http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2021.99.111

Research Article
Efficiency of Red Onion Peel Extract Capsules on Obesity and Blood Sugar

[1]Khaled F. Mahmoud, [2]Amal Z. Hammouda, [1]Hatem S. Ali and [1]Azza A. Amin

[1]Department of Food Science and Technology, National Research Centre, P.O. Box 12622, Giza, Egypt
[2]Department Special Food and Nutrition Department Food Technology Research Institute, Giza, Egypt

Abstract

Background and Objective: Red onions are one of the most consumed vegetable crops in Egypt, their peel is rich in antioxidants that reduce the risk of diabetes and weight is lost. The study aimed to extract bioactive compounds present in Egyptian Red Onion Peels Waste (ROPE), increasing their efficiency and protecting them using nano-encapsulation as new emerging technology. **Materials and Methods:** Extraction of the bioactive compounds in the Egyptian red onion peels was carried out to study their antioxidant activity before and after nano-emulsions and micro-capsules, their physical and morphological characteristics with their different nano-forms and their application in sponge cake products. The biological evaluation was also studied using rats and statistical analysis. **Results:** The results showed that the ethanol extracts high content of bioactive compounds compared to water extract and that the use of nano-technique as a new emerging technology in form of nano-emulsion using sodium alginate with diameter size between 8.3-13.6 nm. Results also indicated that there was an improvement in the efficiency of antioxidant activity at high-temperature degrees during baking, with a melting point of up to 223.64°C, with an improvement in the blood sugar levels of diabetic rats and a significant decrease in body weight. **Conclusion:** Nano treatments had a protective effect on liver, safety towards kidneys, lowering blood sugar, improving the efficiency comparing to the other samples and were more acceptable to the consumer.

Key words: Bioactive compounds, red onion peel, nano-emulsion, micro-encapsulation, application, food additives, sponge cake, physio-chemical properties, biological evaluation

Citation: Mahmoud, K.F., A.Z. Hammouda, H.S. Ali and A.A. Amin, 2021. Efficiency of red onion peel extract capsules on obesity and blood sugar. Pak. J. Biol. Sci., 24: 99-111.

Corresponding Author: Khaled F. Mahmoud, Department of Food Science and Technology, National Research Centre, P.O. Box 12622, Giza, Egypt

Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Onion is a common plant within the Egyptian *Allium* Family. The plant is distributed across temperate areas. They are used medicinally to treat warm intestinal conditions, stomach ulcers, high blood pressure and fever of malaria[1].

Recent research confirms that the outer skins of onions are rich in antioxidant and anti-inflammatory compounds with multiple functional compounds including organosulfur, anthocyanin, flavonoids, quercetin, kaempferol and polyphenols[2].

Tannin a flavonoid-type polyhydroxyphenol is present in plant tissue defensive layers of red onion peels and its antioxidant effects have been reported by Akaranta and Odozi[3] and Kahkonen *et al.*[4].

The food industry produces a large amount of red onion peel as waste aside from the different quantities remaining from the local markets as a result of trading and selling, which makes it necessary to search for possible ways to benefit from it. One way could be to the use of this waste as a new and natural source of high-value functional ingredients, due to the presence of bioactive compounds in onion, which present health benefits. One way to evaluate its potential as a functional ingredient was by providing information on the red onion peel pulp and its antioxidant activity[5].

Pharmacological properties of Red onion peel (*Allium cepa* L.), such as antioxidant, antimicrobial and antitumor ones, reduction of cancer risk and protection against cardiovascular diseases were of highly valued[6,7]. Red onion peel has shown health-promoting effects based on its secondary metabolites, such as flavonoids to which the strong antioxidant properties of onion have been attributed, considering the most health-promoting properties when it is used as raw[8-10].

Red onion peel, which has a higher content of pharmacologically important phytochemicals, has unfortunately not achieved the same prominence as the yellow ones. Red onion peels owe their hue to anthocyanins which are considered to be the most biologically antioxidant active compounds. Anthocyanins are glycosides of 2-phenyl benzopyrylium salts consisting of rhamnose, glucose, xylose, arabinose, rutinose and galactose[11]. Also, acylated and non-acylated anthocyanins such as cyanidin mono-and diglucosides, cyanidin 3-laminaribiose, peonidin mono-and diglucoside, petunidin glucoside and 5-carboxypyranocyanidin 3-glucoside have been documented to occur in red onion peels[12].

Cyanidin derivatives are the most widely recorded anthocyanins that appear in various red onions peel. Epidemiological trials of antioxidant compounds from food plants have shown their beneficial impact on chronic diseases. So, with information on the content of antioxidant compounds in foods from specific regions could improve to obtain high extraction yields which can be capsulated by optimizing various parameters. Since anthocyanins have shown a strong potential to be utilized in nutraceuticals and diverse products, the stability problems of such molecules are becoming critical for functional usage. Analysis experiments have shown that red onion peel extract is affected and oxidized by different factors such as pH, enzymes, temperature, SO_2, UV radiation, chelating metal ions or ascorbic acid[13]. Although thermal degradation kinetics of anthocyanins has been identified as pseudo-first-order kinetics[14], other researchers[15] have shown that the existence of other synergistic antioxidant compounds can provide protective effects against anthocyanin degradation through extracts and foods.

The aim of this study was to extract the bioactive compounds from Egyptian red onion peels (waste), improve their physical and chemical properties by using nanotechnology, preparing them in nano-form capsules and trials using them as a functional food or food supplements aside with their biological evaluation, studying their effect on liver and kidney function, reducing rats body weight and blood sugar.

MATERIALS AND METHODS

Study area: The study was carried out at Nanotechnology Lab and Food Science and Technology Lab, Food Technology Department, National Research Centre, Egypt from May, 2019-October, 2020.

Materials

Red onion peel: Egyptian cultivated Red Onion Peels (ROP) were collected from a local market and used for the extraction of bioactive compounds. The peels were dried in a drying oven (SL SHEL LAB, 1370FX, U.S.A) at 30°C for 24 hrs until the moisture contents were 5% (w/w). The dried Red Onion Peels (ROP) were then ground to a particle size of 5-10 mm using a high-speed mixer (Blender, U.S.A) and were stored at 4°C until used.

Chemicals: 2,2-diphenyl-1-picryl hydrazyl radical (DPPH), Folin-Ciocalteu reagent, Tween 20, ethanol, aluminum chloride, potassium acetate, linoleic acid, gallic acid and quercetin were purchase from sigma Aldrich.

