Antioxidant and antimicrobial activities and UPLC-ESI-MS/MS polyphenolic profile of sweet orange peel extracts

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ABSTRACT

With growing consumer awareness, exploitation of renewable resources is cost-effective and environmentally friendly. This work examines the potential of citrus peels as natural antioxidants and antimicrobials for food preservation. Extraction yield, total soluble phenols and flavonoids of various citrus peels (sweet orange, lemon, tangerine and grapefruit) were optimized by varying the solvent type. While the highest extract yield (~16 g/100g) was obtained from the sweet orange peels in methanol, extraction with ethanol maximized the concentration of total phenols and flavonoids (~80 mg catechol equivalents/100 g dry weight). In addition, sweet orange peel extract showed the highest DPPH, ABTS and hydroxyl radical scavenging values. UPLC-ESI-MS/MS analysis of aqueous and ethanolic extracts of sweet orange peels revealed more than 40 polyphenolic compounds including phenolic acids and flavonoids, some of which have not been previously reported. The predominant polyphenols were narirutin, naringin, hesperetin-7-O-rutinoside naringenin, quinic acid, hesperetin, datiscetin-3-O-rutinoside and sakuranetin. The incorporation of sweet orange peel extract into two vegetable oils enhanced their oxidative stability. In addition, all citrus peel extracts possessed high antimicrobial activity against several food-borne pathogens, and the activity was highest for the sweet orange peel extract. Overall results suggested the great potential of sweet orange peels as natural antioxidant and antimicrobials, which can be efficiently extracted using a simple and low-cost method, for enhancing the storage stability and safety of vegetable oils.

1. Introduction

Citrus fruits are the world’s largest fruit sector with an annual production of >100 million tons. In Egypt, the sixth world producer of orange, there are a variety of citrus fruits in particular oranges (69% of citrus production) such as navel, Baladi, sweet and blood oranges, whose production has been dramatically increased to ~4.27 million tons including 1.34 tons in exports (Omran et al., 2018). Approximately 20% of the total weight of citrus peels is wasted as by-products in conventional food processing, contributing to some environmental pollution (Huang and Ho, 2010). With increasing the industrial citrus waste to more than 40 million tons, many researchers have been trying to convert citrus wastes into valuable products to avoid severe pollution and destruction to the environment (Sharma et al., 2017).

Recently, there is a global interest in extracting beneficial compounds from agro-byproducts for use in food preservation (Shahidi et al., 2019). Although citrus peels are not edible, they possess significant biological activities including antimicrobial, antioxidant and anti-cancer activities (Singh et al., 2020). Recent studies indicated that citrus peels extract has a higher antioxidant capacity than synthetic antioxidants, and strong inhibitory effects on lipid oxidation (Contini et al., 2014). This antioxidant activity is due to the presence of a number of bioactive components in citrus such as phenolic compounds, limonoids, flavonoids and polysaccharides scavenging single oxygen, hydroxyl radicals, and lipid per-oxyl radicals (Shahidi, 1997). Citrus peels were also found to possess high antimicrobial activity against several food-borne pathogens (Lawal et al., 2013).

Lipids including oils and fats are essential ingredients that enhance many of the functional properties and sensory attributes of food products (Awad and Marangoni, 2006). However, susceptibility of lipids to
oxidation during food processing, preparation, and storage limits the quality and shelf-life of food products. Oxidation generates free radicals and toxic metabolites, which deteriorate the food quality, sensory attributes (e.g., color and flavor) and nutritional value, and often cause sickness (Chaiyasit et al., 2007; Aladedunye and Matthaus, 2014). Synthetic antioxidants such as tertiary butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) are commonly added to oils to delay, decrease or inhibit lipid oxidation at low temperatures (Cassarotti and Jorge, 2014). However, they are unstable at high temperatures and have negative impact on health (Chaiyasit et al., 2007). With growing consumer awareness, exploitation of the renewable wastes and by-products of citrus fruits can be a cost-effective and environmentally friendly strategy for producing highly valuable and natural antioxidant and antimicrobial ingredients for various food applications.

The analysis of polyphenols and flavonoids in citrus peels have been typically identified and quantified using high performance liquid chromatography (HPLC) (Ignat et al., 2011; Brito et al., 2014; Silva et al., 2014; Omoba et al., 2015; Sharma et al., 2017; Singh et al., 2020). Recently, only a few reports have utilized ultra-performance liquid chromatography–electrospray ionization–tandem mass spectrometry (UPLC-ESI-MS/MS) (Xing et al., 2017; Ana et al., 2018; Wang et al., 2019), which showed higher sensitivity and resolution, a shorter analysis time and substantially less organic solvent requirement than HPLC.

In this work, attempts were made to optimize the solvent extraction of bioactive components from the peels of various local citrus fruits, and to evaluate their antioxidant and antimicrobial activities. The antioxidant capacity of extracts was evaluated using three standard methods while the antimicrobial activity was tested against both Gram-positive and Gram-negative bacteria as well as fungi. The polyphenol profile in sweet orange peels, which exhibited the highest yield, was chemically identified and quantified using a rapid and sensitive UPLC-ESI-MS/MS method. Moreover, the ability of orange peel extract to protect vegetable oils against oxidation during storage, and its effect on the dietary value of oils were studied.

2. Materials and methods

2.1. Plant materials and chemicals

Four species of citrus namely: grapefruits (Citrus paradisi), sweet oranges (Citrus sinensis), tangerine (Citrus reticulate) and lemons (Citrus limon) were purchased at maturity stage (i.e., just entered for consumer purchase) from a local market in Alexandria, Egypt (January 2018), and the species were authenticated by the Pomology Department, Faculty of Agriculture, Alexandria University, Egypt. The fruits were washed with tap water and the peels were manually cut with a knife and lyophilized for 48 h at −56 °C in a Dura-Dry MP freeze dryer (FTS Process, USA at 0.04 Mbar). Finally, each fruit’s freeze-dried peels were crushed using a mortar and stored for analysis at −20 °C. Soybean oil and sunflower oil without added antioxidants were kindly provided from Alexandria Oil Company (Alexandria, Egypt). All analytical grade chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Extracts preparation

Extract preparation was performed according to (Hegazy and Ibrahim, 2012). Twenty grams of citrus peels were extracted with 200 mL of water, ethanol, methanol, acetone, petroleum ether or hexane (1:10 w/v) at room temperature by Maceration extraction method for 6 h. Extracts were filtered through a Whatman No. 2 filter paper for removal of peel particles. The residue was re-extracted twice under the same condition to ensure complete extraction. Yield of the components in different solvents was estimated by evaporating the organic solvents under vacuum using a rotary evaporator, followed by lyophilization.

