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Research Article

Bioaccessibility of Encapsulated Mango Peel Phenolic Extract and its Application in Milk Beverage

¹Tamer M. El-Messery, ¹Marwa M. El-Said, ¹Heba H. Salama, ²Dina Mostafa Mohammed and ³Gaspar Ros

¹Dairy Department, National Research Centre, Cairo, 12622, Egypt

²Department of Nutrition and Food Sciences, National Research Centre, Dokki, Cairo, 12622, Egypt

³Department of Food Science and Nutrition, Faculty of Veterinary Science, Regional Campus of International Excellence Campus Mare Nostrum, University of Murcia, 30100, Spain

Abstract

Background and Objective: Mango peel is an excellent source of bioactive compounds especially Phenolic Compounds (PC). Due to the difficulty of adding the mango peel to food products as a solution we evaluated the feasibility of encapsulating a phenolic-rich extract from mango peel (MPPE) then added to milk beverage and the therapeutic effectiveness in protecting against oxidative stress was investigated. **Materials and Methods:** The characterization of MPPE microcapsules has been conducted by Scanning Electron Microscopy (SEM) and Encapsulation Efficiency (EE). The MPPE microcapsules were added in different proportions to the milk (1, 2 and 3%), physicochemical properties and the viscosity were evaluated during 14 days of cold storage. *In vitro* simulation digestion process (oral, gastric and intestinal phase) was evaluated for the total phenolic and flavonoid content (TPC and TFC) and antioxidant activity for both Mango Peel Phenolic Extract (MPPE) microcapsules and milk beverage supplemented with MPPE microcapsules. The milk beverage supplemented with microcapsules was investigated against oxidative stress using CCl₄ injected rats. **Results:** The physicochemical properties of the milk beverage supplemented with microcapsule were not significantly affected compared to the control sample. Furthermore, TPC, TFC and antioxidant activity values were increased significantly (2-3 times) at the end of the simulation digestive process for both MPPE microcapsules and milk beverage supplemented with MPPE microcapsules each individually. *In vivo* experiment, the total antioxidant and catalase levels were increased and the lipid peroxide, Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Alkaline Phosphatase (ALP) activities were decreased when used milk beverage supplemented with MPPE microcapsules. **Conclusion:** These results demonstrated that MPPE microcapsules rich in bioactive components can be used as a chemo-preventive agent against oxidative stress disorders in experimental rats.

Key words: Milk beverage, mango peel, phenolic extract, antioxidant activity, *in vitro* simulation digestion, *in vivo* experiment, oxidative stress, microcapsules

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Corresponding Author: T.M. El-Messery, Dairy Department, Food Industries and Nutrition Division, National Research Center, 33 El Bohouth St. Dokki, P.O. Box 12622, Giza, Egypt Tel: 00201009266969

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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

Milk drinks are one of the best refreshing and nutritious beverages as well¹. It is also an excellent choice for the transport of active biomaterials such as phenols after being extracted from their different various^{2,3}. Functional foods are increasing day by day because people are adopting functional foods for health benefits and consumers are very much aware of their health⁴. The flavored milk drinks are a potential food supplement for children and adults who contain phosphorus, calcium, iron and other essential nutrients⁵. It is concluded that milk drinks are proven to be beneficial under various conditions, such as diabetes, anemia, cancer, constipation, eczema, support healthy skin, the common cold and kidney swelling⁶. Mango (*Mangifera Indica* L.) is one of the most commonly traded tropical fruits worldwide. Approximately 20% of the fruit is peel⁷. Mango peels are agricultural waste, which can be used as a means of benefiting from the recycling of agricultural waste using its extracts to support various food products^{8,9}. The mango peel is a source of biologically active substances such as carotenoids, polyphenols, anthocyanins, flavonoids and enzymes and vitamins⁷. The mango peel was used as an anticancer effect because has contained compounds with phenolic compounds and antioxidant activity^{10,11}. The bioactive polyphenols have biological properties and antioxidant activity that enable them to use potential ingredients for nutraceutical formulations¹² so increased in the food and pharmaceutical fields¹³.

Microencapsulation is used to increase thermal and chemical stability and their effects against environmental conditions such as humidity and heat. Moreover, to improve the stability, bioavailability and preserve the health beneficial properties of natural sources are incorporating their extracts rich in polyphenols into polymeric matrices¹⁴. Nonetheless, the encapsulation can improve the delivery systems, controlled release of food ingredients and offer prolonged, this protection also can mask its astringent flavors or strong odours¹⁵. Encapsulated mango peel powder has hepatoprotective, antidiabetic, antiviral, antitumor and gastroprotective properties which are mentioned in many studies¹⁶. Materials used for encapsulating bioactive compounds include the alginate polymer (maltodextrin and whey protein concentrate), which is biocompatible, biodegradable and has a non-toxic nature¹⁷. Maltodextrin (MD) has some advantages such as bland flavor, low cost, good protection against oxidation and low viscosity. Therefore, it is better to use MD in combination with Whey Protein Concentrate (WPC) as surface-active biopolymers for an effective microencapsulation^{18,19}.

This study aims to investigate the effect of encapsulated mango peel phenolic extract in milk beverage on the functional and nutritional properties. As well as studying the impact of these microcapsules on bioactive components release during *in vitro* gastrointestinal simulation and the oxidative stress in the experimental rats.

MATERIALS AND METHOD

Study area: The present study was conducted during September 2020 at the Dairy Department, Food Industries and Nutrition Division, National Research Centre, Egypt.

