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Research Article
Nanoencapsulation of Bioactive Compounds Extracted from Egyptian Prickly Pears Peel Fruit: Antioxidant and Their Application in Guava Juice

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Abstract

Background and Objective: Nanoencapsulation involves coating for bioactive compounds and can strongly affect their mechanical, formative properties, bioavailability and stability. The aim of this study was to benefit the bioactive compounds extracted from Egyptian prickly pear peel fruit in nanoencapsulation formula to protect and improve their stability and bioavailability and evaluate their activity comparing to synthetic ones. **Materials and Methods:** Bioactive compounds were extracted by ethanol, exposed to ultrasonic water bath, then encapsulated in two natural polymers (sodium alginate and chitosan) using nanotechnology techniques aside with their chemical and physical evaluation and application in guava juice. **Results:** No significant differences in DPPH radical-scavenging activity were observed between ethanol extract (EE) and nanoencapsulate formulas (NEE-Chi and NEE-Algi+Chi) of prickly pears peels fruit extract at 15 mg mL^{-1} concentration. While, scavenging activity of prickly pears peels fruit extract in NEE-Alg at the same concentration was also significantly less than other formulas. Nanoencapsulate extract in chitosan (NEE-Chi) and NEE-Alg+Chi formulas had the highest reducing power activity (481.76 and 462.49 µg GAE/100 g DW), respectively. The highest nanoencapsulated yield and efficiency were observed for NEE-Chi formula being 91.74 and 97.81%, respectively. Thermal stability (DSC) of nano-encapsulated increased after nano-encapsulation showing the highest results with NEE-Alg+Chi being 219.53°C. The addition of NEE-Alg+Chi to guava juice showed significantly affected the taste, odor, color, mouth feel, appearance and overall acceptability being 6.9, 6.7, 6.8, 6.8, 6.8 and 6.7, respectively, compared to other samples of guava juice supplemented with prickly pears peel fruit extracts. The highest content of total antioxidant capacity in guava juice samples was found in NEE-Chi formula stored for 120 days at 23°C. **Conclusion:** The impact of this study indicated that nanoencapsulation affects positively the bioactive compounds extracted from prickly pears peels fruit, improves the antioxidant activity, enhancing the quality and stability of bioactive compounds.

Key words: Prickly pears peel fruit, antioxidant activity, nanoencapsulated, guava juice

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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

Opuntia is a genus of the family Cactaceae that grows worldwide and the most usual species of this genus is *Opuntia ficus indica* which is a tropical fruit tree that grows in tropical and subtropical areas. In these areas it is not possible to cultivate the lands with many different crops due to the lack of water and often the salinity of the soils. There are few crops capable of being cultivated in this type of lands and one of them is cactus pears or prickly pears plant[1]. Therefore, it is suitable for this crop to be cultivated in arid and semiarid regions, as food for human and animals[2-4] whereas, it is cultivated with high distribution in the Mediterranean areas, United States especially in California, Texas, Central, South Latin America and South Africa.

In some countries of North Africa such as Egypt, the cactus pears are named (the bridge life) because in the seasons without rain, the cactus pears fruits and pads are only the way to give food, feed and water to their animals[5]. Recently, there are several researches about prickly pears processing, which are usually eaten fresh after peeling in many regions giving many different products such as juices, nectars, dehydrated sheets, marmalades, jelly, jams, wines, natural sweeteners and other alcoholic candies, beverage, frozen fruit, canned and, etc.[6-9].

In Egypt, prickly pears have been grown for many years ago peculiarly in sandy areas in different parts, because it is very drought resistance. The trees are grown not only for their fruits but also as fences and windbreakers[10]. Prickly pears fruits are also very susceptible to microbial spoilage because of low acids and high sugar content of pulp so that storage life of the fruit in the fresh state is limited. The presence of spines makes prickly pears difficult to peel. Minimal processing might be an important way to increase the acceptability of this fruit.

In the last years, the production of Egyptian prickly pears fruits was increased due to the increment in cultivation area (i.e., Abo-Zabal, Sinai, belbis and reclaimed areas at El-Bostan, Elbanger and El-Nubaria) from 1471, 1842 to 2558 acre in 1994, 2001 and 2004, respectively[10]. Therefore, the corresponding production was increased from 10,233-22,974 t in 1994-2003 and 28,024 t in 2004[11]. In 2015 the cultivation area was given acre under the Egyptian condition from 15-22 thousand tons.

The average weight of varies prickly pears fruit was from 100-160 g depending on cultivation the origin site and usable parts (i.e., pulp 48-52% and peel 48-52%). The pulp can be divided into strained pulp and seeds (44-45%), which is the basis for juice products and food as it contains 83-87% water and 6-14% sugars.