2.3. Determination of total phenolic and total flavonoid contents

Total soluble phenols (TPC) were determined according to (Singleton and Rossi, 1965). Briefly, 50 μL of extracts were mixed with 3 mL of deionized water and 250 μL of Folin–Ciocalteu reagent (1 N). After 8 min of equilibration, 750 μL of 20% Na2CO3 and 950 μL of H2O were added to the extracts; after incubation for 30 min at room temperature, the absorbance was read at 765 nm with a UV–Vis spectrophotometer. Concentration of total soluble phenols compound was calculated using a standard curve of aqueous solutions of gallic acid and expressed as mg gallic acid equivalent/100 g dry weight of extract (mg GAE/100 g DW).

Total flavonoid content (TFC) was determined according to methods described by (Gonzalez-Aguilar et al., 2007; Gonzalez-Aguilar et al., 2007). 1 mL from each extracted sample was mixed and equilibrated with 4 mL of deionized water and 300 μL 5% NaNO2 for 5 min. After equilibration, 300 μL of 10% AlCl3 (methanolic solution) were added; the mixture was allowed to sit for 1 min and then 2 mL of 1 M NaOH were added. The last volume was completed to 10 mL with H2O, stirred, and readings were taken. Mixture absorbance was determined at 415 nm, using a UV–Vis spectrophotometer. Concentration of total flavonoids of fruits was calculated using a standard curve of catechol and expressed as mg catechol equivalent/100 g dry weight of extract (mg CE/100 g DW).

2.4. UPLC-ESI-MS/MS analysis of polyphenolic compounds

Sweet orange water and ethanolic extracts were analyzed using an ultra-performance liquid chromatography combined with mass spectrometry (UPLC-ESI-MS/MS) (Waters Acquity H-Class and Xevo G2-XS QTof). The column used was a C18 with 1.7 μm particle size, 2.1 mm by 50 mm (Waters Acquity BEH Column). Mobile phase was a mixture of A: water +0.1% formic acid, and B: acetonitrile +0.1% formic acid. A mobile phase gradient with the following parameters was used: initial condition, 5% B, 0.5 min–4 min: 5% slope to 60% B, 4 min–5.5 min: 80% B, 5.5–6.5 min: 5% B. The flow rate was set at 0.5 mL/min, and the injection volume was 10 μL for all runs. Electrospray ionization (ESI) desolvation was conducted at 600 °C and 1000 L/h of nebulizer gas, with the sampling cone at 130 °C and 50 L/h gas flow, and a capillary voltage of 3000 V. The mass spectrometer was operated in the negative mode and the MS/MS data were acquired in data-independent acquisition (Waters MSMe) mode with Lockspray continuous calibration (Waters MassLynx software suite). Data analysis was performed using the MS-DIAL v.r.8 Software 21 and the complete MSDIAL metabolomics MSP negative-mode spectral library (Tsugawa et al., 2015).

2.5. Antioxidant activity determination

2.5.1. ABTS radical scavenging assay

The ABTS assay, the procedure followed the method of (Re et al., 1999) with slight modifications. The ABTS radical cation (ABTS+) was generated by reaction of 5 mL of aqueous ABTS solution (7 mM) and 88 mL of 140 mM (2.45 mM final concentration) of a potassium persulfate solution. The mixture was held in dark at 29 °C for 14 h before being used, and then it was diluted with ethanol in order to obtain an absorbance of 0.7 ± 0.02 units at 734 nm using a UV–Vis spectrophotometer. Peel extracts (30 μL) or reference substances (BHA), were allowed to react with 3 mL of the resulting blue-green ABTS radical solution in a dark condition. The decrease of absorbance at 734 nm was measured at the end point of 6 min. The ABTS scavenging capacity of the extract was compared with that of BHA and percentage inhibition calculated as:

\[
\text{ABTS radical scavenging activity (\%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100
\]

Where \(\text{Abs}_{\text{control}}\) is the absorbance of ABTS radical + methanol; \(\text{Abs}_{\text{sample}}\) is the absorbance of ABTS radical + sample extract/standard. The activity of extracts was estimated at a minimum of three different concentrations. All the tests were performed in triplicate.
2.5.2. DPPH free radical scavenging activity

The extracts obtained above were used to assess the antioxidant capacity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method according to (Brand-Williams et al., 1995) with some modifications. The solution of DPPH (600 μM) was diluted with ethanol in order to obtain an absorbance of 0.7 ± 0.02 units at 517 nm peel extracts (30 μL) or controls (BHA) were allowed to react with 3 mL of DPPH radical solution for 30 min in dark and the decrease in absorbance from the resulting solution was monitored. The activity of extracts was estimated at a minimum of three different concentrations. All tests were performed in triplicate.

The inhibition of DPPH radical (%): \[
\text{Scavenging activity (％)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{(A_{\text{control}})} \times 100.
\]

Where A_control is Absorbance of the control solution; A_sample is Absorbance of the test extract.

2.5.3. Scavenging of hydroxyl radicals

Hydroxyl radical scavenging (HRS) assays were conducted by a Fenton reaction method (Ihe et al., 2004). Briefly, a reaction mixture containing 1.0 mL of Brilliant Green (0.435 mM), 2.0 mL of FeSO_4 (0.5 mM), 1.5 mL of H_2O_2 (3.0%, w/v), and 1.0 mL of extract in different concentrations was incubated at room temperature for 20 min, and the absorbance was measured at 624 nm. Changes in the absorbance of the reaction mixture indicated the scavenging ability of the extract for hydroxyl radicals. HRS activity was expressed as follows:

Scavenging activity (%) = [(A0 - A)/A0] x 100. Where A0 is the absorbance of the sample, A is the absorbance of the control, and A is the absorbance without the sample or Fenton reaction system.