Materials: Mango (*Mangifera Indica* L.) was purchased from the Egyptian local market. Maltodextrin (MD) and Whey Protein Concentrate (WPC) (80%) were purchased from Alfasol Co., Turkey. Fresh Full cream milk was procured from the Animal Production Research Institute, Agriculture Research Center, Giza, Egypt. Emulsifier mono and diglyceride 60% was obtained from Misr for Food Additives (MISAD), Giza, Egypt. Pectin was obtained from Sisco Research Laboratories (SRL) Mumbai, India. Commercial grade granulated cane sugar produced by Sugar and Integrated Industries Co. at Hawamdia was obtained from the local market. All chemicals and solvents were purchased from MERCK, USA.

Methods

Preparation of Mango Peel Phenolic Extract (MPPE): The mango fruits were selected without defects, then washed with running water and peeled. Ten gram of mango peel was added to 200 mL of ethanol (80%) and an ultrasonic bath was used for 20 min at room temperature. The extract was collected and centrifuged then the filtrate was separated. The extraction process was repeated 3 times. Ethanol was removed using a rotary evaporator (Büchi R20, Switzerland) and the concentrate was lyophilized using a freeze dryer (Christ Alpha 1-2LD plus, Germany). The powder was collected and kept at -20°C until it was used. MPPE microcapsules formulations is illustrated in Table 1.

Polyphenols HPLC analysis: HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using the Eclipse C18 column (4.6×250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 1 mL min⁻¹. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A), 0-5 min (80% A), 5-8 min (60% A), 8-12 min (60% A), 12-15 min (85% A) and 15-16 min (82% A). The multi-wavelength detector was monitored at 280 nm. The

Table 1: Mango peel phenolic extract (MPPE) microcapsules formulations

Formulations	Wall materials		MPPE* powder: Wall materials
	MD* (g)	WPC* (g)	
F ₁	100	00	1:10
F ₂	100	00	1:20
F ₃	80	20	1:10
F ₄	80	20	1:20
F ₅	60	40	1:10
F ₆	60	40	1:20

*MD: Maltodextrin, WPC: Whey protein concentrate, MPPE: Mango peel phenolic extract

injection volume was 10 µL for each of the sample solutions. The column temperature was maintained at 35 °C²⁰.

Preparation of MPPE microcapsules: MPPE microcapsules were prepared according to Farrag¹³. Whey Protein Concentrate (WPC) and Maltodextrin (MD) were used as wall materials (Table 1).

Measurements of particle size distribution and zeta potential: The particle size and zeta potential were determined with a dynamic light scattering instrument (Nano ZS, Malvern Instruments and Worcestershire, UK).

Encapsulation characterizations

Encapsulation Efficiency (EE): The encapsulation efficiency of MPPE microcapsules was calculated according to Eq. 1 as described by Ades²¹ and Fernandes²²:

$$EE = \left(\frac{TPC - SPC}{TPC} \right) \times 100$$

where, TPC is the total phenolic content and SPC is the surface phenolic compounds

Surface morphology analysis: The particle structure of MPPE microcapsules was evaluated by Scanning Electron Microscopy (Quanta FEG 250 SEM) (Thermo Fisher Scientific, Oregon, USA).

Determination of Total Phenolic and Flavonoid Content (TPC and TFC) and antioxidant activity: The total phenolic content was determined according to the method of Naczk and Shahidi²³ and the results were expressed as mg of catechin equivalent per gram. The method illustrated by Leong and Shui²⁴ was used to determine DPPH radical-scavenging activity and the results were expressed as µmol Trolox equivalent per gram. Total Flavonoid Content (TFC) was measured according to Djeridane²⁵ and the results were

expressed as mg Rutin equivalents per gram. Ferric Reducing Antioxidant Power (FRAP) was evaluated as stated by Benzie and Strain²⁶ and the results were expressed as µmol Trolox equivalent per gram.

Manufacture of milk beverage supplemented with MPPE microcapsules: The milk beverage formulations were prepared as follows:

Control : Plain milk without MPPE microcapsules

T₁ : Milk+MPPE microcapsules 1% (v/w)

T₂ : Milk+MPPE microcapsules 2% (v/w)

T₃ : Milk+MPPE microcapsules 3% (v/w)

Each formulation was contained sucrose 5% (w/w), pectin 0.1% (w/w) and monoglyceride 0.1% (w/w). The milk beverage formulations were heated to 90 °C per 5 min then cooled. The mango flavor and food-grade yellow color was added (1 and 0.1%, respectively). All milk beverage formulations were packed into sterilized bottles and stored at 5 ± 2 °C for 15 days.

Physicochemical analysis: The total solids, protein, fat and ash were measured by using methods described by Ling²⁷. pH value was measured using a Jenway 3510 pH meter. The Titratable Acidity (TA) was measured according to Krisnaningsih²⁸.

The viscosity of all samples has been measured using a dynamic viscometer at a speed of 50 rpm (Brookfield Model-LV, Brookfield Engineering Laboratory, Stoughton, USA).

In vitro gastrointestinal digestion: *In vitro* digestion for each MPPE microcapsules and milk beverage supplemented with MPPE microcapsules using the method of McDougall²⁹ with some modifications. An initial pepsin/HCl digestion for 2 hrs at 37 °C was followed by digestion with bile salts/pancreatin for 2 hrs at 37 °C to simulate gastric and small intestine conditions, respectively. The simulated stomach solution was prepared with pepsin, NaCl and pH was adjusted to 2.0. Total 20 mL stomach solution was mixed with 5 g of encapsulated powder dispersions then incubated in a shaking water bath for 2 hrs at 37 °C at 100 rpm. Then, the mixture was cooled down immediately and 2 mL aliquots of the Post-Gastric (PG) digestion were collected. After the addition of 4.5 mL of 4 mg mL⁻¹ pancreatin and 25 mg mL⁻¹ bile salt mixtures, a segment of cellulose dialysis tubing containing sufficient NaOH to neutralize the titratable acidity was placed into the beaker. After incubation in a shaking water bath for

2 hrs at 37°C and 100 rpm, the solution in the dialysis tubing was collected and stored at -80°C until further analysis. Before analysis, samples were thawed and centrifuged at 18,000 rpm for 10 min.