It is known for a long time that prickly pears fruit is used in traditional medicine e.g., (glaucoma, ulcer and dyspnea) in addition to liver, fatigue and wounds[12,13]. Different studies using European and Asian various types of prickly pears fruit have shown high antioxidant activities that significantly reduce the oxidative stress in patients and may protection chronic pathologies. Some authors also reported that the prickly pears fruit and its peels are good source of fiber that helps to reduce plasma cholesterol and blood sugar levels[14,15]. The full prickly pears fruit could be considered a functional food, this feature has been attributed to its bioactive compounds such as vitamin C and E, flavonoid, carotenoids, polyphenols (isorhamnetin, quercetin and kaempferol), pigments and taurine[16,17].

So, the prickly pears appear to be a very promising new source of pectin and mucilage, which is extracted from cladodes and fruits. In addition they have good physico-chemical properties that make their applications in food, medicine, pharmacy, dietary supplements, and others commercial sources possible[18].

Bioactive compounds extracted from plants lose their antioxidants i.e. (phenolic compounds) when exposed to high temperatures, oxidation and light and decrease their yields, so it must be protected by encapsulating techniques[19].

Chavez-Santoscoy *et al.*[20] found that the juices of Moroccan prickly pears contained higher phenolic amount compared to that of Mexican prickly pears ranging from 55.4-226.3 μg GAE g^{-1} of juice.

The encapsulation methods enable the protection of the bioactive ingredients extracted from peels by a carrier (matrix or polymers) for transportation and release of the active compounds in a controlled manner[21]. Also, the encapsulation could be used to extend the expiration date for functional substances when ingested in the intestine undergoing a series of physiological conditions[22].

Wang *et al.*[23], Kouassi *et al.*[24] and Bilia *et al.*[25] reported that micro-or nano-encapsulation involves coating of the emulsion droplets in fluidic dispersions in the microsize or nanosize system (below 100 nm dimension), protect the active compounds against environmental factors (e.g., oxygen, light, moisture, and pH) and take advantage for dramatically increased the surface area to volume output and is an alternative for overcoming these problems but additionally, due to the sub cellular size, may increase the cellular absorption mechanisms and increasing bio-efficacy. So, when brought into a system, nanomaterials can strongly affect the mechanical and formative properties like elasticity and stiffness.

The interest of bioactive compounds extracted from plants and their application in food preservation have been amplified in the last few years by an increasingly negative consumers perspective for synthetic preservatives, where studies have proven that the synthetic antioxidants unlike the natural antioxidants cannot be reused by the organism after they have donated their electron to satiate free radicals and therefore become harmful metabolic derivatives that increase instead of decreasing the total of oxidative stress[26,27].

This study is part of an ongoing research aimed to discover new plant extracts with bioactive potentials.

The study concentrate and benefit from the bioactive compounds (e.g., antioxidants) extracted from the peels of Egyptian prickly peers fruit (*Opuntia ficus indica* L.) as a residue in nanoparticle capsulation formula to protect and improve their bioavailability and stability, evaluate their activity as preservatives comparing to synthetic ones and their application in guava juice.

MATERIALS AND METHODS

Raw materials: The cultivars of the Egyptian prickly pears peels fruit (*Opuntia ficus indica* L.) were collected from street vendors and local markets in Egypt (Fig. 1).

The collected prickly pears fruit peel (4 kg) (95% yellow cultivar and 5% red cultivar) was washed abundantly with water tap to remove the pulp attached, sand, seeds and clay, then dried at 40°C on air oven for 48 h. to obtain 500 g dry sample which was later coarsely powdered in bladed mill to 60-mesh size and used for solvent extraction.

Chemicals: Ascorbic acid, ferric trichloride, Folin-Ciocalteu, potassium ferricyanide [K_3Fe $(CN)_6$], 2,20-diphenyl-1-picrylhydrazyl (DPPH), sulphuric acid, sodium phosphate, ammonium molybdate and sodium hydroxide (NaOH), trifluoroacetic acid, ammonium molybdate were purchased from Sigma Co. (St. Louis, MO, USA).

Methods

Extract preparation: For sample preparation, 50 g were extracted with 100 mL of ethanol 95% (two times) at 25°C for 48 h, exposing to ultrasonic water bath[28] for 2 h and then concentrated using a rotary evaporator under reduced pressure at 40°C to obtain 9.3% ethanol extract (EE). The aqueous fraction, 50 g dry sample was extracted with 100 mL of distilled water (two times); heating at 90°C for 2 h, exposing to ultrasonic water bath for 2 h and then concentrated using rotary evaporator to obtain water extract (WE) soluble residual aqueous fraction yielding 12.5%. At the end of the process; both fractions EE and WE were subjected to evaluate their antioxidants.