2.6. Storage stability of vegetable oils

To test the effectiveness of sweet orange peels extract in preventing oil oxidation during storage, 100 g of vegetable oils (sunflower seeds or soybeans oils) incorporated with 50 mg of peel extract. A synthetic antioxidant (BHT) was added as their limit of 200 mg/kg as a positive control (Duh and Yen, 1997). Oil samples were placed into airtight glass bottles without headspace and stored for 15 min and stored for 28 days at room temperature (~24 °C). Oils without added antioxidants were considered as blank controls. Oil samples were stored after 15 min (for the Rancimat test), and every 7 days for measuring the peroxide value (PV). Other oil samples were taken on day 0 and 28 for measuring their fatty acid composition.

2.7. Rancimat test

The oxidative stability of the investigated oil samples was studied on a rancimat device (Model 743, Metrohm). Oil samples (3 g) were transferred into a reaction vessel, and the temperature and aeration rate were set to 120 °C and 20 L/h, respectively. Results were expressed as induction time (IT), which is the time (in hours) required to decompose oil-oxidized hydroperoxides (Laubli, 1988).

2.8. Determination of peroxide value (PV)

PV was determined by the improved ferrous oxidation–xylenol orange (mFOX) method (Dermij et al., 2012). Oil samples with and without sweet orange peel ethanol extract equivalent to 0.2 g were mixed with 9.8 mL chloroform-methanol, 7:3 ratio, in screw capped vials on a vortex mixer for 5 s. Then, 100 μL of 10 mM xylene orange was mixed and vortexed for 5 s. Then, 50 μL of 36 mM iron (II) solution was added and the sample was mixed on a vortex mixer for 5 s. After 5 min of incubation at room temperature, the absorbance of the samples was determined at 560 nm by a UV-visible spectrophotometer (T80 UV/Vis spectrometer PG Instruments LTD, United Kingdom).

2.9. Fatty acid composition

Fatty acids of oils were converted into their methyl esters (FAMEs) before gas chromatography (GC) analyses with a modification according to the AOCS Official Method Ce 1h-05 (Firestone, 2009) were performed. Oils were dissolved in 0.5 mL hexane and converted into FAMEs and 100 μL methanolic KOH (2 M) was added. Hydrochloric acid (2 M) was added until methyl orange indicator changed to pink and the mixture was allowed to settle. Then, 10 μL of the organic layer was injected into an Agilent 7890 GC (Agilent Technologies, Santa Clara, CA) equipped with a flame ionization detector. A stainless-steel column (30 m × 0.25 mm) packed with 70% cyanopropyl polysilphenylene siloxane was used. The oven temperature was held at 100 °C after sample injection and increased to 225 °C with a rate of 5 °C/min. The injector and detector temperatures were 260 °C and 280 °C, respectively. Helium was used as the carrier gas (3 mL/min), split ratio of 1:100 and the injection volume was 1 μL. The concentration of FAMEs in samples was determined using fatty acids standards including myristic, palmitic, stearic, oleic, linoleic, linolenic and arachidic acid (Sigma Aldrich, UK).

2.10. Antimicrobial activity

The antimicrobial activity of citrus peels extract was performed by the agar well diffusion method (Shehata et al., 2017). Eight species known to be pathogenic to human including Bacillus cereus ATCC 49064, Staphylococcus aureus NCTC 10788, Listeria monocytogenes ATCC 19116, Escherichia coli BA 12296, Salmonella senftenberg ATCC 8400, Yersinia enterocolitica ATCC 23715, Aspergillus carbonarius ITEM 5010 and Aspergillus parasiticus ITEM 11 were used. One hundred μL of the inoculum (1 × 10^8 cfu/mL) was mixed with specific media of each microorganism and poured into Petri plates. One hundred μL of the test compound was introduced into the well. The plates were incubated overnight at 37 °C for bacteria and 28 °C for fungi, and the diameter (mm) of the resulting zone of inhibition was measured.

2.11. Statistical analysis

All analytical results were expressed as mean of triplicates ± SD. The data were statistically analyzed using the software package SPSS v16 (SPSS Inc., Chicago, USA). One-way analysis of variance was performed to identify significant differences according to Duncan's multiple range test at significance level 5% (P < 0.05).

3. Results and discussion

3.1. Extraction yield

Recently, there is a worldwide interest in safe extraction of natural bioactives from industrial waste and by-products such as plant peels for functional foods and nutraceutical applications (Tunchayaphum et al., 2013). To enhance the yield of bioactive components from freeze-dried citrus peels (sweet orange, lemon, tangerine and grapefruit), they were extracted using various solvents including water, ethanol (70%), methanol (70%), acetone, petroleum ether and hexane. Results showed the extraction yield varied by the fruit type. About 8.23–15.56, 3.26–8.93, 8.66–13.56 and 7.43–12.73 g/100 g were extracted from sweet orange, lemon, tangerine and grapefruit peels, respectively. As shown in Table 1, the highest extraction yield was obtained from the sweet orange peel methanol extract (15.56 ± 0.60 g/100 g) followed by the tangerine peel aqueous extract (13.56 ± 0.81 g/100 g) and the grapefruit peel hexane extract (12.73 ± 0.80 g/100 g). The lowest extraction yield was 3.26 ± 0.15 g/100 g for the lemon peel acetone extract. Additionally, acetone extracts exhibited the least percent yield for sweet orange, lemon and grapefruit peels. These results thus indicate that methanol and ethanol are more efficient in extracting phytochemicals from citrus peels than other organic solvents (hexane, petroleum ether and acetone). A
moderate yield was obtained using water extraction for all citrus peels. In agreement, greater extraction yields from dried Citrus (C. unshiu) peels in water (~40%) and ethanol (~55%) were reported (Kim, 2013). Shie and Lay (2013) also reported higher yields (66.47–23.67%) in the methanol extract of C. limon fruits from Taiwan (Shie and Lay, 2013). However, other studies have shown lower yields from the ethanol extracts of C. sinensis (~7%) and C. limon (~16%) peels (Kumar et al., 2011). These differences can be due to various factors such as citrus varieties, efficacy of extraction, amount of soluble components and type of the solvent used (Hsu et al., 2006). The present study indicated that methanol is the most effective solvent for the extraction of bioactive compounds from sweet orange peels. Nevertheless, due to its biodegradability, bio-solvent and lower toxicity characteristics, ethanol is preferred for the extraction of antioxidant compounds (Karadeniz et al., 2005).