In vivo experiment: Thirty male Sprague-Dawley rats (150-200 gm) were maintained on a standard laboratory diet and water for 1 week before the experiment for acclimatization and to ensure normal growth and behavior. The animals were distributed and housed in individual solid bottom cages in a temperature controlled ($23 \pm 10^\circ\text{C}$), 40-60% relative humidity and artificially illuminated (12 hrs dark/light cycle) room free from any source of chemical contamination. The basal diet is for the control groups (-ve and +ve) added by plain milk beverage (without MPPE microcapsules) and the other groups fed on the basal diet supplemented with milk beverage and different concentrations of MPPE microcapsules according to Ezzat³⁰.

Experimental design: Thirty rats were divided into 5 groups (6 for each) where 24 rats were induced by received CCl_4 in olive oil (1:3) intragastrically (0.5 mL kg^{-1}) twice a week for 2 weeks as follows:

- **Control (-ve):** Normal rats fed on the basal synthetic diet that served as -ve control
- **Control (+ve):** Induced rats fed on the basal synthetic diet and plain milk beverage every day by oral intubation for 2 weeks that served as +ve control
- **Group 1:** Induced rats fed on the basal synthetic diet and T_1 by oral intubation for 2 weeks
- **Group 2:** Induced rats fed on the basal synthetic diet and T_2 by oral intubation for 2 weeks
- **Group 3:** Induced rats fed on the basal synthetic diet and T_3 by oral intubation for 2 weeks

The blood samples were collected from retro-orbital venous plexus under diethyl ether anesthesia where serum and plasma were separated by centrifugation at 3000 rpm for 15 min and stored at -20°C to measure the biochemical parameters.

Statistical analysis: The results of the experiments were expressed as mean values \pm standard deviation for at least three replicates. The statistical analysis of data was performed using Minitab 18 (Minitab Ltd., Coventry, UK).

RESULTS AND DISCUSSION

Identification of phenolic and flavonoid compounds by HPLC:

The Phenolic Compounds (PC) of Mango Peel Powder (MPP) was determined by the HPLC method (Table 2), the list of phenolic compounds used as a standard is twelve compounds. The highest retention time peak was Gallic acid (3.116 min) then followed by Chlorogenic acid (3.68 min), Catechin (4.267 min), Methyl gallate (5.049 min), Syringic acid (5.787 min), Rutin (6.799 min), Ellagic acid (7.566 min), Coumaric acid (8.253 min), Ferulic acid (9.359 min), Naringenin (9.934 min), Cinnamic acid (13.96 min) and Kaempferol (14.419 min), respectively. The phenolic compounds in MPP were arranged descending depend on the concentration in MPP ($\mu\text{g g}^{-1}$) as follows: Gallic acid 3601.75, Coumaric acid 1160.41, Naringenin 1034.57, Catechin 604.28, Syringic acid 533.68, Methyl gallate 210.64, Ferulic acid 110.82, Ellagic acid 64.94, Kaempferol 60.15, Rutin 56.36, Chlorogenic acid 40.27 and Cinnamic acid 9.37. The major PC in MPPE was Gallic acid ($3601.75 \mu\text{g g}^{-1}$) and these results are in agreement with Velderrain-Rodríguez³¹.

Table 2: Phenolic and flavonoid compounds of mango peel phenolic extract powder

Phenolic compounds	Retention time (min)	Concentration ($\mu\text{g g}^{-1}$)
Gallic acid	3.116	3601.75
Coumaric acid	8.253	1160.41
Naringenin	9.934	1034.57
Catechin	4.267	604.28
Syringic acid	5.787	533.68
Methyl gallate	5.049	210.64
Ferulic acid	9.359	110.82
Ellagic acid	7.566	64.94
Kaempferol	14.419	60.15
Rutin	6.799	56.36
Chlorogenic acid	3.680	40.27
Cinnamic acid	13.96	9.37

Table 3: Characterizations of MPPE microcapsules

Formulation**	Particle size D_{32} (nm)	Zeta potential (mV)	Encapsulated Efficiency (%)
F ₁	202.25 \pm 9.56	-14.57 \pm 0.54	60.75 \pm 0.46
F ₂	215.12 \pm 25.3	-13.62 \pm 0.36	65.89 \pm 1.56
F ₃	303.4 \pm 35.2	-24.76 \pm 0.09	80.99 \pm 0.31
F ₄	300.7 \pm 25.5	-20.66 \pm 0.97	74.13 \pm 0.57
F ₅	382.1 \pm 15.36	-22.23 \pm 0.35	74.25 \pm 2.31
F ₆	336.1 \pm 23.1	-21.35 \pm 0.52	74.23 \pm 0.24

Mean values (\pm S.D.), (n = 3). MPPE = Mango peel phenolic extract, *F₁ = MPPE powder: wall materials (MD 100%) 1:10, F₂ = MPPE powder: wall materials (MD 100%) 1:20, F₃ = MPPE powder: wall materials (MD:WPC 80:20) 1:10, F₄ = MPPE powder: wall materials (MD:WPC 80:20) 1:20, F₅ = MPPE powder: wall materials (MD:WPC 60:40) 1:10, F₆ = MPPE powder: wall materials (MD:WPC 60:40) 1:20