Fig. 1: Egyptian Prickly pears fruit (*Opuntia ficus indica* L.)

Antioxidant assays: Each extract sample was dissolved in 95% ethanol or distilled water to make a concentration of 1 mg mL^{-1} and then diluted to prepare the series concentrations for antioxidant assays. Reference chemicals (Ascorbic acid as natural antioxidant and BHT as synthetic antioxidant) were used for comparison in all assays.

DPPH radical scavenging activity assay: The free radical scavenging activity of the EE, WE and three encapsulation formulas (NEE-Alg, NEE-Chi and NEE-Alg+Chi) were measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described earlier by Brand-Williams *et al.*[29] and Bursal and Gulcin[30]. The stock solution was prepared by dissolving 24 mg DPPH with 100 mL ethanol and stored at 20°C until required. The working solution was obtained by diluting DPPH solution with ethanol to attain an absorbance of about 0.98±0.12 at 517 nm using the spectrophotometer. A 3 mL aliquot of this solution was mixed with 0.1 mL of the sample at various concentrations (5-35 mg mL^{-1}). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm.

The control was prepared as above without any sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as follow:

$$\text{Scavenging effect (\%)} = \frac{\text{Control absorbance-Sample absorbance}}{\text{Control absorbance}} \times 100$$

Total antioxidant capacity: The total antioxidant capacity of the fractions was determined by phosphomolybdate method using ascorbic acid and BHT as a standard[31]. An aliquot of 0.1 mL of both extract samples and encapsulation formulas

(WE, EE, NEE-Alg, NEE-Chi and NEE-Alg+Chi) was mixed with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 765 nm against a blank. A typical blank contained 1 mL of the reagent solution and the appropriate volume of the solvent and incubated under the same conditions. Ascorbic acid and BHT was used as standard. The antioxidant capacity was estimated as follow:

$$\text{Antioxidant effect (\%)} = \frac{\text{Control absorbance-Sample absorbance}}{\text{Control absorbance}} \times 100$$

Reducing power capacity: The reducing power capacity of Egyptian prickly pears fruit peel extracts and encapsulated formulas was determined according to Yildirim *et al.*[32] with slight modifications[33]. Briefly, 1.25 mL phosphate buffer (0.2 M, pH 6.6) and 1.25 mL potassium ferricyanide (1% in water, w/v) were mixed with 0.1 mL prickly pears fruit peel extract and its encapsulated formulas (from 3-30 mg mL^{-1}). After 20 min incubation at 50°C, 1 mL trichloroacetic acid (10% in water, w/v) was added to the reaction mixture. After centrifugation for 10 min at 3000 rpm, 1.5 mL were taken from the supernatant, then mixed with 1.5 mL distilled water and 100 µL fresh ferric trichloride (0.1% in water, w/v). The reducing power capacity was determined by measuring the absorbance at 700 nm against a blank (without extract). A higher absorbance is relevant of high reducing capacity. The ascorbic acid and BHT (ranging from 5-30 mg mL^{-1}) was used as reference.

Total phenolic content: The total phenolic content was determined by the spectrophotometric method[34]. In brief, 1 mg mL^{-1} sample of prickly pears fruit peel extract was mixed with 1 mL of Folin-Ciocalteu's phenol reagent. After 5 min, 10 mL of a 7% Na_2CO_3 solution was added to the mixture followed by the addition of 13 mL of deionized distilled water and mixed thoroughly.

The mixture was kept in the dark for 90 min at 25°C, after which the absorbance was read at 750 nm with a model UV-2401 PC spectrophotometer. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution as standard. The estimation of the phenolic compounds was carried out in triplicate. The total phenolic content in prickly pears fruit peel extract was expressed as µg of gallic acid equivalents (DW) per g of sample extract.

Preparation of nano-capsule formulas: Egyptian prickly pears peels fruit extracts were encapsulated in two natural polymers (sodium alginate and chitosan) using nanotechnology techniques. Both the sodium alginate and chitosan solutions were prepared by dissolving in distilled water and 1% acetic acid respectively according to the method of Gazori *et al.*[35] with some modifications to produce three formulas as follow:

The first formula was prepared by using sodium alginate to produce nano-capsule of prickly pears peels fruit ethanol extract in sodium alginate (NEE-Alg). Briefly, 4 g of sodium alginate was dissolved in 100 mL deionized water using a magnetic stirrer at 2000 rpm for 1 h and kept the solution overnight in a shaking water bath. One milliliter of prickly pears peel fruit extract was added to 5 mL sodium alginate solution (1:5 v/v ratio) 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.