3.2. Total phenolic (TPC) and total flavonoid (TFC) contents

Today, it has been well established that the bioactive polyphenolic compounds such as phenolic acids and flavonoids in citrus fruits have essential roles in promoting human health and delaying/preventing the incidence of many chronic diseases (Ma et al., 2020). The remarkable free radical scavenging activity of polyphenols protects fruits and vegetables against pathogen infection, predators and photodestructive damage by UV radiation (Ignat et al., 2011). Polyphenols in citrus peels possess exceptional health-promoting activities including antioxidant, anti-inflammatory, anti-proliferative, anti-allergic, antiviral, anticarcinogenic, neuroprotective and antimicrobial, which protect against oxidative stress related diseases (Ma et al., 2020) (Oboh and Ademoun, 2012).

Previously, citrus peels have been shown to contain higher TPC levels than the other citrus fruit parts (Guiraudies et al., 2010). In this work, the freeze-dried sweet orange peel extract exhibited the highest content of total soluble phenols (from 153.26 to 345.23 mg GAE/100 g DW) among all studied citrus peels regardless of the extraction solvent (Table 1). TPC extractability from sweet orange peels varied based on the solvent type in the order of ethanol > methanol > acetone > water > petroleum ether > hexane. This confirmed the effectiveness of polar solvents (methanol, ethanol, and water) in extracting phenolic compounds from citrus peels compared to organic solvents (hexane and petroleum ether). In comparison, a study of 21 varieties of citrus showed that the total amount of phenolics in fresh citrus peels ranges from about 188.2 to 766.7 mg GAE/100 g FW, which were extracted for a long time (3 d) and in methanol at 4 °C (Ramful et al., 2010). Despite their longer extraction time and use of methanol, the ethanol extracts from sweet orange or other citrus fruit peels were still higher. The TPC values were also much higher than those reported for orange peel extracted in ethanol, methanol and acetone (1.39–1.85 mg GAE/g) (Park et al., 2014), yet close to that reported recently for Spanish orange peel extract (3.9 mg GAE/g DW) (Gómez-Mejía et al., 2019). Importantly, the TPC obtained from the sweet orange peels was significantly higher than the TPC extracted by ethanol and with the aid of non-conventional methods such as high-power ultrasound (275.8 mg GAE/100 g) (Khan et al., 2010) and High hydrostatic pressure (136.85–288.16 mg GAE/100 g) (Casquete et al., 2014, 2015). The lowest TPC (90.63 ± 1.47 mg GAE/g DW) was from the hexane extract of the tangerine peel.

Flavonoids are a widespread group of health promoting polyphenolic compounds with high antioxidant activity, which may protect against oxidative stress related diseases (Benavente-García et al., 1997). The content of total flavonoids (TFC) obtained from the peels of citrus fruits using different solvents ranged from ~19 to 80 mg catechol (CE)/100 g dry weight (Table 1). The highest TFC was for the orange peels ethanol extract (79.54 ± 0.95 mg CE/100 g). In addition, the extraction of flavonoids from sweet orange peel also depended on the type of solvent used, in the order of ethanol > acetone > water and methanol > petroleum ether > hexane. Sweet orange peels also showed the highest TFC in the other solvents except for the grapefruit peel extract in methanol (~60 mg CE/100 g), which was slightly higher than sweet orange peel in the same solvent (52 mg CE/g). Other workers reported very small TFC yield from various citrus peels extracts including orange peels by methanol (~28 μg QE/g) and ethanol (~30 μg QE/g) (Hegazy and Ibrahim, 2012). These differences in TFC are probably due to variations in citrus species and origin and extraction solvent characteristics (e.g., polarity).

Overall, this simple and sustainable extraction method allowed to obtain flavenoids of polyphenolic compounds and flavonoids from citrus peels. By avoiding aggressive solvents and using environment friendly solvents (e.g., water and ethanol), this simple, eco-friendly recovery method can provide a cost-effective and safe source of bioactive ingredients from citrus fruit wastes for use in food processing and preservation. This extraction method, which also avoids the need for expensive equipment or high energy, can be very beneficial for small food companies, especially in developing countries.

Table 1
Extract yield, total phenolics (TPC), and total flavonoids (TFC) of citrus peels.

| Solvents       | Citrus peels | Yield (g/100 g) | TPC (mg GAE/100 g) | TFC (mg catechol/100 g) |
|----------------|--------------|-----------------|--------------------|-------------------------|
| Water          | Sweet        | 9.40 ± 0.78     | 255.86 ± 1.77      | 52.06 ± 1.74            |
|                | orange       | 12.56 ± 0.81    | 135.83 ± 1.25      | 40.33 ± 2.02            |
|                | Tangerine    | 11.53 ± 0.62    | 149.83 ± 2.25      | 30.20 ± 0.36            |
|                | Grapefruit   | 8.36 ± 0.36     | 150.16 ± 2.45      | 39.43 ± 0.70            |
| Ethanol        | Sweet        | 10.90 ± 0.26    | 345.23 ± 1.45      | 79.54 ± 0.95            |
|                | orange       | 6.40 ± 0.79     | 196.16 ± 2.25      | 31.03 ± 0.98            |
|                | Tangerine    | 11.02 ± 0.45    | 212.66 ± 3.05      | 39.43 ± 0.70            |
|                | Grapefruit   | 11.46 ± 0.55    | 237.50 ± 2.67      | 60.33 ± 1.75            |
| Methanol       | Sweet        | 15.56 ± 0.80    | 320.43 ± 1.28      | 58.20 ± 2.49            |
|                | orange       | 3.26 ± 0.15     | 150.50 ± 2.43      | 52.00 ± 1.16            |
|                | Tangerine    | 10.23 ± 0.92    | 160.83 ± 1.65      | 27.40 ± 2.20            |
|                | Grapefruit   | 7.43 ± 0.04     | 257.20 ± 2.43      | 51.80 ± 1.96            |
| Acetone        | Sweet        | 10.16 ± 0.35    | 190.46 ± 3.03      | 45.33 ± 1.16            |
|                | orange       | 5.70 ± 0.55     | 150.50 ± 1.80      | 29.20 ± 1.11            |
|                | Tangerine    | 10.10 ± 0.45    | 112.80 ± 2.55      | 27.36 ± 2.87            |
|                | Grapefruit   | 9.80 ± 0.79     | 161.43 ± 3.04      | 25.46 ± 1.30            |
| Petroleum ether| Sweet        | 11.80 ± 0.26    | 153.26 ± 1.41      | 39.53 ± 0.94            |
|                | orange       | 7.55 ± 0.32     | 130.90 ± 2.35      | 23.96 ± 1.61            |
|                | Tangerine    | 8.66 ± 0.45     | 90.63 ± 1.47       | 18.96 ± 1.47            |
|                | Grapefruit   | 12.73 ± 0.28    | 100.60 ± 2.33      | 26.33 ± 0.80            |