Extraction techniques

Red onion peel ethanols extract (ROPEE): Fifty grams of ROP dried powders were extracted with 250 mL of ethanol (72 %) in a shaker (dark place) at 150 rpm for 48 hrs[16]. The mixture was subjected to a water bath ultrasound (Ultrasonic Cleaner MTI Corporation, Model UD150SH3.8LQ, U.S.A) transaction intended to increase the output of the extract and skated in an ultrasonic water bath at 35°C for 30 min[17]. The mixtures were filtered by Whatman filter paper (No. 1) and solvent was evaporated at 40°C to obtain 19.4% of ROPEE.

Red onion peel water extract (ROPWE): For ROPWE preparation, 50 g of ROP powder was extracted with 250 mL of distilled water (w/v) (two times) at 25°C for 1.5 h and the mixture was exposed to an ultrasound water bath for 2 hrs[18], filtrated using Whatman filter paper (No. 1), then concentrated using a rotary evaporator under reduced pressure at 40°C to obtain water extract (ROPWE) soluble residual aqueous fraction yielding 26.8%.

Preparation of various forms of ROPEE: ROPEE was encapsulated in three natural polymers separately (chitosan, sodium alginate and gelatin) using nanotechnology techniques. Sodium alginate 3% (w/v) were prepared by dissolving in distilled water using a magnetic stirrer at 2000 rpm for 1 h and kept the solution overnight in a refrigerator at 4°C.

Chitosan solutions were prepared by dissolving in 1% acetic acid according to the method of Tarane *et al.*[19] and gelatin solution was prepared 3% by dissolving 3 g in 100 mL distilled water and stirring to produce nano-emulsion and microencapsulation of ROPEE:

- **Nano emulsion forms:** Ten mL of ROPEE was added to 50 mL chitosan solution, then in sodium alginate solution and finally in gelatin solution (1:5 v/v ratio) separately and the mixture was homogenized using the ultrasonic probe (Ultrasonic probe, CAUTION, Sonics Vibra-cell, VCX-750, NEWTOWN, CT, U.S.A) with a diameter of 3.8 mm by applying 160 W with 50% pulse for 20 min to produce three red onion peel ethanol extract nanoemulsion forms in chitosan (ROPENEc), in sodium alginate (ROPENEs) and gelatin (ROPENEg), respectively
- **Microencapsulation forms:** The micro-capsule forms were prepared by using sodium alginate and chitosan to produce nano-capsule of ROPEE in chitosan, sodium alginate and gelatin solutions, separately. After preparation of the polymers solution and get the previous emulsions forms, afterward, 50 mL of each emulsion separately was then sprayed into a collecting water bath containing calcium chloride solution (2 w/v %) using an Inotech Encapsulator (Encapsulator B-390/B-395 Pro, two nozzles BUCHI, USA) with a 450 m nozzle. The resulting microcapsules were allowed to harden in cross-linking solutions for 3 hrs. The ROPEE loaded polymer beads were collected from the cross-linking solutions using a sieve. Finally, three forms of red onion peel ethanol extract microcapsule were obtained in chitosan (ROPEMCc), in sodium alginate (ROPEMCs) and gelatin (ROPEMCg)

Determination of total phenolics and flavonoids contents of rope forms: Total phenolics contents were determined by using Folin-Ciocalteu assay[20]. The ROPE forms samples (1 mg mL^{-1}) were dissolved separately in 1.0 mL methanol and then mixed with 2 mL of 2% aqueous sodium carbonate solution. After 3 min, 100 µL of 50% Folin-Ciocalteu's reagent was added to the mixture. After 30 min of standing, the absorbance was measured at 750 nm with a spectrophotometer (2120 UV, Optizen, Daejeon, Korea). The results were derived from a calibration curve ($y = 9.57x - 0.14$, $R^2 = 0.995$) of gallic acid (0-250 µg mL^{-1}) and expressed in Gallic Acid Equivalents (GAE) per gram extract weight.

The total flavonoid contents were determined by aluminum chloride colorimetric method[21]. The ROPEE forms samples (1 mg mL^{-1}) were dissolved separately in 1.0 mL methanol were mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. After incubation at 25°C for 30 min, the absorbance was measured at 415 nm. The results were derived from the calibration curve ($y = 0.0058+0.0126$, $R^2 = 0.9981$) of quercetin (0-100 µg mL^{-1}) and expressed in Quercetin Equivalents (QE) per gram extract weight.

Antioxidant activity of ROPEE forms

DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity: DPPH radical scavenging activity measures the reducing capacity of natural antioxidants toward DPPH radical for ROPEE included free, nano-emulsions and microcapsules forms upon reduction, the color of the DPPH solution fades. The DPPH free radical scavenging abilities of red onion peel extracts were measured by a modification of Chithiraikumar *et al.*[22]. The optical density was measured at 517 nm using UV-Vis Spectrophotometer.

Table 1: Sponge cake formula components

	Sponge cake formula samples			
Ingredients (g)	Control	ROPEE	ROPENEs	ROPEMCs
ROPEE forms	0	5 g	10 g	10 g
Cake flour	100	95	90	90
Whole egg	72	72	72	72
Sucrose	72	72	72	72
Sunflower oil	57	57	57	57
Whey	4	4	4	4
Nonfat dry milk	2	2	2	2
Baking powder	2	2	2	2
Vanilla	0.5	0.5	0.5	0.5
Water	30	30	30	30

Where: ROPEE is red onion peel ethanol extract, ROPENEs is red onion peel ethanol extract nano-emulsion with sodium alginate, ROPEMCs is red onion peel ethanol extract microcapsule with sodium alginate

Physical properties of ROPEE forms

Differential scanning calorimetric (DSC): The thermal stability of ROPEE formulas (free, microencapsulated and nano-emulsion) was determined using a Differential Scanning Calorimeter (DSC), model 823E from Mettler Toledo. A dynamic scan was performed at a heating rate of 20°C min^{-1} over a temperature ranged from 20-300°C. Evaporation enthalpies were calculated by peak area integration of DSC profiles[23].

Scanning electron microscopy (SEM): Microcapsules were observed in a Scanning Electron Detector microscope with Energy Dispersive X-ray, SEM and EDX Leo 440i 6070 (LEO Electron Microscopy, Oxford, England) operating at 15 kV and electron beam current of 100 pA. Image acquisition was performed by the LEO software, version 3.01[24].