Characterizations of MPPE microcapsules

Zeta potential and particle size: The magnitude of repulsion between the particles or the charge attraction was measured by zeta potential (Table 3). The zeta potential of MPPE microcapsules was ranged from -13.62 to -24.76 mV. The zeta potential of F₁ and F₂ were lowest compared to other formulations that contained WPC. This may due to the carboxylate groups of WPC being the only charged functionalities present in its globular so, the zeta potential of their always negative independently of pH³². The presence of this potential helps the stability of the solution and prevents droplet coalescence. The diameter of microcapsules varied from 202.25 to 382.1 nm. Thus, the particle size of formulations contained WPC (F₃-F₆) was 300.7 to 382.1 nm, compared to the formulations contained MD (F₁ and F₂ were 202.25 and 215.12 nm, respectively) because WPC was easy to interact with phenolic compounds and form a complex which leads to the coalescing of droplets faster¹³. Also, using MD as a wall material increased the viscosity of the solutions, which was followed by an increase in the size of the particles where the large droplets formed during atomization and therefore, obtained large powder particles³³.

Encapsulated Efficiency (EE): Through the results presented in Table 3, it was noted that the highest EE was observed in F₃ to F₆ compared to F₁ and F₂, where, F₃ was the highest EE (80.99%). The surface-polyphenol content of capsules was needed to calculate the encapsulation efficiency. In a high efficient encapsulation process, the smaller amount of polyphenol content remains on the surface. This means that the presence of WPC with MD improved MD efficiency as a carrier of polyphenols constituents¹³.

Microcapsules morphology: The result of Fig. 1 shows the surface morphology of spray-dried MPPE microcapsules captured with SEM. It could be observed that microcapsules have irregular shapes, mostly spherical structures with small

surface indentations and wrinkles. It was found that the type of wall materials is the most important factor which affects the appearance of capsules. The MPPE microcapsules which contained MD as a wall material (Fig. 1a, b) appeared mostly spherical structure and more smooth than the used combination of MD and WPC while images of Fig. 1c-f appeared spherical shape and apparent cracks. This was indicated to low in the permeability of MPPE microcapsules to gases which provide extra protection and retention of the core material (polyphenols). These results are in agreed with our previous finding El-Messery³⁴. Moreover, the variable sizes are a typical characteristic of particles produced by spray drying. Similar morphological characteristics of spray-dried microcapsules were found in previous research of Eratte³⁵.

Physicochemical properties of milk beverage: The total solids, protein, fat, ash, pH and acidity of supplemented milk beverage with MPPE microcapsules during 2 weeks of storage are presented in Table 4. The total solids of milk beverage samples were 16.66, 17.450, 18.540 and 19.700% for control, T₁, T₂ and T₃, respectively. The protein content of milk beverage samples was 5.550, 5.520, 5.325 and 5.575% for control, T₁, T₂ and T₃, respectively. The fat content of milk beverage samples was 6.160, 6.260, 6.275 and 6.370% for control, T₁, T₂ and T₃, respectively. The ash content of milk beverage samples were 0.935, 1.025, 1.145 and 1.225% for control, T₁, T₂ and T₃, respectively. It is evident from the results, the supplemented milk beverages had a higher content of total solids, protein, fat and ash as a result of MPPE microcapsules addition than the control sample.

It is due to the presence of WPC and MD used in the microencapsulation process. There was no great difference in the chemical composition of all samples after two weeks of storage compared to 0 days. These results are in agreement with Salama *et al.*³⁶.

Thus, measuring pH values are important for the supplemented milk beverage samples because the pH affects the physicochemical stability. The pH values of milk beverage

Table 4: Physicochemical properties of milk beverage supplemented with MPPE microcapsules

Treatments*	Storage period	Total solids%	Protein %	Fat %	Ash %	pH	Titrateable acidity%
Control	Fresh	16.66±0.11	5.550±0.13	6.16±0.04	0.935±0.04	6.055±0.06	0.185±0.07
T ₁		17.450±0.13	5.520±0.08	6.260±0.02	1.025±0.02	6.470±0.03	0.216±0.04
T ₂		18.540±0.07	5.325±0.10	6.275±0.03	1.145±0.05	6.445±0.07	0.227±0.02
T ₃		19.700±0.10	5.575±0.05	6.370±0.08	1.225±0.03	6.245±0.02	0.242±0.04
Control	2 Weeks	16.690±0.13	5.555±0.04	6.025±0.08	0.940±0.04	6.250±0.01	0.245±0.07
T ₁		17.50±0.07	5.570±0.02	6.165±0.1	1.060±0.02	6.320±0.01	0.263±0.03
T ₂		18.630±0.10	5.545±0.01	6.235±0.06	1.130±0.02	6.245±0.03	0.274±0.06
T ₃		19.575±0.04	5.605±0.04	6.285±0.02	1.240±0.07	6.045±0.05	0.295±0.06

The mean values (± S.D.), (n = 3). MPPE = Mango Peel Phenolic Extract. * Control = Plain milk without MPPE microcapsules, T₁ = Milk + MPPE microcapsules 1% (v/w), T₂ = Milk+ MPPE microcapsules 2% (v/w) and T₃ = Milk+ MPPE microcapsules 3% (v/w).