* Dry weight. Results are expressed as means ± standard error of three measurements. Means in the same column between citrus peels and regardless of the solvent used in the extraction followed by different superscripts are significantly different according to Duncan’s multiple range test at significance level P < 0.05.
3.3. Antioxidant activity

Antioxidant capacities of the citrus peel extracts were determined by DPPH, ABTS and hydroxyl radical scavenging assays. Results showed significant differences between citrus peels within the same assay (p < 0.0001) (Table 2). Antioxidants react with DPPH converting it to 1,1-diphenyl-2-picryl hydrazine, due to its rapid hydrogen accepting ability, which intercepts the spread of free radical oxidation chain forming stable end products that do not cause further lipid oxidation (Yamaguchi et al., 1998). In the DPPH assay, the greatest antioxidant capacity was obtained for the ethanol extract of sweet orange peel (79.32 ± 1.05%) whereas the hexane extract of lemon peel had the lowest antioxidant capacity (42.43 ± 0.59%). In fact, sweet orange peel extract had the highest antioxidant activity regardless of extraction solvent in all assays. These results are in agreement with a previous work, which found that orange peel extracts possess a comparatively higher activity than other peels in all assays (Hegazy and Ibrahim, 2012). These outcomes are likely associated with the peel content of phenolics. Previous work assessed the DPPH radical scavenging ability of different citrus species, and indicated that the antioxidant activity and phenolic content are strongly affected by the species as well as extraction solvent (Zahoor et al., 2016). Same study also found that the extraction of red blood peel (C. sinensis ‘Maltaise Sanguine’) with methanol yielded the highest antioxidant activity, in agreement with the current results (Table 2).

ABTS + assay-measured antioxidant activity for various citrus peels and extraction solvents exhibited similar trends as the DPPH method. The scavenging ability of hydroxyl radicals by orange peels was also powerful compared with other citrus peels (Table 2). In the ABTS’ reduction assay, the antioxidant activity was quantified in terms of reduction in ABTS’ radicals by antioxidants. Among all citrus peels, the highest antioxidant capacities were determined for the ethanolic sweet orange peel extract (68.36 ± 0.72%) followed by the peels of lemon (64.16 ± 0.90%), grapefruit (57.43 ± 1.46%) and tangerine (50.41 ± 0.90%). Methanolic sweet orange peels extracts also exhibited high antioxidant activities, whose values were statistically similar to those of the ethanolic extract. Hydroxyl radicals are well known for abstracting membrane lipid hydrogen atoms and leading to lipid peroxidation. Apparently, the capacity of the extracts to quench hydroxyl radical seems to be directly related to the avoidance of lipid peroxidation propagation process (Halliwell and Gutteridge, 1984). Among the different peel extracts, methanol extracts (70.30 ± 1.35%) obtained from sweet orange peels possessed the highest activity, followed by the ethanol extract obtained from this fruit peel (66.56 ± 1.46%). In all assays, lowest antioxidant activity was for the tangerine extracts in ethanol, methanol and acetone, and for the grapefruit extracts in petroleum ether and hexane. These variations in the antioxidant activity between extracts can be explained by the differential solubilization of antioxidant compounds, as was reported for citron blood orange (Jayaprakasha and Patil, 2007). The antioxidant capacity of citrus peels might be related to the presence of phenolic compounds and flavonoids. Multiple compounds such as flavonanes, flavanone glycosides and polymethoxylated flavones are special to citrus, which are comparatively uncommon in other plants (Li et al., 2006). Based on these results, the ethanolic sweet orange peel extract was selected for further analysis and application as potential natural antioxidant for vegetable oils.

3.4. Polyphenolic profile of sweet orange peel extracts

An ultra-performance liquid chromatography combined with mass spectrometry (UPLC-ESI-MS/MS) was used to identify the phenolic compounds in the aqueous and ethanolic extracts of sweet orange peels. Tables S1 and S2 summarize the 22 (aqueous extract) and 32 (ethanolic extract) compounds identified by UPLC-ESI-MS/MS and their characteristics including the retention time, detected accurate masses in negative ionization mode, molecular formula, percentage of each compound and characterization references. As shown in Table S1, the polyphenolic compounds detected in the aqueous extract belong to 6 different phenolic families; phenolic acids, flavanones, flavones, flavonols, anthocyanin and scopoletin. The major polyphenolic compounds were narirutin (~20%), hesperetin-7-O-rutinoside naringenin (~15.9%), naringin (15.7%), quinic acid (~12%), datiscetin-3-O-rutinoside (11.5%), and flavone base + 30 1MeO C-Hex-Hex (6.6%). There were also moderate (~2-4%) to low (~<1%) concentrations of other polyphenolic compounds such as cymaroside A, isoorientin, flavanone base + 30, C-Hex, diasmetin-7-O-rutinoside and didymin. In addition, a few non-polyphenolic classes such as terpenes (sylviside) and cardioxyls (citrate) were detected. On the other hand, the 32 compounds contained in the ethanolic sweet orange peel extract (Table S2) belonged to 5 phenolic families; simple phenols, phenolic acids, flavanones, flavones and flavonols, while the major compounds were narirutin (~20%), naringin (~18.2%), hesperetin (~11.8%), datiscetin-3-O-rutinoside (11.5%) and sakuranetin (~6%). Compounds detected at low concentrations (~2–4%) include cymaroside A, isoorientin, flavanone base + 30, C-Hex, diasmetin-7-O-
rutinose and didymin, and some compounds represented ~1% or less. Anthocyanin and scrophulein were not detected in the ethanolic extract. Non-polyphenolic classes such as unsaturated fatty acids were detected in the ethanolic extract but not in the aqueous extract. Table 3 summarizes the concentrations (μg/g) of the phenolic content from the aqueous and ethanolic extracts of sweet orange peels quantified by UPLC-ESI-MS/MS. The predominant compounds were naringin (~29 μg/g), naringenin (~27 μg/g), hesperetin-7-O-rutinoside naringenin (~15 μg/g), quinic acid (~13 μg/g), hesperetin (~17 μg/g), dactisicetin-3-O-rutinoside (~11 μg/g) and sakuranetin (~9 μg/g). In comparison, a recent study that also used UPLC for identifying the phenolic compounds in sour orange extracts in aqueous ethanol (96 and 50%) as well as water reported a lower concentration of naringenin (3–14 μg/g) (Ana et al., 2018). Other workers attempted to enhance the total phenols and flavonoids from fresh orange peel by using pulsed electric field, which enhanced the yield of naringin and hesperidin from 1 to 3.1 and from 1.3 to 4.6 mg/100 g FW, respectively. This is almost the same yield of naringin obtained from the peels of sweet oranges in this study without the use of non-conventional methods. These results thus indicated that sweet orange peel is a rich natural source of several phenolic compounds that are well known for their antioxidant and antimicrobial activities. To the best of our knowledge, this is the first study that detected sinapoylhexoside (isomer of 995), dactisicetin-3-O-rutinoside and others in orange or its peel extracts.