Application

Sponge cake preparation: Sponge cake with four different samples was prepared by the addition of 0 g ROPEE (control), 5 g ROPEE, 10 g ROPENEs and 10 g ROPEMCs replacement of cake flour and the sponge cake formulas were shown in Table 1. The cooled cakes[25] were packed in polypropylene bags at room temperature before Physico-chemical and sensory evaluation analysis.

Physical examinations of cake: The height, size and weight of sponge cake samples were measured by calculating the height of the cake according to Fatin *et al.*[26].

Sensory evaluation: The sensory evaluation was conducted with the assistance of 20 specialists in baking technology in the Department of Food Technology, National Research Centre, Egypt. Sensory evaluation was performed in sponge cake to evaluate crumb color, porosity, flavor, texture and overall acceptability of sponge cake samples. The objective sensory quality of a sponge cake is described by its sensory profile consisting of sensory features according to Lawless and Heymann[27].

Biological evaluation

Preparation of diets: Male albino rats weighing 150-200 g, purchased from the National Research Centre (NRC), Giza, Egypt, adopting guidelines from the National Institutes of Health Manual for Laboratory Animals Care and Use (Publication No. 85-23, revised 1985).

Balanced and diabetes diet meals were prepared as in Table 2. The diabetes diet meals were prepared according to Al-Okbi *et al.*[28], with some modifications.

Determination of Feed Intake (FI), body weight gain (DWG) and Feed Efficiency Ratio (FER): Daily Feed Intake (FI) per group was calculated throughout the experimental period (14 days). The biological values of different diets were assessed by the determination of body weight gain percent (BWG %) which was calculated at the end of the experimental period as well as Feed Efficiency Ratio (FER) was calculated twice a week, according to the method of Chapman *et al.*[30].

Oral glucose tolerance test (OGTT): The Oral Glucose Tolerance Test (OGTT) was performed on overnight fasting normal rats for all groups except for the balanced group, while the 3rd, 4th and 5th groups dealt with the basic meal, sponge cake samples containing ROPEE (2 mg kg^{-1}), ROPEMCs (2 mg kg^{-1}) and ROPENEs (2 mg kg^{-1}), respectively.

Glucose (2 g kg^{-1}) was fed 30 min after pretreatment. Blood glucose levels were measured at 0, 30, 60, 120 and 240 min after glucose load to access the effect of the extract on blood glucose levels of the glucose loaded animals. The blood glucose was measured using blood glucose test strips and glucometer (Accu-Chek Advantage Test Strips).

Table 2: Diet meals composition (g/100 g)

			Groups feeding on cake samples		
Ingredients	Balanced diet group(g)	Diabetic diet group (g)	ROPEE (g)	ROPEMCs (g)	ROPENEs (g)
Casein	12	12.00	12	12	12
Glucose	-	0.25	0.25	0.25	0.25
Sucrose	-	40.00	40.00	40.00	40.00
Starch	68.5	28.00	8.00	8.00	8.00
Cellulose	5	5.00	5	5	5
Cholesterol	-	-	-	-	-
Bile salt	-	0.25	0.25	0.25	0.25
Salt mixture	3.5	3.5	3.5	3.5	3.5
Vitamin mixture	1	1.00	1	1	1
Sunflower oil	10	10.00	10	10	10
Cake samples	-	-	20	20	20
Total (g)	100	100.00	100	100	100

*Casein contains 90% protein as determined by AOAC[29], ROPEE is red onion peel ethanol extract, ROPEMCs is red onion peel ethanol extract microcapsule with sodium alginate, ROPENEs is red onion peel ethanol extract nano-emulsion with sodium alginate

Sub-acute toxicity test: Male rats, 6 per group, were treated orally for 42 days. ROPEE, ROPEMCs and ROPENEs forms were administered at 10, 20 and 20 mg kg^{-1}, respectively. Liver Function was estimated through the determination of plasma activity of Transaminases represented by AST and ALT[31]. Plasma and urinary creatinine were assessed by the method of Tobias *et al.*[32], with the calculation of creatinine clearance as a determinant of kidney function.

Statistical analysis: The results of the research were analyzed using the Statistical Ready Program, using Duncan[33] and the package of social sciences (SPPSS). The substantive and non-significant variations between the variable values were ($p<0.05$).

RESULTS AND DISCUSSION

Total phenolic content (TPC): Results in Table 3 showed that the water extraction yield was higher compared to the ethanol extract and despite this, the TPC is completely different from the percentage of extraction yield.

Results also showed that the TPC of ROPWE in this study was 136.18 and 384.62 mg GA g^{-1} in water extract and ethanol extract, respectively. These results are in agreement with that obtained by Brahma *et al.*[34] but higher than that obtained by Narashans *et al.*[35], who reported that TPC of onion skin ranged from 14.55±0.41 mg GAE g^{-1} dw to 288.74±1.27 mg GAE g^{-1} dw.

After conducting the packaging operations with micro-capsules and nano-emulsions of red onion peel extract, the total percentage of phenolic compounds was decreased by a small amount according to the packaging method used and its efficiency. As for sodium alginate, the decreased was 342.48 mg GA g^{-1} followed by gelatin-coated extract and chitosan being 307.88 and 194.27 mg GA g^{-1}, respectively.

Although the total content of phenols in water extract coated with nano-emulsions sodium alginate was the highest being 131.14 mg GA g^{-1} and the lowest content was observed in water extract micro-capsules coated with chitosan.

Total flavonoids contents (TFC): Total Flavonoid Contents (TFC) of red onion peel extracts and its various nano and micro-forms are shown in Table 4. Results showed that the ethanol extract was higher in TFC than water extract being 196.32 and 68.13 mg QE g^{-1} extract, respectively. This result is higher than that obtained by Narashans *et al.*[35], being 168.77 mg QE g^{-1}.

Results also showed that all forms of packaging decreased compared to the non-coated except for ethanol and water extracts coated with sodium alginate ROPENEs being 184.36 and 62.18 mg QE g^{-1}, respectively and which could be due to the efficiency of the packaging process.

As a result the high efficiency of sodium alginate emulsions and capsules, the following of experiments will be continued with both previous samples.

Antioxidant analysis

Dpph radical scavenging activity: Antioxidant activity based on the results of DPPH free radical scavenging activity in Table 5, all 2 red onion peel extracts (ROPEE and ROPWE) acted as an antioxidant by donating their electron to scavenge radicals rather than inhabitation the formation of lipid peroxide during oxidation. The antioxidant activity of ROPEE and ROPWE extracts was 89.21 and 43.87%, respectively and the highest DPPH radical scavenging was observed in ROPEE.

