plates then backed in an oven at 160°C for 30 min. Cooking foil was also put in the oven for sterilization. After baking, plates were covered with the sterile cooking foil and transferred to the laminar flow bench. Then they were cut into 5×5 cm square pieces and packed in polypropylene films to prevent drying before analyses. Cakes were prepared to provide five samples: The first sample without antioxidant agent (control). The second sample was prepared by adding 200 ppm BHT (synthetic antioxidant). While, the other samples were prepared by adding nanoencapsulated antioxidant at four different formula of antioxidant OPE, LPE, encapsulated OPNE and LPNE.

Extraction of lipid from cake samples: Cakes lipid was extracted according to the method described by Baiano and Del Nobile (2005). About 100 g of cakes were ground roughly and placed in a closed flask with 200 mL ethanol. The flask was shaken for 1 h and then filtered through filter paper (Whatmann No. 1). The solvent was removed from the extracted lipids by rotary evaporator at 50°C. The extracted lipids were stored at room temperature for subsequent determination.

Sensory evaluation of cakes: The sensory evaluation of cakes containing encapsulated OPNE or LPNE at different levels, BHT and control was performed using 20 trained panelists. Each panelist was presented with individual cake samples that served in a randomized order according to the methods described by Chaiya and Pongsawatmanit (2011) and Wu *et al.* (2013). Each panelist was asked to rate the liking of quality attributes according to appearance, color, odor, taste, texture (hardness and softness) and overall acceptability for each sample using a 9-point hedonic scale (1 = Dislike extremely and 9 = Like extremely).

Storage of cake: The processed cakes were cooled and removed from the pan after 1 h. The cooled cake were packaged in aluminum foils and kept at 5°C for 60 days up to analyze every 10 days to determined shelf life of cake.

Statistical analysis: One way analysis of variance (ANOVA) with multiple ranges significant difference (LSD) test ($p<0.05$, $p<0.01$) were carried out by Statistical Package for the Social Sciences (SPSS) version 16.0 software (SPSS Inc., Chicago, USA). Data are reported as mean value±standard deviation (SD) for three replicates.

RESULTS AND DISCUSSION

Total Phenolics Content (TPC): Table 1 showed that ethanol extract of orange peels (OPEE) characterized with its higher TPC (52.28 mg gallic acid g^{-1}) compared to Lemon Peels Ethanol Extract (LPEE) of (27.14 gallic acid g^{-1}). While, water was able to extract 35.35 and 46.31 gallic acid g^{-1} from orange and lemon peels, respectively. This result agreed with those found by Cai *et al.* (2004).

Total Flavonoids Content (TFC): Plants phenolic compounds consist of several categories; one of the most important among these is the flavonoids which have potent antioxidant activities. Total Flavonoids Content (TFC) in OPE and LPE were determined and presented in Table 2. The obtained results indicated that ethanol was able to extract 41.53 and 20.64 μg g^{-1} from orange and lemon peels, respectively. Also, TFC in (OPWE) and (LPWE) reached to 22.14 and 27.02 μg g^{-1}, respectively. This result is higher than those reported by Hegazy and Ibrahium (2012), where they found that TFC in ethanolic solution extract of orange peels (OPEE) was 29.75 μg g^{-1} by ethanolic solution extraction.

Table 1: Total Phenolics Content (TPC) of orange and lemon peels extract

	Total phenolics contents (mg gallic acid g^{-1})	
Samples	Water Extract (WE)	Ethanol Extract (EE)
Orange peels (OPE)	35.35±1.2	52.28±2.1
Lemon peels (LPE)	46.31±1.4	27.14±1.5

Table 2: Total Flavonoids Content (TFC) of orange and lemon peels extract

	Total flavonoids content (μg g^{-1})	
Samples	Water Extract (WE)	Ethanol Extract (EE)
Orange peels (OPE)	22.14±0.9	41.53±1.6
Lemon peels (LPE)	27.02±1.1	20.64±1.2

Antioxidant activity: There is no universal method that can measure the antioxidant capacity of cake accurately and quantitatively. Clearly, matching radical source and system characteristics to antioxidant reaction mechanisms is critical in the selection of appropriate assessing antioxidant capacity assay methods, as is consideration of the end use of the results (Prior *et al.*, 2005). In this way, to screen the antioxidant properties of the samples, four different *in vitro* assays were performed: Antioxidant capacity, reducing power, ferrous ion chelating, scavenging hydrogen peroxide and DPPH radical scavenging capacity.