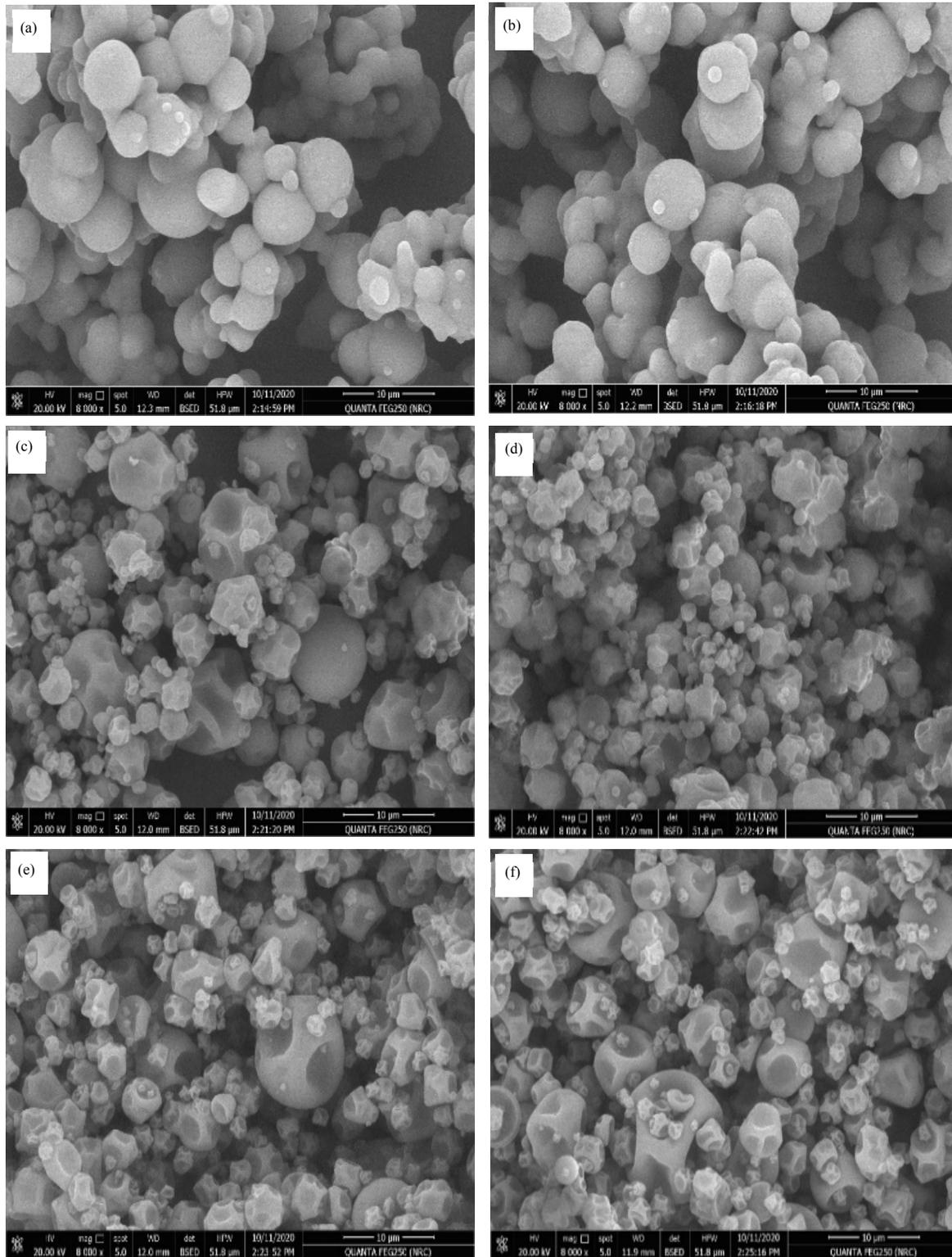


Fig. 1(a-f): Surface morphology of MPPE microcapsules

MPPE: Mango peel phenolic extract, A: MPPE powder: wall materials (MD 100%) 1:10, B: MPPE powder: wall materials (MD 100%) 1:20, C: MPPE powder: wall materials (MD:WPC 80:20) 1:10, D: MPPE powder: wall materials (MD:WPC 80:20) 1:20, E: MPPE powder: wall materials (MD:WPC 60:40) 1:10, F: MPPE powder: wall materials (MD:WPC 60:40) 1:20

Table 5: TPC, TFC and antioxidant activity (DPPH and FRAP) of MPPE microcapsules and milk beverage supplemented with MPPE microcapsules during *in vitro* gastrointestinal digestion (oral, gastric and intestinal phase)