Table 3: Extraction yield, total phenolic content of ROPE, ROPEMC and ROPENE coated with chitosan, sodium alginate and gelatin

		Total phenolic contents of onion peel extract forms mg of gallic acid/g of extract (mg GA g^{-1})						
			Chitosan		Sodium alginate		Gelatin	
Extracts	Extraction yield (%)	Free (ROPE)	Nano-emulsion (ROPENEc)	Micro-capsule (ROPEMCc)	Nano-emulsion (ROPENEs)	Micro-capsule (ROPEMCs)	Nano-emulsion (ROPENEg)	Micro-capsule (ROPEMCg)
ROPEE	5.12±0.7	384.62±9.5	241.05±5.4	194.27±5.3	371.55±8.2	342.48±8.2	329.40±7.1	307.88±5.9
ROPWE	9.75±1.2	136.18±4.8	46.22±3.7	32.75±2.1	131.14±3.9	117.06±3.2	95.28±2.8	84.31±2.6

Where: ROPEE is red onion peel ethanol extract; ROPWE is red onion peel water extract

Table 4: Total flavonoid contents of ROPE, ROPEMC and ROPENE in chitosan, sodium alginate and gelatin

	Total flavonoids contents of onion peel extracts (mg of quercetin g^{-1} of extract)							
		Chitosan		Sodium alginate		Gelatin		Concentration of quercetin
Extracts	Free (ROPE)	Nano-emulsion (ROPENEc)	Micro-capsule (ROPEMCc)	Nano-emulsion (ROPENEs)	Micro-capsule (ROPEMCs)	Nano-emulsion (ROPENEg)	Micro-capsule (ROPEMCg)	(mg g^{-1} dry weight)
ROPEE	196.32±5.6	103.28±4.1	89.54±3.4	184.36±5.2	162.33±4.3	158.41±4.0	134.73±3.9	61.93±2.8
ROPWE	68.13±2.3	31.52±2.1	24.49±1.9	62.18±3.1	51.08±2.8	49.80±2.4	40.61±2.3	24.82±2.5

Where: ROPEE is red onion peel ethanol extract; ROPWE is red onion peel water extract

There was a significant statistical difference between free ROPE and its forms (nano- and micro-). the results indicated that there was a decreased in DPPH in the nano-form for both two extracts but nano-emulsions had the higher contents in both of them and that the sodium alginate encapsulation in the form of nano-emulsion (ROPENEs) for ethanol extract was 86.53%, followed by the micro-capsules extract (ROPEMCs) being 78.55%. The lowest result was the extract of chopped red onion peels with chitosan, while the gelatin capsule was medium between the previous two cases.

These findings are in agreement with Amarowicz *et al*.[36] and Hussain *et al*.[37], who showed that the DPPH radical scavenging in the United Arab Emirates (UAE) is much more than maceration and supercritical fluid extraction techniques.

From the previous results, the ethanol extract of red onion peel can be chosen due to the high content of the antioxidant activity in addition to its tow nano sodium alginate forms i.e., microcapsule and nano-emulsion to complete this study.

Physical characterization of ROPE and its sodium alginate forms

Scanning Electron Microscopy (SEM): Scanning Electron Microscopy (SEM) for ROPE and its sodium alginate forms are shown in Fig. 1.

SEM micrographs and their cross-section of free red onion peel ethanol extract (ROPEE) surface were shown in (Fig. 1a). According to SEM analysis, all obtained particles appeared dispersed, well-formed, spherical and that some of the nano-capsule particles were different in size and shape. The approximate sizes measured during the SEM analysis of all slurries range between 54.3-116.3 nm and are on the micro-scale.

The morphology of micro- and nano-encapsulated ethanol extract encapsulated with sodium alginate as wall material (ROPEMCs) with particle size distributions are shown in Fig. 1b. Changes in the core-to-wall ratio, conductivity and viscosity of the feed solution have significant influences on particle size. An increase in viscosity and reduced conductivity of the feed solution (1: 5) lead to particle size reduction. Results showed that microencapsulate was pseudospherical with a round shape and that agglomerations were observed ranging from 1.241-6.521 μm which may be due to corrugated surfaces obtained by freeze-dryer. As for microencapsulates, a smooth surface without any holes was observed. Their particle size was quasi-spherical shape and uniform size with slight agglomeration, possibly due to low core-to-wall ratios and the hydrophobic nature of the red onion peel extract increasing. These results confirmed that reduced size and compact structures of microencapsulates through encapsulation by sodium alginate give better encapsulation efficiency.

In Fig. 1c, the morphological results showed that all nano-emulsions red onion peel extract coated with sodium alginate ROPENEs are sparse, spherical and uniform in shape and sizes located between 8.3-13.6 nm and distributed well within emulsions.