Antioxidant capacity: Table 3 showed the effect of OPE and LPE on antioxidant activity compared to BHT and α-tocopherol at different concentrations levels. The obtained results indicated that antioxidant activity of OPWE was higher than of LPWE at different concentration level. The highest antioxidant activity of OPWE and LPWE reached to 63.866 and 52.741% at extract concentration 120 mg mL^{-1}. On the other hand, antioxidant activity of ethanol extract was higher than water extract for orange and lemon peels at different concentration levels, i.e., antioxidant activity of OPWE and LPWE at concentration 10 mg mL^{-1} was 8.403 and 5.793%, respectively while, they were increased to reach 19.637 and 12.641% in case of using ethanol extract at the same concentration level. The highest antioxidant activity of OPEE and LPEE reached to 81.347 and 61.534% in case of using ethanol extract at 120 mg mL^{-1} extract concentration.

Table 3: Antioxidant activity of OPWE, LPWE, OPEE and LPEE compared to BHT and α-tocopherol

	Antioxidant activity (%)					
	Water extract		Ethanol extract			
Concentration (mg mL^{-1})	Orange peels (OPWE)	Lemon peels (LPWE)	Orange peels (OPEE)	Lemon peels (LPEE)	BHT	α-tocopherol
10	8.403±0.5	5.793±0.7	19.637±1.3	12.641±1.5	43.63±2.1	29.06±1.5
30	15.966±1.2	10.504±1.1	31.606±2.8	18.917±2.3	79.54±2.5	61.57±2.4
60	31.092±2.4	22.053±2.6	38.860±2.6	25.884±2.8	88.36±2.1	69.27±2.1
100	49.579±3.1	43.860±3.8	64.767±3.1	54.462±3.2	92.60±3.5	79.73±3.3
120	63.866±3.7	52.741±4.0	81.347±4.2	61.534±4.6	96.95±3.4	86.42±3.6

Table 4: Reducing power of OPWE, LPWE, OPEE and LPEE compared to BHT and α-tocopherol

	Reducing power (%)					
	Water extract		Ethanol extract			
Concentration (mg mL^{-1})	Orange peels (OPWE)	Lemon peels (LPWE)	Orange peels (OPEE)	Lemon peels (LPEE)	BHT	α-tocopherol
5	19.06±1.3	23.37±2.0	29.04±2.4	32.28±3.4	42.35±3.6	27.39±1.9
10	31.47±2.2	35.61±2.8	37.87±3.1	47.51±4.8	64.81±4.5	41.64±3.5
30	66.62±5.4	72.58±6.1	79.13±5.9	83.49±5.2	110.27±9.0	69.04±7.0
60	112.87±7.9	98.91±8.3	108.93±10.3	158.68±11.5	178.39±10.7	151.51±12.2
100	146.50±12.0	171.45±13.5	186.55±14.8	237.12±15.3	251.76±16.3	218.36±15.8
120	167.84±14.7	206.36±16.2	225.43±17.1	256.54±19.2	282.25±21.2	239.45±18.6

Butylated hydroxytoluene (BHT) is a synthetic antioxidant, widely used by the food industry as preservatives. Replacing natural antioxidant instead of synthetic antioxidant is a good thing. Alpha-tocopherol is a form of vitamin E that is preferentially absorbed and accumulated in humans and is found naturally in a variety of foods. Therefore, antioxidant activity of OPE and LPE were compared with one of the most common synthetic antioxidant (BHT) and other of the natural antioxidant (α-tocopherol). The obtained results showed that antioxidant activity of BHT at concentration level of 10 mg mL^{-1} reached to 43.63%, while α-tocopherol, OPEE and LPEE increased to 79.73, 64.767 and 54.462% at concentration level of 100 mg mL^{-1}, respectively. The fruit is rich in their content of flavonoids and alonthusianinat which we participate effectively in antioxidant (Garcia-Alonso *et al*., 2004).