Experiments	TPC (mg catechin g ⁻¹)			TFC (mg rutin g ⁻¹)			DPPH (μmol trolox g ⁻¹)			FRAP (μmol Trolox g ⁻¹)		
	Oral	Gastric	Intestinal	Oral	Gastric	Intestinal	Oral	Gastric	Intestinal	Oral	Gastric	Intestinal
MPPE microcapsules*												
F ₁	1.43±0.01	1.81±0.00	2.72±0.01	5.89±0.10	6.11±0.83	12.22±0.41	0.93±0.09	1.45±0.02	1.49±0.38	3.79±0.08	4.13±0.38	5.15±0.36
F ₂	1.42±0.04	1.75±0.01	2.66±0.02	3.69±0.14	5.39±1.73	13.29±0.79	1.13±0.01	1.41±0.07	1.66±0.01	2.47±0.09	3.94±0.38	5.01±0.33
F ₃	1.50±0.05	1.93±0.05	2.81±0.02	4.65±0.78	6.41±0.44	17.08±0.20	1.03±0.08	1.00±0.27	3.66±0.05	3.73±0.66	4.05±0.29	5.21±0.19
F ₄	1.50±0.01	1.51±0.00	2.75±0.02	6.93±1.53	7.86±1.40	14.85±0.56	1.03±0.17	1.13±0.17	3.62±0.05	2.49±0.45	4.00±0.20	4.99±0.17
F ₅	1.50±0.01	1.53±0.03	2.83±0.02	5.63±0.09	12.41±1.72	15.92±2.36	1.09±0.06	1.42±0.25	3.57±0.04	3.39±0.22	4.16±0.23	5.12±0.26
F ₆	1.48±0.01	1.53±0.04	2.78±0.00	6.57±0.09	14.84±1.86	14.87±1.15	1.03±0.03	1.98±0.15	3.64±0.37	2.89±0.18	3.93±0.20	4.75±0.80
Milk beverage**												
T ₁	2.10±0.17	3.18±0.77	5.13±0.68	5.44±2.00	47.51±7.75	60.51±0.86	0.20±0.26	1.30±0.03	1.66±0.13	0.21±0.10	2.15±0.55	3.78±0.41
T ₂	2.19±0.09	3.45±0.99	7.65±0.21	7.91±2.55	45.44±3.51	66.93±0.43	1.17±0.02	1.32±0.03	1.67±0.15	0.53±0.11	3.04±0.48	3.42±0.82
T ₃	2.42±0.06	6.36±0.07	8.25±0.29	6.62±0.06	46.53±4.05	64.61±1.03	1.10±0.00	1.34±0.00	1.67±0.02	0.85±0.11	3.52±0.21	3.87±0.17

Mean values (±S.D.), (n = 3). MPPE: Mango peel phenolic extract; TPC: Total flavonoid content; TFC: Total phenolic content; DPPH: 2, 2'-diphenyl-1-picrylhydrazyl radical; FRAP: Ferric reducing antioxidant power, *F₁ = MPPE powder: wall materials (MD:WPC 100%) 1:10; F₂ = MPPE powder: wall materials (MD:WPC 80:20) 1:20; F₃ = MPPE powder: wall materials (MD:WPC 60:40) 1:10; F₄ = MPPE powder: wall materials (MD:WPC 60:40) 1:10; F₅ = MPPE powder: wall materials (MD:WPC 60:40) 1:10; F₆ = MPPE powder: wall materials (MD:WPC 60:40) 1:20; **Control = Plain milk without MPPE microcapsules, T₁ = Milk+MPPE microcapsules 1% (v/w), T₂ = Milk+MPPE microcapsules 2% (v/w), T₃ = Milk+MPPE microcapsules 3% (v/w)

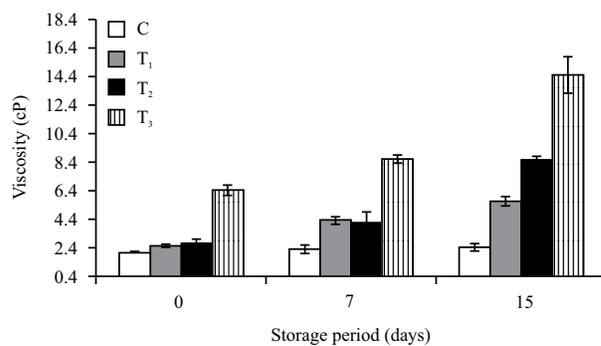


Fig. 2: The viscosity of milk beverage supplemented with MPPE microcapsules

samples were 6.055, 6.470, 6.445 and 6.24 for control, T₁, T₂ and T₃, respectively. The titratable acidity values of milk beverage samples were 0.185, 0.216, 0.227 and 0.242% for control, T₁, T₂ and T₃, respectively. The supplemented milk beverages had lower pH values and higher acidity compared to the control sample. After 2 weeks of storage, the pH values of all samples were decreased while the acidity concurrently increased. These results are in agreement with Hassan *et al.*³⁷.

Viscosity is an important measure of beverages and one of the most important factors that attract the consumer and evaluate the extent of his acceptance. The result of Fig. 2 presented the viscosity of milk beverages containing different concentrations of MPPE microcapsules (1, 2 and 3%).

The viscosity of milk beverage samples was 2.027, 2.50, 2.70 and 6.4 cP for control, T₁, T₂ and T₃, respectively in 0 days while after 15 days of storage the viscosity was 2.40, 5.64, 8.50 and 14.47 cP for control, T₁, T₂ and T₃, respectively. The viscosity of all samples whether fresh or during storage increased dramatically as a result of the addition of MPPE microcapsules. This is due to an increase in the total solids (as presented in Table 1 where MD and WPC were used as wall materials that can bind water, which increases the viscosity of the medium. These results are agreed with Salama *et al.*³⁶.

In vitro gastrointestinal digestion: *In vitro*, gastrointestinal digestion models are useful for evaluating the biological activity and the stability of phenolic compounds and other endogenous factors. The stability of Polyphenols and subsequently their bioaccessibility can be influenced by the method of food processing, microbiota and digestive enzymes³⁸.

Phenolic and flavonoid compounds: The data of Table 5 showed the impact of *in vitro* gastrointestinal digestion

simulation on TPC and TFC of MPPE microcapsules and milk beverage samples. It is observed that the TPC increased strongly after the intestinal digestion phase for MPPE microcapsules (2.72, 2.66, 2.81, 2.75, 2.83 and 2.78 mg catechin g^{-1} for F₁ to F₆, respectively) and milk beverage samples (5.13, 7.65 and 8.25 mg catechin g^{-1} for T₁, T₂ and T₃, respectively) compared to the oral phase. The previous trend was observed in the evaluation of TFC which increased after the intestinal digestion phase for MPPE microcapsules (12.22, 13.29, 17.08, 14.85, 15.95 and 14.87 mg Rutin g^{-1} for F₁ to F₆, respectively) and milk beverage samples (60.51, 66.93 and 64.61 mg Rutin g^{-1} for T₁, T₂ and T₃, respectively) compared to the oral phase. This is due to the short time that the samples stay in the oral phase (about 2 min), insufficient solubility in the saliva fluid and therefore the amount released from phenolic compounds is small³⁹. However, the phenolic compounds were released in a large amount in the milk beverage samples after the intestinal phase compared to the MPPE microcapsules. Hence, this result means that the milk beverage samples are protected against the condition changes of digestion such as pH variations and the type of enzyme. These results are in agreement with our previous study Farrg¹³, the effect of total phenolic compounds and antioxidant activity in the grape were studied during *in vitro* gastrointestinal digestion.