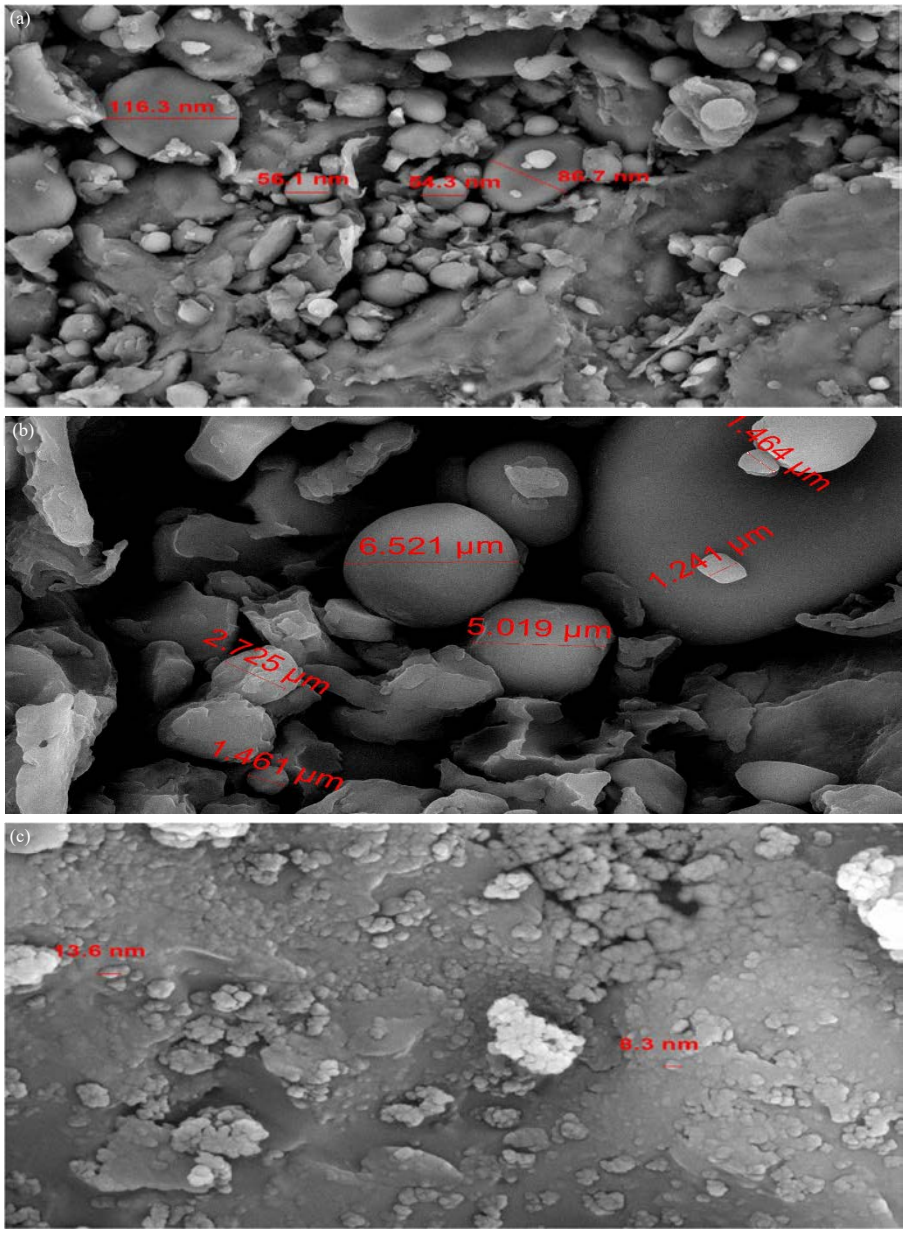

Fig. 1(a-c): (a) SEM morphology of free red onion peel extract (ROPEE), (b) Micro-capsule of red onion peel extract form (ROPEMCs) and (c) Nano-emulsion red onion peel extract from (ROPENEs)

Thermal stability of ROPEE, ROPEMCs and ROPENEs: The thermal stability of the ROPEE, ROPEMCs and ROPENEs was measured using Differential Scanning Calorimetry (DSC) under normal nitrogen conditions. Heat flow versus temperature was obtained via DSC characterization and results are shown in Fig. 2a. Results indicated that there was one endothermic peak observed for ROPEE (as the temperature increased from 20-300°C) starting from 54.86-77.13°C and the melting point was at 66.34°C.

Results in Fig. 2b for ROPEMCs, indicated two endothermic peaks appeared in the heating process at 66.34 and 162.68°C. The peak at 66.34°C was attributed to the ROPEE residue during the microencapsulation technique by sodium alginate and encapsulation efficiency. The endothermic peak appeared at 148.34 and 174.72°C and its melting point was increased to 162.68°C compared to ROPEE due to the extract protection i.e., antioxidant activity by microencapsulation process.