Reducing power: The reducing power could be used as a significant marker of the antioxidant activity. The reducing powers of the water and ethanol extracts of orange and lemon peels at different concentrations levels were determined and compared with the reducing power of synthetic antioxidant (BHT) and natural antioxidant (α-tocopherol) at the same concentration level. Table 4 showed that the reducing power of LPWE was higher than OPWE of at different concentration level. The highest reducing power of LPWE and OPWE reached to 206.36 and 167.84% at 120 mg mL^{-1} extract concentration, respectively. Furthermore, reducing power of ethanol extract was higher than LPWE and OPWE for at different concentration levels, where reducing power of LPWE and OPWE at concentration 10 mg mL^{-1} was 35.61 and 31.47%, respectively while, they were increased to reach 47.51 and 37.87% in ethanol extract at the same concentration level. The highest reducing power of LPEE and OPEE in ethanol extract, BHT and α-tocopherol reached to 256.54, 225.43, 282.25 and 239.45% at 120 mg mL^{-1} concentration. These result agreed with Chun *et al*. (2005) who reported that the vehicles flavonoids and alonthusianinat in fruits have the ability to shorthand ferric ion Fe^{+3} in a complex Potassium Ferricyanide ($KFe(CN)_6$) to ferrous ion Fe^{+2} and the intensity of the color blue or greenish-blue sign of increased strength reductive.

Ferrous ion chelating: The ability of peel fruits extracts to connect ion ferrous shown in Table 5. In this assay, the transformation of Fe^{3+} into Fe^{2+} in the presence of various fractions was measured. The antioxidants present cause the reduction of Fe^{3+}/ferricyanide complex ($FeCl_3/K_3Fe(CN)_6$) to the ferrous form (Fe^{2+}). The results of statistical analysis on the significant differences between the samples and percentage chelating of OPEE were 56.2% and LPEE 51.7% at concentration 4 mg mL^{-1}. The highest proportion of connecting of OPEE (72.4%) was less than EDTA and citric acid and there are significant differences between water and ethanol extract, the highest percentage of the trap into the OPWE 54.9% showed the LPWE lowest susceptibility to iron trap is 39.8%. The results showed increased the viability of water and ethanol extracted and all samples to link up the ion focus.

Table 5: Ferrous ion chelating effect of OPWE, LPWE, OPEE and LPEE compared to BHT citric acid and EDTA

	Reducing power (%)					
	Water extract		Ethanol extract			
Concentration (mg mL^{-1})	Orange peels (OPWE)	Lemon peels (LPWE)	Orange peels (OPEE)	Lemon peels (LPEE)	Citric acid	EDTA
1	10.6±1.2	9.7±0.9	9.2±1.1	32.6±2.4	29.0±2.3	43.9±2.4
2	13.0±1.5	18.3±1.3	13.8±1.6	39.7±2.6	36.5±2.8	68.1±3.5
3	29.4±2.6	22.8±2.1	37.9±1.9	43.9±3.5	51.6±3.1	73.5±3.7
4	43.2±3.4	26.4±2.5	56.2±2.8	51.7±3.4	57.3±4.2	85.7±4.4
5	54.9±3.9	39.8±2.3	72.4±3.7	64.5±4.2	72.8±4.6	99.4±6.3

Table 6: Ability of orange and lemon peels extract to scavenging hydrogen peroxide compared to ascorbic acid and rutin

Scavenging H_2O_2 (%)					
Orange peels		Lemon peels			
Water extract (OPWE)	Ethanol extract (OPEE)	Water extract (LPWE)	Ethanol extract (LPEE)	Ascorbic acid	Rutin
75.25±3.2	89.03±4.6	73.11±5.1	86.52±6.5	83.41±6.8	78.63±5.3

Scavenging hydrogen peroxide: Table 6 Indicates to susceptibility extracts ethanol and water of peel fruits studied to capture the hydrogen peroxide, as the percentage of scavenging of OPEE and LPEE 89.03 and 86.52%, respectively, since the superiority orange morally of lemon and also ascorbic acid and a compound routine in its ability to proselytism, decreased susceptibility scavenging decreased slightly in water extracted was capture the proportion of hydrogen peroxide in OPWE 75.25% and the lowest rate in LPWE 73.11%. These results obtained greater than those obtained by Kim (2013).