Antioxidant activity: The results of antioxidants activity in both the DPPH and FRAP assays for MPPE microcapsules and supplemented milk beverage during gastrointestinal digestion are shown in Table 5. The Antioxidant Activity (AA) values using the DPPH method in MPPE microcapsules after the intestinal digestion phase were 1.49, 1.66, 3.66, 3.62, 3.57 and 3.64 $\mu\text{mol Trolox } g^{-1}$ for F₁-F₆, respectively while The AA values of the supplemented milk beverage samples after the intestinal digestion phase were 1.66, 1.67 and 1.67 $\mu\text{mol Trolox } g^{-1}$ for T₁, T₂ and T₃ respectively. The FRAP values in MPPE microcapsules after intestinal digestion were 5.515, 5.01, 5.21, 4.99, 5.12 and 4.75 $\mu\text{mol Trolox } g^{-1}$ for F₁ to F₆, respectively while the FRAP values in milk beverage samples after intestinal digestion were 3.78, 3.42 and 3.87 $\mu\text{mol Trolox } g^{-1}$ for T₁, T₂ and T₃, respectively compared to the oral phase.

It was observed that the antioxidant activity (DPPH and FRAP) in MPPE microcapsules was higher compared to the supplemented milk beverage during the oral, gastric and intestinal phases. Also, the antioxidant activity of both MPPE microcapsules and supplemented milk beverages were increased after the oral phase. This is could be attributed to the high releasing of bioactive compounds, with scavenging properties, from the samples under the acidic

conditions of gastric digestion. Thereby, during the digestive process, antioxidant compounds could be more reactive particularly at acidic pH (gastric medium) than at neutral pH (intestinal medium), which could increase with pH in other compounds⁴⁰. Furthermore, the phenolic compounds have a chemical structure that plays a role in the free radical-scavenging activity (DPPH) and Ferric-Reducing Antioxidant Power (FRAP) which is mainly dependent on the position and number of hydroxyl groups on the phenolic molecules aromatic rings⁴¹. These results are in agreement with Correa-Betanzo⁴² and Chen⁴³, which observed increasing the DPPH and FRAP values after the gastric phase of digestion for blueberry extracts and fruit seeds, respectively.

In vivo experiment: The liver is considered as the fundamental organ where detoxified toxins, drugs and nourishments or metabolized⁴⁴. It is associated with numerous functional proteins, essential vitamins, lipids and lifesaving antibodies creation^{45,46}. In this study, carbon tetrachloride was used to incite liver dysfunction in rat model⁴⁷, where it can harm typical metabolic pathways by enacting the ROS framework. It was noticed that there was a slight decrease in total food intake in groups 1, 2 and 3 (439, 441 and 442 g, respectively) and there wasn't a massive difference in the initial body weight (177.8, 178.5 and 176.5 g, respectively) final body weight (196.8, 195.5 and 198.5 g, respectively) and body weight gain (19, 17 and 22 g, respectively) compared to animal control (+ve) (Table 6). Also, it was observed a decrease in food efficiency in groups 1 (0.043) and 2 (0.039) compared to animal control (+ve) (0.049). These results are agreement with findings Yao⁴⁸.

The lipid peroxide level was high in groups 1, 2 and 3 (80.7, 83.3 and 85.98 nmol mL^{-1} , respectively) compared to the animal's control (+ve) (75.17 nmol mL^{-1}) (Table 7). This increase is considered the important underlying cause of the oxidative stress initiation related to various tissue injuries, cell death and the progression of acute and chronic diseases⁴⁹⁻⁵¹. This result is in agreement with Gamal⁵² and Sabina⁵³. Also, the lipid peroxide level increased in the animal control (+ve) compared to the normal control. The total antioxidant was higher in groups 1, 2 and 3 (0.83, 0.84 and 0.90 mM L^{-1} , respectively) compared to animal control (+ve) (0.79 mM L^{-1}) and there wasn't a massive difference between the animal control (+ve) and the normal control (0.77 mM L^{-1}).

The catalase level was decreased in animal control (+ve) (15.26 U min^{-1}) compared to the normal control while an increase of catalase level was observed in groups 1, 2 and 3 (23.33, 25.17 and 30.21 U min^{-1} , respectively) compared to

Table 6: Effect of milk beverage supplemented with MPPE microcapsules on the body weight, body gain, total food intake and food efficiency

Group*	Initial body weight (g)	Final body weight (g)	Body gain (g)	Total food intake (g)	Food efficiency
Control (-ve)	173.5±1.15	195.2±2.45	21.7±1.3	443±2.65	0.049±0.491
Group (+ve)	175.32±2.11	197±2.31	21.68±0.2	435±2.5	0.049±0.08
Group 1	177.8±1.41	196.8±2.48	19±1.07	439±1.25	0.043±0.856
Group 2	178.5±1.35	195.5±1.91	17±0.56	441±1.61	0.039±0.349
Group 3	176.5±1.41	198.5±2.11	22±0.7	442±1.61	0.049±0.435