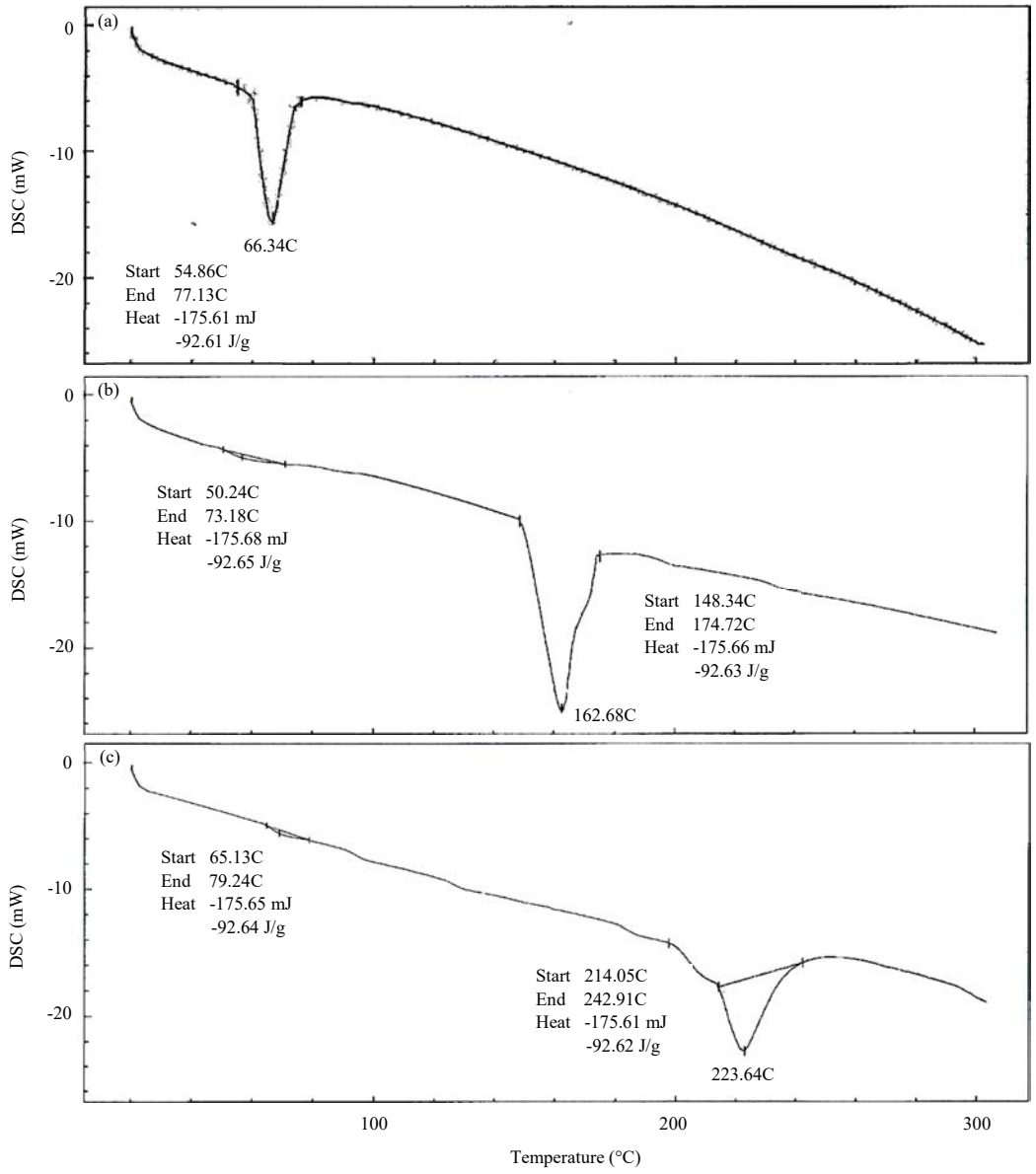

Fig. 2(a-c): (a)DSC of ROPEE, (b) ROPEMCs and (c) ROPENEs for thermal stability

Table 5: DPPH free radical scavenging activity of different antioxidants of ROPE, ROPEMC and ROPENE in chitosan, sodium alginate and gelatin

		DPPH free radical scavenging activity (%)					
		Chitosan		Sodium alginate		Gelatin	
Extracts	Free (ROPE)	Nano-emulsion (ROPENEc)	Micro-capsule (ROPEMCc)	Nano-emulsion (ROPENEs)	Micro-capsule (ROPEMCs)	Nano-emulsion (ROPENEg)	Micro-capsule (ROPEMCg)
ROPEE	89.21±2.8	43.65±2.0	39.11±1.9	86.53±2.6	78.55±2.4	62.98±2.3	58.13±2.1
ROPWE	43.87±2.2	26.17±1.4	24.08±1.4	39.74±2.1	36.22±1.8	31.85±1.5	29.43±1.3

Where: ROPEE is red onion peel ethanol extract, ROPWE is red onion peel water extract

The DSC measurements for the ROPENEs sample gained more stability in the form of nano-emulsion (Fig. 2c).

Results also indicated the appearance of tow peaks one was located at almost the same temperature between 66.13 and 79.24°C which was attributed to the un-sheathed red onion peel extract remaining after the nano-emulsion process. The other peak appeared for the nano-emulsion of red onion peel extract (ROPENEs) was between 214.05 and 242.91°C and

Table 6: Physical properties of sponge cake samples

	Cake samples			
Physical properties	Control	ROPEE	ROPEMCs	ROPENEs
Volume (cm^3)	11.52	26.15	34.43	37.44
Size (cm)	6.40±0.9	8.3±1.1	9.7±1.6	10.4±1.8
Weight (g)	124.11±2.5	132.52±2.5	133.94±2.6	138.27±2.6

Where: ROPEE is red onion peel ethanol extract, ROPEMCs is red onion peel ethanol extract microcapsule with sodium alginate, ROPENEs is red onion peel ethanol extract nano-emulsion with sodium alginate

Table 7: Sensory properties of sponge cake

Cake samples	Crumb color	Porosity	Flavor	Texture	Overall acceptability
Control	6.824±0.9^{b}	7.513±1.1^{ab}	7.933±0.9^{b}	6.772±0.8^{b}	7.261±0.9^{ab}
Cake+ROPEF	7.446±0.8^{ab}	7.832±1.2^{ab}	8.413±0.8^{ab}	7.245±0.9^{ab}	7.734±1.2^{ab}
Cake+ROPEMCs	8.228±0.6^{ab}	8.941±1.6^{a}	9.121±1.3^{a}	9.253±1.2^{a}	8.886±1.1^{a}
Cake+ROPENEs	9.215±0.7^{a}	9.057±1.8^{a}	9.305±1.2^{a}	9.548±1.4^{a}	9.281±1.5^{a}

ROPEE is red onion peel ethanol extract, ROPEMCs is red onion peel ethanol extract microcapsule with sodium alginate, ROPENEs is red onion peel ethanol extract nano-emulsion with sodium alginate

its melting point was increased to 223.64°C compared to the two other samples (ROPEE and ROPEMCs) which gave it more stability. These results indicated that the nano-emulsification process protects the extract against heat and increases its heat-resistance.

Therefore, red onion peel extract nano-emulsion could be successfully added to bakery products as it retained its antioxidant activity and remained stable at this high-temperature.

Application: The effect of adding ROPEE, ROPEMCs and ROPENEs to sponge cake compared to the control sample and their physical properties and sensory evaluation are shown in Table 6 and 7, respectively.

Results in Table 6 and Fig. 3 showed that the weight of the sponge cake samples supplemented with red onion peel extract did not have any significant differences between them being 132.52, 133.94 and 138.27 g for ROPEE, ROPEMCs and ROPENEs respectively, while a significant difference appeared compared to the control sample, which was 124.11 g. This is due to the ability of the packing material to swell and retain moisture for a long time after the baking process, protects the vital active compounds in the extract and improves the properties of the final product, especially in reducing the phenomenon of bread staling which occurs in bakery products.

There were significant differences in size between the different cake samples compared with the control and the high size samples were in the sponge cake sample supplemented with ROPENEs as it was 10.4 cm, while there are significant differences in the cake samples volume compared to the control sample and that the most significant result was 37,44 cm^3 in the sample supplemented with ROPENEs.

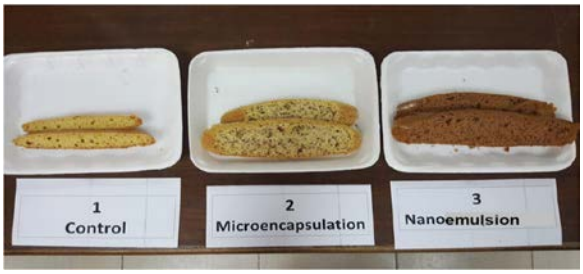

Fig. 3: Picture of sponge cake samples

Sensory evaluation: Measuring sponge cake samples product liking and preference are shown in Table 7. Statistically significant differences evaluated by the trained consumers were found in the crumb color, porosity, flavor, texture and overall acceptability scores in comparison with the control sample. The best result was obtained with a sponge cake sample containing ROPENEs that show the highest results in color, porosity, flavor, texture and overall acceptance being 9.215, 9.057, 9.305, 9.548 and 9.281, respectively. It was darker due to the red color present in the cake in the form of nano-emulsions and good distribution inside the dough. However, the sensory characteristics of the control sample were lower than those of the other cakes and no statistically significant differences were found between the control sample and the sponge cake sample containing ROPEE. The sensory characteristics liking results pointed out that a partial replacement of cake flour with up to 5% ROPENEs in sponge cakes is satisfactory.