Nanoencapsulation of orange and lemon peels extract: Ultrasonication probe used to breaking down molecules for four formulas individualize the material and increases the surface area and efficiency. Ultrasonication process with high amplitude accompanied by a time increasing will produce a high energy that used for cavitation process and will affect into gradual decreasing of particle size, the use of ultrasonic waves for the formation of nanoparticles is one of the effective methods. Ultrasonic frequency ranging from 20 kHz-1 MHz is widely used as waves for the formation of nanoparticles.

The application of ultrasonics to nanomaterials (maltodextrin and arabic gum) has manifold effects. The most obvious is the dispersing of materials in liquids in order to break particle agglomerates. Generally, this leads to smaller particles and increased size uniformity. Ultrasonic cavitation improves the material transfer at particle surfaces. Also, improve surface functionalization of materials (maltodextrin and arabic gum) having a high specific surface area. Finally, the formulas were homogenization by homogenizer was disintegration methods top-down technologies like' pearl milling involving wet milling for four formulas that provide more efficient size reduction than the conventional size reduction techniques (Williams *et al*., 2013).

Thermal stability by Differential Scanning Coliremetry (DSC): Orange peel ethanol extract (OPEE) was evaluated before and after nano-encapsulation (OPNE) using Differential Scanning Coliremetry (DSC). Figure 1 showed that the melting point of OPEE was 73.6°C, while nano-encapsulation (OPNE) increased melting point to reach 231.8°C before degradation. Therefore, nano-encapsulation can improve the stability of orange peels extract (OPEE) against high thermal transactions during food processing. This result agreed with Giles *et al*. (2012) and could be used in cake as antioxidants which, exposed to high temperature during processing.

Transmission Electron Microscope (TEM): The TEM was used to determine the particle size (Fig. 2) before and after encapsulation and re-dispersion in distilled water. The particle sizes of all treatments were kept below 38 nm before encapsulation (Fig. 2b) and raised a minimum of 4.2 nm after encapsulation (Fig. 2a) and re-dispersion. Particle sizes of all treatments decreased after encapsulation and re-dispersion, indicating that some of the nano particles formed by dispersion aggregate during homogenization. The samples were still remained less than (10 nm) which, ensured good solubility and application in water solution.

Encapsulation Efficiency (EE): Four formulas of nano-encapsulated lemon and orange peels extract (MD+AG/OPE$_1$ or LPE$_1$), (MD+AG/OPE$_2$ or LPE$_2$), (AG/OPE or LPE) and (MD/OPE or LPE) were prepared and