Mean ± SE, *Control (-ve) = Normal rats fed on the basal synthetic diet that served as -ve control, Control (+ve) = Induced rats fed on the basal synthetic diet and plain milk beverage every day by oral intubation for 2 weeks that served as +ve control. Group 1 = Induced rats fed on the basal synthetic diet and T₁ by oral intubation for 2 weeks, Group 2 = Induced rats fed on the basal synthetic diet and T₂ by oral intubation for 2 weeks, Group 3 = Induced rats fed on the basal synthetic diet and T₃ by oral intubation for 2 weeks

Table 7: Effect of milk beverage supplemented with MPPE microcapsules on lipid peroxide, total antioxidant, catalase, ALT, AST, ALP and creatinine

Group**	Lipid peroxide (nmol mL ⁻¹)	Total antioxidant (mM L ⁻¹)	Catalase (U min ⁻¹)	ALT* (U L ⁻¹)	AST* (U L ⁻¹)	ALP* (U L ⁻¹)	Creatinine (mg dL ⁻¹)
Control (-ve)	40.51±1.73	0.77±0.032	35.88±3.11	25.5±2.85	30.3±4.22	55.43±4.55	0.55±0.25
Group (+ve)	75.17±2.55	0.79±0.049	15.26±1.19	132.15±10.12	98.78±7.15	153.28±9.15	0.95±3.22
Group 1	80.7±2.71	0.83±0.032	23.33±1.08	65.28±3.1	65.24±4.23	125.29±6.23	0.69±0.07
Group 2	83.33±1.39	0.84±0.081	25.17±1.25	53.5±1.33	50.25±3.78	95.33±3.28	0.55±0.14
Group 3	85.98±1.35	0.90±0.051	30.21±1.45	44.3±1.08	40.39±3.65	64.19±2.31	0.45±0.11

Mean ± SE, *ALT = Alanine aminotransferase, AST = Aspartate aminotransferase and ALP = Alkaline phosphatase, **Control (-ve) = Normal rats fed on the basal synthetic diet that served as -ve control. Control (+ve) = Induced rats fed on the basal synthetic diet and plain milk beverage every day by oral intubation for 2 weeks that served as +ve control. Group 1 = Induced rats fed on the basal synthetic diet and T₁ by oral intubation for 2 weeks, Group 2 = Induced rats fed on the basal synthetic diet and T₂ by oral intubation for 2 weeks, Group 3 = Induced rats fed on the basal synthetic diet and T₃ by oral intubation for 2 weeks

animal control (+ve). From the results, it is clear MPPE microcapsules had potential protection against oxidative stress by reestablishment the endogenous antioxidants in the tissues⁵⁴. The antioxidant and anti-lipid peroxidation properties of MPPE microcapsules could be attributed to its constituent of flavonoids and other polyphenolics⁵⁵.

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) levels were higher in the animal control (+ve) (132.15, 98.78 and 153.28 U L⁻¹, respectively) compared to the normal control (25.5, 30.3 and 55.43 U L⁻¹, respectively) while ALT, AST and ALP levels decreased in groups 1, 2 and 3 compared to animal control (+ve). AST and ALT levels were decreased in the animal control (+ve) which attributed to the damaged structural integrity of the liver⁵⁶.

Creatinine level was higher in the animal control (+ve) (0.95 mg dL⁻¹) compared to the normal control (0.55 mg dL⁻¹) while the Creatinine level was lower in groups 1, 2 and 3 (0.69, 0.55 and 0.45 mg dL⁻¹, respectively) compared to animal control (+ve). This may due to CCl₄ which destroys the membrane and led to massive hepatic enzyme leakage (AST, ALT and ALP) associated with immune cell infiltration, massive centrilobular apoptosis, ballooning degeneration and cell death⁵⁷. The reduction of elevated liver marker enzyme activity would be beneficial for liver stability⁵⁸.

The results showed the possibility of using Mango Peel Phenolic Extract (MPPE) is a rich source of phenolic and flavonoids compounds and antioxidants in microcapsules to fortify a milk beverage product with them, which hadn't an

immense effect on the chemical and physical properties. Fortification of the milk beverage with MPPE protected them during the simulation digestive process as well as reduced the oxidative stress damage in the experimental rats. Moreover, the use of this waste is an efficient strategy in developing novel functional food, with improved quality attributes and functional roles.

CONCLUSION

Mango peel is a by-product of the mango beverage industry, which is a rich source of bioactive compounds. Since these compounds are difficult to add to the food product due to the speed of damage as well as their instability during digestion, the process of encapsulation is the optimum solution to use it. Through the results, the MPPE microcapsules were more stable during the digestion process as well as the milk beverage supplemented with these capsules. *In vivo* study, the MPPE microcapsules prevented oxidative stress and inflammation in a rat model. Our data had shown that group 3 was the best treatment that improved the hepatic changes produced by CCl₄ administration and could be used in treating patients who are susceptible to the oxidative stress that leads to liver function disorders.

SIGNIFICANCE STATEMENT

This study reveals the possibility of using mango peel to obtain bioactive compounds that have promising effects in therapeutics. Also, the optimal combination of whey protein

concentrate and maltodextrin were detected to obtain the highest efficiency of the encapsulation process of the bioactive compounds extracted from mango peel. By simulating the digestion process, the amount released from these compounds in the capsule was known at each digestion stage (oral, gastric and intestinal). Also, it was discovered that the milk beverage supplemented with these capsules has a positive effect to reduce the oxidative stress in the experimental rats. Thus, a new theory on supplemented dairy product as therapeutic may be arrived at.

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