Biological evaluation

Effect of ROPEE and its forms inside the sponge cakes on FBW, BWG, TFI and FER: Effect of three types of sponge cake samples (containing ROPEE, ROPEMCs and ROPENEs, each

Table 8: Nutritional parameters of different experimental groups

	Parameters				
Rat groups	Initial body weight IBW (g)	Final body weight FBW (g)	Body weight gain BWG (g)	Total food intake TFI (g)	Food efficiency ratio FER (%)
G_1: Natural control	132.23±2.51[a]	227.45±6.41[a]	95.22±7.28[a]	563.12±25.17[b]	0.169±0.016[a]
G_2: Diabetic	132.51±3.62[a]	251.66±8.63[a]	119.15±9.36[a]	514.25±20.52[b]	0.232±0.014[b]
G_3: ROPEE	132.43±2.94[a]	246.41±7.91[a]	113.98±6.88[a]	498.16±17.64[ab]	0.229±0.012[b]
G_4: ROPEMCs	132.39±3.06[a]	239.29±7.15[a]	106.90±5.47[a]	486.22±15.81[ab]	0.219±0.013[ab]
G_5: ROPENEs	132.28±3.11[a]	233.38±6.95[a]	101.10±5.32[a]	469.67±14.95[ab]	0.215±0.011[ab]

Where: ROPEE is red onion peel ethanol extract, ROPEMCs is red onion peel ethanol extract microcapsule with sodium alginate, ROPENEs is red onion peel ethanol extract nano-emulsion with sodium alginate, Each value is the Mean±SD, Mean values in each column having different subscript (a, b, c, d) are significantly different at $p<0.05$

sample separately) on Final Body Weight (FBW), Body Weight Gain (BWG), Total Feed Intake (TFI) and Feed Efficiency Ratio (FER) in diabetic rats are presented in Table 8. Group 2 (diabetic group) had a significant increase in FBW, BWG and FER compared to the normal rat's group 1 (natural control group) by 251.66 g, 119.15 g and 0.232%, respectively.

Rats were fed on sponge cake samples containing ROPEE, ROPEMCs and ROPENEs separately were significantly decreased for FBW, BWG and TFI compared with the diabetic group (G_2). The decreases in BWG in G_3, G_4 and G_5 were 113.98, 106.90 and 101.10 g for ROPEE, ROPEMCs and ROPENEs in sponge cake samples, respectively. While, the decreases in TFI of rat's weight in G_3, G_4 and G_5 were 498.16, 486.22 and 469.67 g for ROPEE, ROPEMCs and ROPENEs in sponge cake samples, respectively. On the other hand, rats were fed with cake samples containing ROPEE, ROPEMCs and ROPENEs, FER significantly decreased by 0.229, 0.219 and 0.215%, respectively compared to G_2.

The results indicated that the diabetic rats had significantly increased in food consumption, BWG and FER compared with the other feed on G_1.

The weight of the mice increased positively during the feeding period on a ration containing a sponge cake supplemented with different red onion peel extracts in all groups except for the positive control group.

The increase in body weight gained in rats and the significant increase in the ratio of food efficiency ratio to rats fed on sponge cake diet samples rich in red onion peel extract compared to a balanced diet without significant change in total food intake may be attributed to higher calories in the diet achieved by higher carbohydrate levels intake.

Among the previous results in Table 8, the FBW of rats decreased significantly in G_5 compared to the other groups except for G_1 control. These results indicate the ability of the red onion peel extract coated with nanometric emulsions to reduce the weight and protecting the group rich in phenols, flavonoids and antioxidants maintaining its activities from any possible environmental change that might happen to them. There were no studies available on the benefits of red onion peels specifically for weight loss but in general, onion peels may contribute to reducing the risk of obesity. It was found that the consumption of nutritional supplements of peel extracts rich in quercetin is associated with a decrease in body weight and the proportion of fat in the body as indicated by a preliminary study conducted on obese or overweight mice, the results of which were published in Nutrition research and practice in 2016.

Effect of ROPEE, ROPEMCs and ROPENEs on blood glucose rats: The effect of different forms of ROPEE inside sponge cake samples on the blood glucose levels is illustrated in Table 9.

Results showed that the G_2 had a significant increase in blood glucose level compared to G_1 by 231.58 mg dL^{-1}. Rats were fed on ROPEE, ROPEMCs and ROPENEs in a separate way in groups showed that G_3, G_4 and G_5 significantly decreased blood glucose level when compared to G_2, the decreases in blood glucose level in G_3 fed a sponge cake containing ROPEE after 30, 60, 120 and 240 min from taking a glucose dose were 205.37, 178.64, 166.82 and 152.95 mg dL^{-1}, respectively.

Whereas, the decrease in the blood sugar level of rats in groups 4 and 5 was lower as a result of coating the red onion peel extract with a layer of sodium alginate, either in the form of nano-emulsions or in the form of micro-capsules as they protect them from any changes in anti-oxidant properties and consequently spread their ability to reduce blood sugar in mice with diabetes. Results showed that G_4 which is fed on sponge cake contains ROPEMCs decreased by 162.52, 146.08, 128.53 and 91.33 mg dL^{-1} after 30, 60, 120 and 240 min from taking a glucose dose. While there was more reduction in blood glucose observed in G_5 fed on sponge cake contains ROPENEs after 30, 60, 120 and 240 min being 143.18, 135.31, 121.67 and 85.94 mg dL^{-1}, respectively.