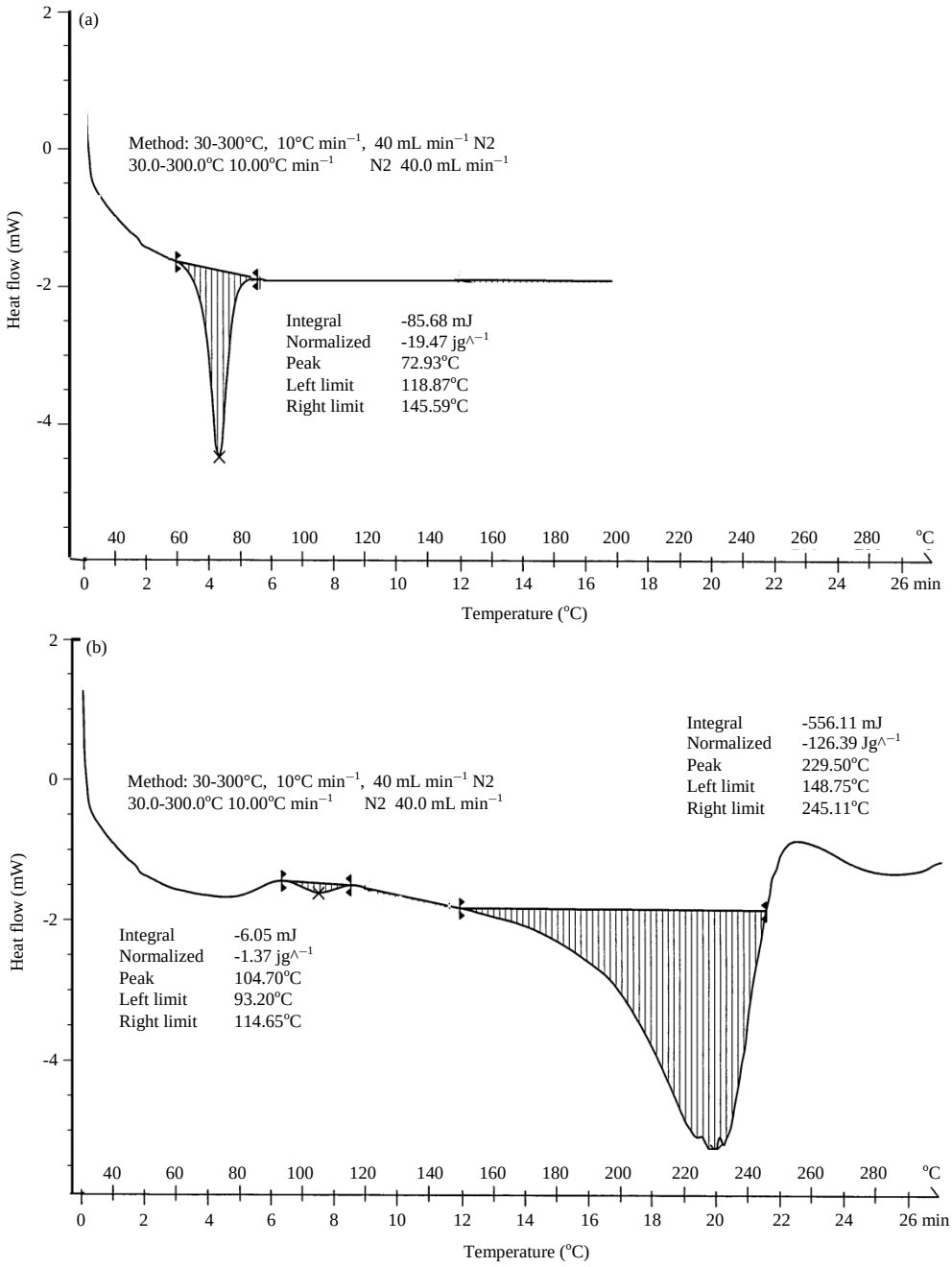

Fig. 1(a-b): DSC of extracted (OPEE) and nanoencapsulated (OPNE) of orange peel, (a) Before encapsulation and (b) After encapsulation

evaluated as illustrated in Fig. 3. The encapsulation efficiency similar to those achieved with LPEE was 61.42, 74.38, 53.97 and 64.05%, respectively. While, the encapsulation efficiency of all formulas orange peel encapsulated (OPNE) were increased to 87.65, 97.62, 63.81 and 72.32%, respectively. The difference in the encapsulation efficiency is assumed to the nanoencapsulation approach also provides for good dispersion properties in formulas and homogeneous distribution of the peels extract into the matrix. The partical size of the nanoencapsulated powder may be as well a significant factor in encapsulation efficiency which indicated that AG/LPE had the smallest particle size. From previous results it means that the surface area increased and more of OPE could have adhered to its exterior causing an efficiency reduction.

These results were higher than that obtained by Beristain *et al*. (2001) by using spray drying technique was 83.6%.