3.6. Orange peel extract enhances oxidative stability of vegetable oils

3.6.1. Peroxide value (PV)

The PV of soybean and sunflower oils increased significantly during the 28 days of storage both with and without the addition of antioxidants (Fig. 1). At the end of the 28 days storage period, both native (no additives) oils had considerably greater PV (p < 0.05) than oils incorporated with either sweet orange peel extract or synthetic antioxidant (BHT). The PVs of sunflower oil incorporated with orange peel extract and BHT were 19.53 ± 1.42 and 21.43 ± 0.94 meq/kg, while those of soybean oil were 17.63 ± 0.75 and 22.30 ± 1.20 meq/kg, respectively. Therefore, the addition of sweet orange peel extract enhanced the antioxidant activity of the two vegetable oils, which reduced the rate of oil oxidation. These results are consistent with the PVs of rosemary extract oils that were significantly lower (P < 0.05) than native oils and synthetic antioxidant incorporated oils (Yang et al., 2016).

3.6.2. Rancimat analysis

The Rancimat test has been popularly utilized in assessing the antioxidant abilities of natural and synthetic antioxidants. The Rancimat analysis works by measuring differences in conductivity produced by low molecular weight volatile organic acids such as formic and acetic acids generated from oil oxidation at high temperatures (Cordeiro et al., 2013). The cycle of induction is the time it takes to generate secondary oxidation products and is used to describe the resistance to oxidation of the oil. The longer the time of induction the greater is the oil stability. Fig. 2 shows the effect of sweet orange peel extract and synthetic antioxidant (BHT) on the oxidative stability of vegetable oils. It is not surprising that oils without the addition of any antioxidants were the easiest to oxidize, as stated by the smallest induction period (IP) values, which correlated well with the highest PV values and lowest oxidative stability of these oils. Results showed that the IP values of oils with added sweet orange peel extract or BHT are greater than that of the native oils. The sunflower oil initially showed a significantly higher IP value (3.6 h) than soybean (3.1 h). This might be due to the powerful antioxidant capacity reported for orange peel extract (Zahoor et al., 2016). The findings reported here agree with the IP of rosemary extract oils that were significantly higher (P < 0.05) than native oils and oils with added synthetic antioxidant (Yang et al., 2016). The IP values for the two forms of oils with incorporated sweet orange peel extract were higher than those for oils with added BHT, demonstrating that sweet orange peel extract is more effective in stabilizing oil against oxidative degradation than synthetic antioxidants.

### Table 3

Phenolic content (%) from aqueous and ethanolic extracts of sweet orange peels by UPLC-ESI-MS/MS.

| Compounds                        | Extract conc. (%) |
|---------------------------------|-------------------|
|                                 | Aqueous           | Ethanol         |
|                                 | (μg/g)            | (μg/g)          |
| Feruloylquinic acid             | 0.71 ± 0.01       | ND               |
| D(-)-Quinic acid                | 14.21 ± 0.03      | ND               |
| Hydroxyquinol                   | ND                | 0.20 ± 0.01     |
| 2,5-dihydroxybenzoic acid 2-O-beta-D-glucoside | 1.64 ± 0.03 | 1.29 ± 0.03 |
| Catechol                        | ND                | 0.11 ± 0.02     |
| Coumaroyl + C_6H_5O_3 (isomer of 843, 844, 846) | ND         | 1.55 ± 0.01     |
| Citrate                         | 0.72 ± 0.03       | 0.53 ± 0.02     |
| Cisimarinin                     | 0.11 ± 0.03       | ND               |
| Cynaroside A                    | 1.99 ± 0.02       | 2.28 ± 0.01     |
| Benzyl alcohol ÷ Hex-Pen        | ND                | 0.38 ± 0.02     |
| Isoorientin                     | 3.69 ± 0.01       | 3.82 ± 0.01     |
| Sinapoyl D-glucoside            | 0.61 ± 0.02       | 0.99 ± 0.02     |
| Isohamnetin-3-O-galactoside-6′-rhamnoside | ND   | 6.15 ± 0.01     |
| Flavonone base + 30, C-Hex      | ND                | 3.87 ± 0.03     |
| Flavone base + 30, C-Pen-Hex    | ND                | 1.89 ± 0.02     |
| Flavone base + 30, 1MeO, C-Hex-Hex | 6.62 ± ND      | ND               |
| Dactisicetin-3-O-rutinoside     | 11.50 ± 0.01      | 1.93 ± 0.01     |
| NP-0000626                      | 1.80 ± ND         | ND               |
| Isohamnetin-3-O-rutinoside      | ND                | 0.78 ± 0.01     |
| Naringenin                      | 0.98 ± 0.03       | 2.04 ± 0.03     |
| Narirutin                       | 19.40 ± 0.02      | 19.86 ± 0.02    |
| Sakuranetin (S)-5-hydroxy-2-(4-hydroxyphenyl)-7-methoxychroman-4-one | 0.31 ± ND | ND               |
| Kaempferol-4-methyl ether       | ND                | 0.24 ± 0.01     |
| Diosmetin-7-O-rutinoside        | ND                | 2.64 ± 0.02     |
| Naringin                        | 15.72 ± 0.03      | 18.21 ± 0.01    |
| Naringoside                     | 0.11 ± 0.01       | ND               |
| Hesperetin                      | 3.10 ± 0.02       | 11.79 ± 0.01    |
| Hesperetin-7-O-rutinoside       | 15.86 ± 0.03      | ND               |
| Azelaic acid                    | ND                | 1.2 ± 0.03      |
| Sakuranetin                     | ND                | 6.16 ± 0.00     |
| Sylviside                       | 0.11 ± ND         | ND               |
| Cyanidin-3-O-alpha-arabinoside   | 0.06 ± ND         | ND               |
| Isoakuranetin-7-O-norbesperidose | ND                | 3.18 ± 0.02     |
| Isoakuranetin-7-O-rutinoside    | 0.71 ± 0.01       | 1.17 ± 0.01     |
| Naringenin                      | ND                | 0.12 ± 0.02     |
| Kaempferol-3-O-arabinoside      | 0.05 ± ND         | ND               |
| Vitexin                         | ND                | 0.06 ± 0.03     |
| 9-Octadecenoic acid             | ND                | 3.41 ± 0.02     |
| 9-Octadecenedioic acid          | ND                | 0.76 ± 0.02     |
| Hexadecanediol acid             | ND                | 0.79 ± 0.01     |
| Hydroxylinoleic acid            | ND                | 2.58 ± 0.03     |