Among the results obtained from the previous table, we find that the diabetic rats fed on the red onion peel extract G_4 and G_5 after 240 min consuming 1% glucose dose could

Table 9: Fasting blood glucose and glucose administration of different experimental groups (mg dL^{-1})

	Blood glucose mg dL^{-1}				
Rat groups	Fasting blood glucose (0 min)	After (30 min)	After (60 min)	After (120 min)	After (240 min)
G_1: Natural control	82.63±1.95^{a}	129.51±2.78^{ab}	118.2±2.15^{a}	109.41±2.30^{a}	91.04±2.11^{a}
G_2: Diabetic	83.44±2.14^{a}	231.58±5.63^{bc}	197.28±3.84^{ab}	171.92±4.08^{ab}	124.26±3.17^{a}
G_3: ROPEE	81.98±2.01^{a}	205.37±6.45^{bc}	178.64±4.52^{ab}	166.82±3.94^{b}	152.95±3.56^{bc}
G_4: ROPEMCs	82.33±1.96^{a}	162.52±4.07^{b}	146.08±3.51^{b}	128.53±3.18^{b}	91.33±2.88^{a}
G_5: ROPENEs	82.79±2.05^{a}	143.18±3.54^{b}	135.31±3.02^{b}	121.67±2.31^{b}	85.94±2.26^{a}

Where: ROPEE is red onion peel ethanol extract, ROPEMCs is red onion peel ethanol extract microcapsule with sodium alginate, ROPENEs is red onion peel ethanol extract nano-emulsion with sodium alginate

Table 10: Parameters reflecting kidney and liver function in different experimental groups

	Parameters			
	Kidney function		Liver function	
Rat groups	Plasma creatinine (mg dL^{-1})	Urinary creatinine (mg dL^{-1})	AST (U L^{-1})	ALT (U L^{-1})
G_1: Natural control	0.28±0.02^{a}	13.81±0.8^{a}	30.28±1.86^{a}	12.05±1.66^{a}
G_2: Diabetic	0.39±0.06^{bc}	14.02±0.9^{a}	46.67±2.68^{bc}	23.48±2.14^{bc}
G_3: ROPEE	0.34±0.04^{ab}	15.45±1.2^{ab}	38.49±2.31^{ab}	19.06±1.92^{ab}
G_4: ROPEMCs	0.29±0.02^{a}	18.84±1.5^{ab}	34.09±2.17^{b}	14.23±1.71^{a}
G_5: ROPENEs	0.29±0.02^{a}	19.35±1.3^{ab}	33.47±2.13^{b}	13.82±1.69^{a}

Where: ROPEE is red onion peel ethanol extract, ROPEMCs is red onion peel ethanol extract microcapsule with sodium alginate, ROPENEs is red onion peel ethanol extract nano-emulsion with sodium alginate

return blood sugar to the normal level close to G_1, which could be attributed to the efficiency of the extract coated with nano-emulsions (ROPENEs) and microencapsulation (ROPMCs) in reducing blood sugar.

These results could be similar to that obtained by Enas[38], who found that the diabetic rats treated with aqueous of pomegranate leaves for 4 weeks displayed significantly lowered blood glucose level and augmentation in insulin level.

Results of the glucose rating test (Table 9) showed no change in fasting blood glucose among all groups after 0 min. Half an hour after taking glucose, blood glucose had reached the highest level for all groups, except for G_2 that showed the highest blood glucose level being 231.58 mg dL^{-1} which differed significantly from the control group. The red onion peel extract coated with nano-emulsions was the only treatment that significantly decreased the blood glucose level after half an hour to 143.18 mg dL^{-1} compared to G_3 and G_4. After one hour, Blood glucose showed little change when comparing G_3 and G_4. The 4 hrs glucose level for G_4 and G_5 showed a significant decrease than G_3.

This indicates the effect of using glucose, which may be associated with a catalytic effect on insulin. It has been previously reported that red onion peel extract has an anti-oxidant effect attributed to its bioactive components as it is an extraordinarily rich source of polyphenols and anthocyanin antioxidants that can enhance insulin secretion, especially when protected by encapsulating them with nano-emulsions or micro-capsules and improving glucose tolerance.

Effect of ROPEE, ROPEMCs and ROPENEs on kidney and liver function in diabetic rats: Effects of ROPEE, ROPEMCs and ROPENEs on the liver function which expressed alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in diabetic rats are presented in Table 10. The untreated G_2 had a significant increase ($p<0.05$) in the levels of AST and ALT enzymes compared to G_1 by 46.67 and 23.48 U L^{-1}, respectively. On the other hand, rats were fed with sponge cake samples containing Red Onion Peel Extract, Either not coated (ROPEE) or coated with micrometer capsules (ROPEMCs) or nano-emulsions (ROPENEs) significantly decrease ($p<0.05$) of ALT and AST compared to G_2. The decreases of ALT in rats groups 3, 4 and 5 that containing ROPEE, ROPEMCs and ROPENEs were 19.06, 14.23 and 13.82 U L^{-1}, respectively.

Whereas, the deficiency of AST in G_3, G_4 and G_5 were 38.49, 34.09 and 33.47 U L^{-1} for ROPEE, ROPEMCs and ROPENES, respectively. ALT and AST are sensitive indicators of liver damage[39].

Also, the results in Table 10 showed that there was a significant increase in plasma creatinine for group 2 compared to the control sample, being 0.39 and 0.28 mg dL^{-1}, respectively. While the groups fed on a sponge cake, rich in

red onion peel extracts in different form ROPEE, ROPEMCs and ROPENEs decreased the level of plasma creatinine significantly being 0.34, 0.29 and 0.29 mg dL^{-1}, respectively.

On the other hand, there was an increase in urinary creatinine in all groups, the most significant increase and improvement in kidney function were observed in rats consuming sponge cake containing red onion peel extract coated with nano-emulsions (ROPENEs) was 19.35 mg dL^{-1}.

From the previous results, the study indicates the application of adding red onion peel coated with micro-capsules or with nano-emulsions in bakery products to improve their properties of color, size and prolong the shelf life, especially after ensuring their safety on liver and kidney function as the results of biological evaluation showed on mice. Also, the study recommends encapsulating bioactive compounds, especially when added to bakery products that are exposed to high temperatures or to juices that are subjected to pasteurization processes to protect them from temperatures, acidity, oxidation and light.

CONCLUSION

The extract of red onion peel is one of the richest food sources in antioxidant flavonoids and phenols. The results showed the inability of these uncoated extract to withstand baking temperatures during the preparation of the sponge cake product due to the demolition of the bioactive compounds. Thereby, the use of new emerging technology to protect the higher content of antioxidant compounds from external oxidizing agents is urgent in ethanol extract than water extract. This nanotechnology could be achieved by coating them with layer of nano-emulsion or micro-capsule using natural sodium alginate material, which has proven its efficiency than chitosan and gelatin.

SIGNIFICANCE STATEMENT

This study discovers the importance of the bioactive compounds found in red onion peels extract that can be beneficial for lowering blood sugar levels and reducing weight. This study also can be help researchers to uncover the importance of the nano-encapsulation food wastes bioactive compounds as new emerging technology to be prepared and used in food by consumers in the near future. Also, it had a major role in improving the properties of the cake samples, such as color and size as nutritional enhancers added to bakery products as natural enhancers.

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