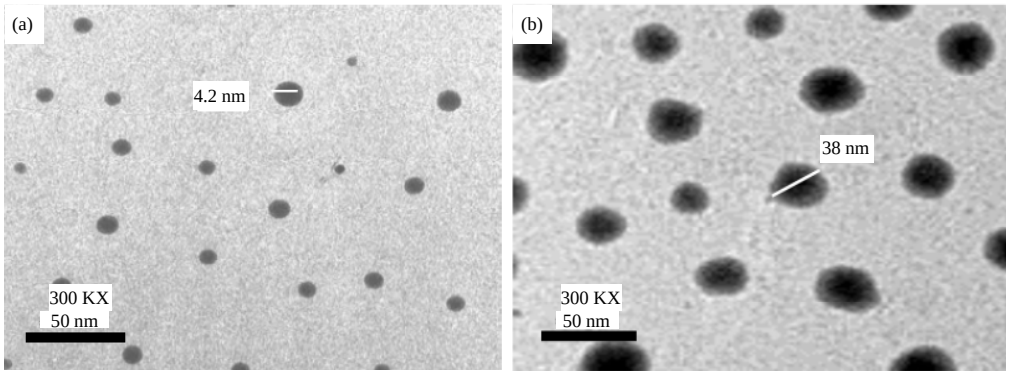

Fig. 2(a-b): TEM of Orange Peels Ethanol Extract (OPEE), (a) After nano-encapsulation (OPNE) and (b) Before nano-encapsulation (OPNE)

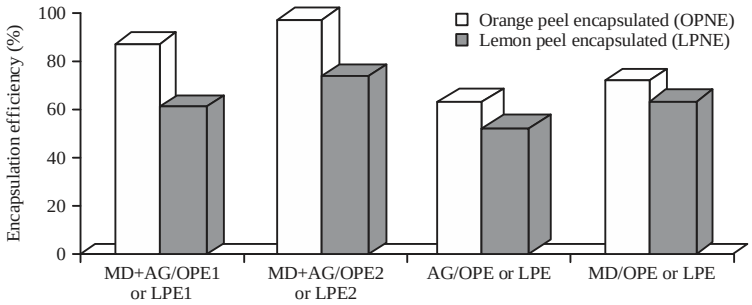

Fig. 3: Encapsulation efficiency of orange and lemon peels extract of all formulas

Table 7: Phytochemical properties of orange and lemon peel extract and their nano-encapsulated

Samples	DPPH radical scavenging (%)	Reducing power (mg BHT equivalent g^{-1} powder)	Total phenolic content (µg gallic acid equivalent g^{-1} powder)
OPEE	64.11±3.51	225.43±9.40	52.28±3.54
LPEE	41.09±3.18	256.54±10.25	27.14±2.81
OPNE	82.34±5.64	274.14±8.51	59.31±3.15
LPNE	53.97±4.26	261.78±9.82	31.02±2.94
BHT	92.60±2.11	282.25±6.27	--

OPEE: Orange peel ethanol extract, LPEE: Lemon peel ethanol extract, OPNE: Orange peel nanoencapsulated and LPNE: Lemon peel nanoencapsulated

Also, nanoencapsulation increased the antioxidant activity in all cases, meaning that less OPEE can be used to obtain the same antiradical activity, which improves the quality of the OPNE.

Phytochemical properties of nano-encapsulated powder of orange or lemon peels extract: The phytochemical properties of the nano-encapsulated powder of orange (OPNE) or lemon (LPNE) peel extracts were compared with orange (OPEE) and lemon (LPEE) peels extract and presented in Table 7. The DPPH test is based on the ability of the stable free radical 2,2-diphenyl-1-picrylhydrazyl to react with hydrogen donors including phenols. The DPPH radical scavenging was increased from 64.11-82.34% after nano-encapsulation processing. Radical scavenges can be reacted and quench with peroxide radicals to terminate the peroxidation chain reaction and improve the quality and stability of food product (Brand-Williams *et al*., 1995). Also, to understand their antioxidant mechanism each orange and lemon peels oil nano-encapsulated were increase than orange and lemon extract. The reducing power of the nano-encapsulated (OPNE) increased from 225.43-274.14 mg BHT equivalent g^{-1}. However, among these encapsulated of orange peel oil has shown the highest reducing power 274.14 mg BHT equivalent per gram. Also, TPC of nano-encapsulated (OPNE) increase from 52.28-59.31 µg GA per gram. Therefore, nano-encapsulated (OPNE) is a potent source of antioxidant.