ND: not detected. Results are expressed as means ± standard error of three measurements.
Without sweet orange peel extract or synthetic antioxidants (BHT), the was significantly lower than that in control soybean oil (15.92%).

Table 4 displays the fatty acid composition of sunflower and soybean oils with and without antioxidants (sweet orange peel extract and BHT) after storage for 28 days. Both oils contained varying concentrations of different fatty acids. Linoleic acid (C18:2) was the dominant fatty acid in both sunflower (58.6%) and soybean (48.6%) oils. The saturated fatty acids (SFA) content in control (without extract) sunflower oil (12.01%) was significantly lower than that in control soybean oil (15.92%). Without sweet orange peel extract or synthetic antioxidants (BHT), the concentration of C16:0, C18:0, C18:1 and C20:0 fatty acids increased after the storage period (28 days) for both control oils. With the addition of sweet orange peel extract, the concentration of C18:1 and C18:2 increased further compared with the control oils after storage, which may indicate that the oxidative stability of unsaturated fatty acids in the oils were enhanced by the antioxidants in orange peel extract. Although the same effect was obtained with BHT, it was less effective. Other workers have also found that the incorporation of rosemary extract into oils enhanced the levels of unsaturated fatty acids such as C18:1 and C18:2 and C18:3 compared to blank oils and synthetic antioxidant incorporated oils (Yang et al., 2016). The SFA content of control sunflower oil (17.61%) and soybean oil (24.76%) were higher after storage compared to their initial values. However, the SFA content in the two oils was more stable in oils mixed with sweet orange peel extract or BHT. With the incorporation of sweet orange peel extract, SFA decreased slightly from 12.1 to 11.59% for sunflower oil and from 15.92 to 15.66% for soybean oil after storage. Using BHT, in comparison, SFA increased to 15.66% and 20.65% for sunflower oil and sunflower oil (24.76%) were higher after storage compared to their initial values. However, the SFA content in the two oils was more stable in oils mixed with sweet orange peel extract or BHT. With the incorporation of sweet orange peel extract, SFA decreased slightly from 12.1 to 11.59% for sunflower oil and from 15.92 to 15.66% for soybean oil after storage. Using BHT, in comparison, SFA increased to 15.66% and 20.65% for sunflower and soybean oils, respectively. These results strongly indicated that sweet orange peel extract is slightly more effective than synthetic antioxidants in stabilizing the content of both unsaturated and saturated fatty acids during long term storage. Sun-waterhouse et al. (2011) also noted the stability of encapsulated olive oil with caffeic acid stored at 20 and 37 °C (Sun-Waterhouse et al., 2011). It is also known that the unsaturated fatty acids of oil get easily oxidized, and that increasing the double bonds of unsaturated fatty acids decreases the oxidative stability of oils (Choe and Min, 2006). In this study, while the amount of PUFAs was significantly reduced in control oils at the end of storage, the incorporation of sweet orange peel extract into sunflower oil increased.

**Fig. 1.** Change in the peroxide value (meq/kg) of (A) soybean oil and (B) sunflower oil during storage. Blank, oil without antioxidant; Extract, oil incorporated with sweet orange peel ethanol extract; BHT, oil incorporated with synthetic antioxidants (BHT).

**Fig. 2.** Induction period of the Soybean and sunflower oils obtained from the Rancimat test. Blank, oil without antioxidant; Extract, oil incorporated with sweet orange peel ethanol extract; BHT, oil incorporated with synthetic antioxidants (BHT).

### 3.6.3. Fatty acid composition

Table 4 displays the fatty acid composition of sunflower and soybean oils with and without antioxidants (sweet orange peel extract and BHT) after storage for 28 days. Both oils contained varying concentrations of different fatty acids. Linoleic acid (C18:2) was the dominant fatty acid in both sunflower (58.6%) and soybean (48.6%) oils. The saturated fatty acids (SFA) content in control (without extract) sunflower oil (12.01%) was significantly lower than that in control soybean oil (15.92%). Without sweet orange peel extract or synthetic antioxidants (BHT), the concentration of C16:0, C18:0, C18:1 and C20:0 fatty acids increased after the storage period (28 days) for both control oils. With the addition of sweet orange peel extract, the concentration of C18:1 and C18:2 increased further compared with the control oils after storage, which may indicate that the oxidative stability of unsaturated fatty acids in the oils were enhanced by the antioxidants in orange peel extract. Although the same effect was obtained with BHT, it was less effective. Other workers have also found that the incorporation of rosemary extract into oils enhanced the levels of unsaturated fatty acids such as C18:1 and C18:2 and C18:3 compared to blank oils and synthetic antioxidant incorporated oils (Yang et al., 2016). The SFA content of control sunflower oil (17.61%) and soybean oil (24.76%) were higher after storage compared to their initial values. However, the SFA content in the two oils was more stable in oils mixed with sweet orange peel extract or BHT. With the incorporation of sweet orange peel extract, SFA decreased slightly from 12.1 to 11.59% for sunflower oil and from 15.92 to 15.66% for soybean oil after storage. Using BHT, in comparison, SFA increased to 15.66% and 20.65% for sunflower and soybean oils, respectively. These results strongly indicated that sweet orange peel extract is slightly more effective than synthetic antioxidants in stabilizing the content of both unsaturated and saturated fatty acids during long term storage. Sun-waterhouse et al. (2011) also noted the stability of encapsulated olive oil with caffeic acid stored at 20 and 37 °C (Sun-Waterhouse et al., 2011). It is also known that the unsaturated fatty acids of oil get easily oxidized, and that increasing the double bonds of unsaturated fatty acids decreases the oxidative stability of oils (Choe and Min, 2006). In this study, while the amount of PUFAs was significantly reduced in control oils at the end of storage, the incorporation of sweet orange peel extract into sunflower oil increased.
their PUFA levels from 48.78% (blank oil) to 53.62% indicating an increase in oxidative stability. Similarly, the PUFA content in soybean oil incorporated with sweet orange peel extract increased slightly from 38.8 (blank oil) to 49.7%, which was still higher than soybean oil treated with BHT (44.79%). Therefore, incorporating sweet orange peel extract into sunflower and soybean oils can be an efficient strategy for enhancing their oxidative stability during storage as well as dietary PUFA.

3.7. Antimicrobial activity

Previous studies have shown that sweet orange and lemon peel extracts possess high antimicrobial activity against several food-borne pathogens (Lawal et al., 2013). To confirm the antimicrobial potential of the freeze-dried citrus peel extracts, they were tested against several Gram-positive and Gram-negative pathogenic bacterial strains as well as fungi. The inhibition zones (mm) of the various citrus peel extracts on selected microorganisms are given in Table 5. As shown, strain Bacillus cereus ATCC 49064 was more sensitive to the extracts of citrus peels than other tested pathogenic strains. In addition, only sweet orange and lemon peel extracts could inhibit the growth of Yersinia enterocolitica (ATCC 23715), as no zone of inhibition was observed for the tangerine and grapefruit peel extracts. Sweet orange peel extract (0.5 mg/mL) demonstrated the highest antimicrobial activity against all the microorganisms tested except for Aspergillus carbonarius ITEM 5010, which showed resistance to all citrus peels even at a higher concentration (1 mg/mL) (not shown). Compared with sweet orange peel, lemon peel had a lower antimicrobial activity against same pathogens. Grapefruit peel extract also exhibited exceptional antimicrobial activity against both Gram-positive and Gram-negative bacteria with the exception of Yersinia enterocolitica ATCC 23715 and the fungus Aspergillus carbonarius ITEM 5010. Although the tangerine peel demonstrated the lowest antimicrobial activity against most target microorganisms, it was more effective against Staphylococcus aureus (NCTC 10788) than lemon and grapefruit peel extracts. Dubey et al. reported strong antibacterial activity for orange peel extract against Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Shigella flexneri, Staphylococcus epidermidis and Pseudomonas aeruginosa (Dubey et al., 2011). Citrus peel extracts have shown more impact on foodborne pathogens due to their contents of phenolic compounds. Several evidences suggested that the interactions of phenolic compounds (e.g., rutin, quercetin and naringenin) lead to increasing the permeability of bacterial cell membrane, decreasing the production of ATP, binding to metabolic enzymes and disruption of membrane integrity, which lead to destruction of bacterial cell membranes (Bordes et al., 2019). Flavonoids, a large group of polyphenolic compounds, can inhibit the metabolism and syntheses of DNA and RNA in bacteria (Mirzoeva et al., 1997).

4. Conclusions

In this work, attempts were made to explore the potential of citrus peels as natural antioxidants and antimicrobials. The yield and content of total polyphenols and flavonoids in sweet orange, lemon, tangerine and grapefruit were dependent on the extraction solvent. Sweet orange peel had the highest yield (~16 g/100g) when extracted in methanol, however extraction in ethanol maximized its total phenolic compounds (~345 mg GAE/100 g DW) and flavonoids (~80 mg CE/100 g DW). Ethanolic extract of sweet orange peel exhibited the highest DPPH and ABTS values while those extracted in methanol had the highest hydroxy radical scavenging value. All citrus peel extracts showed great antimicrobial activities against both Gram-positive and Gram-negative bacteria as well as fungi. Sweet orange peel extract also exhibited the highest antimicrobial activity. UPLC-ESI-MS/MS analysis of sweet orange peels showed that the predominant phenolic compounds were narirutin (~29 µg/g), naringin (~27 µg/g), hesperetin-7-O-rutinoside naringenin (~15 µg/g), quinic acid (~13 µg/g), hesperitin (~17 µg/g), datiscetin-3-O-rutinoside (~11 µg/g) and sakuranetin (~9 µg/g), which are known for their antioxidant and antimicrobial activities. The supplementation of sweet orange peel extract into edible oils effectively prevented rancidity while maintained the oils dietary PUFA levels, suggesting its great potential as a great natural preservative. Overall, the present study showed that freeze-dried sweet orange peels are good sources of antioxidant and antimicrobial flavonoids and other polyphenolic compounds, which can effectively protect oils and other food products against oxidation and spoilage by food-borne pathogens.

Supporting information

Additional Supporting Information may be found in the online version of this article.

Table S1. HPLC-ESI-MS/MS characterization for the identified polyphenol compounds in aqueous extract of sweet orange peels by HPLC-ESI-MS/MS.

Table S2. HPLC-ESI-MS/MS characterization for the identified polyphenol compounds in ethanolic extract of sweet orange peels.

CRediT authorship contribution statement

Mohamed G. Shehata: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. Tarek S. Awad: Validation, Methodology, Formal analysis, Investigation, Writing – original draft. Dalal Asker: Conceptualization, Validation, Formal analysis, Investigation, Visualization, Preparing Artwork, Writing – original draft. Sobhy A. El Sohaimy: Conceptualization, Supervision, Validation, Visualization, Formal analysis, Investigation. Nourhan M. Abd El-Aziz: Methodology, Data curation, Formal analysis, Visualization, Writing – review & editing, Writing – original draft. Mohammed M. Youssef: Supervision, Validation, Investigation, Visualization, Writing – review & editing.
Declaration of competing interest
The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcrfs.2021.05.001.

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