Table 8: Efficiency of antioxidant properties of OPEE or LPEE before and after nano-capsulation in cake during storage at 4°C for 60 days

	DPPH radical scavenging assay (mg mL^{-1})			
	Non-encapsulated		Encapsulated	
Storage period (No. of days)	Orange peels (OPEE)	Lemon peels (LPEE)	Orange peels (OPNE)	Lemon peels (LPNE)
0	91.05±2.71	83.15±2.56	90.23±2.68	82.11±2.84
10	90.27±3.64	79.18±2.26	88.74±3.51	80.74±3.22
20	87.46±2.75	75.35±2.84	87.82±3.22	78.55±2.94
30	83.31±2.92	68.09±3.16	87.24±2.97	77.42±2.87
40	76.18±2.85	64.82±2.90	86.52±3.15	73.58±3.25
50	72.00±2.65	57.91±2.29	85.34±3.03	71.69±3.48
60	69.55±3.89	52.44±3.41	84.84±2.54	68.21±2.97

Table 9: Effect of using natural antioxidants of OPEE or LPEE before and after nanocapsulation on sensory properties of cake compared to cake containing synthetic antioxidant (BHT)

	Organoleptic properties					
Cake samples	Appearance	Color	Odor	Taste	Texture	Overall acceptability
Control	7.666±1.3	7.066±1.2^{ab}	7.066±0.9^{b}	7.266±1.0^{bc}	7.600±0.8^{cd}	7.333±0.9^{bc}
BHT	7.400±1.5	6.733±1.2^{b}	5.200±1.7^{c}	6.933±1.2^{c}	7.333±0.7^{d}	6.720±1.0^{c}
OPEE	7.866±1.2	7.933±1.2^{ab}	7.800±0.8^{ab}	7.733±0.8^{ab}	7.933±0.8^{bcd}	7.853±0.8^{ab}
LPEE	7.800±1.3	8.133±1.3^{a}	7.800±0.8^{ab}	8.133±0.9^{a}	8.133±0.8^{abc}	7.946±0.9^{ab}
OPNE	8.400±1.1	8.533±1.0^{a}	8.466±0.9^{a}	8.400±1.0^{a}	8.666±0.8^{a}	8.493±0.8^{a}
LPNE	8.266±1.1	8.466±0.9^{a}	8.400±0.9^{a}	8.200±0.9^{a}	8.466±0.9^{ab}	8.360±0.7^{a}
LSD	NS	0.868	0.81138	0.756	0.6231	0.679

OPEE: Orange peel ethanol extract, LPEE: Lemon peel ethanol extract, OPNE: Orange peel nanoencapsulated and LPNE: Lemon peel nanoencapsulated

Antioxidant properties of nano-capsulated orange and lemon peels extracts on cake: Efficiency of antioxidant property of orange oil and lemon peels extract before and after nano-encapsulation were evaluated on cake during storage for 60 days at 4°C. Table 8 showed that different OPEE or LPEE before and after nano-encapsulation provides antioxidant benefits to cakes storage periods. Nano-encapsulated of OPNE was demonstrated the most potent effect in terms of antioxidative activity in cake where, its DPPH reached to 84.84 mg mL^{-1} after 60 days, while treated cake with orange, lemon, nano-encapsulated of LPNE decreased to 69.55, 52.44 and 68.21 mg mL^{-1}, respectively. Therefore, it is suggested nano-encapsulated of OPNE be used to extend the shelf life of cakes for its effectiveness as natural antioxidant agent.

Sensory evaluation: The effects of using natural antioxidant compounds (orange or lemon peels extract) before and after nano-encapsulation were evaluated sensorially in cakes and compared with one of the most common synthetic antioxidant (BHT). Table 9 showed that, appearance of all cake samples not affected significantly before or after using synthetic, nature, or nano-encapsulated nature antioxidant of lemon or orange peels extract. While, treating cake with BHT led to slightly decrease acceptability of cake color, odor, taste, texture and overall acceptability compared to LPEE and OPEE before or after nano-encapsulation.

CONCLUSION

This study concluded that orange and lemon peels extract provides antioxidant activity, especially after nano-encapsulation. Therefore, this natural nano-encapsulated product proved benefits to cakes during room temperature storage. Therefore, it is suggested that orange and lemon extract nano-encapsulated could be used to extend the shelf life of cakes for effectiveness as natural antioxidant agent.

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