The second formula was prepared by using chitosan to produce nano-capsule of prickly pears peels fruit ethanol extract in chitosan (NEE-Chi). Four gram of chitosan was dissolved in 100 mL of 1% acetic acid (4 wt%) and stirring for 60 min at 2,000 rpm using magnetic stirrer. After 24 h incubation at 4°C, 1 mL of prickly pears peels fruit extract was added to 5 mL mixture solution and the mixture was homogenized by ultrasonic probe.

The third formula was prepared by using sodium alginate and chitosan to produce nano-capsule of prickly pears peels fruit ethanol extract in sodium alginate+chitosan (NEE-Alg+Chi). With respect to Alg/Chi ratio a certain amount of sodium alginate stock solution was diluted with 10 mL of filtered deionized water. Then 1 mL of calcium chloride solution adjusted to Ca/Alg% ratio was added drop wise to above solution while stirring. The prepared Ca/Alg was stirred for a further 10 min. Then 1 mL of prickly pears peels fruit extract was added to 5 mL of Alg/Chi solution. It was stirred for 10 min to be complexed. In cases where the mixture had large aggregates these were broken up using bath sonicator. The pH of solution was adjusted to 5.3 using 0.1 N NaOH solutions, and was stirred for further 30 min. the mixture were centrifuged at 1100 rpm for 15 min to remove any large aggregates prior to analysis. Centrifugation under these conditions allowed aggregates to formula pellet, leaving nanoparticles suspended in the supernatant. The particle suspension was then centrifuged at 25°C in the Amicon Ultra-15 (Ultracel-100 K) centrifuge tube with 100 kDa cut off at 5000 rpm for 20 min to separate free polymers from nanoparticles.

Characterization of encapsulated prickly pears peels fruit extract formulas

Transmission electron microscope (TEM): The size and morphology of three encapsulated formulas i.e., (NEE-Alg, NEE-Chi and NEE-Alg+Chi) were evaluated by transmission electron microscopy (TEM) (ZEISS 902 A, Zeiss, Germany). Twenty micro-liters of diluted samples was placed on a film-coated 200-mesh copper specimen grid for 10 min and the fluid excess was eliminated using filter paper. The grid was then stained with one drop of 3% phosphotungstic acid and allowed to dry for 3 min. The coated grid was dried and examined under the TEM microscope. The samples were observed by operating at voltage 160 kV[36].

Encapsulation efficiency (%): The encapsulation efficiency of the encapsulated formulas was determined through an indirect method in triplicate by estimating antioxidant activity of prickly pears peel fruit ethanol extract. One gram of the encapsulated formulas was dissolved in absolute ethanol to extract the bioactive compounds in capsule and centrifuge at 2000 rpm for 15 min[37]. One gram sample of encapsulating nanoparticles prickly pears peel fruit extract was dissolved in 10 mL ethanol in an ultrasonic bath for 1 h and then filtered with a 1 mm syringe filter before spectrophotometric assay at 437 nm. The encapsulation efficiency (EE) of bioactive compounds was determined as follow:

$$\text{Encapsulation efficiency (\%) (g/100 g)} = \frac{\text{Mass of extract in nanoparticles}}{\text{Mass of extract added}} \times 100$$

Differential scanning calorimetry: The Differential Scanning Calorimetry (DSC) profile was determined according to Pijpers *et al.*[38]. Ten milligram of EE, NEE-Alg, NEE-Chi and NEE-Alg+Chi samples was placed in aluminum crucibles. The samples were analyzed under a flow of nitrogen gas (40 mL min^{-1}). A dynamic scan was performed at a heating rate of 10°C min^{-1} over a temperature range of 0-300°C. Evaporation enthalpies were calculated by peak area integration of DSC profiles and the results were compared with the estimated vaporization enthalpy of extract major compounds.

Application of prickly pears fruit peels extracts formulas in guava juice: Guava juice was composed of 40% puree, 50% potable water and 10% sucrose. Then preservatives were added as formulations, comparing to other juices and juice without any preservatives addition as a control.

Sensory evaluation: An acceptance test of guava juice samples with or without addition of prickly pears peel fruit extract and their formulas was carried out at the Sensory Analysis Laboratory of the Food Science and Technology Department, National Research Center (NRC) with the participation of 15 volunteer members of the NRC community from both sexes and of all ages. Volunteers were selected based on their interest and availability to take part in the tests. Samples were evaluated through tests of preference and intention to purchase in accordance with Larmond[39]. The testers assessed the samples using a 9-point structured hedonic scale, as follows: 1: Disliked very much, 2: Disliked quite a lot, 3: Fairly disliked, 4: Slightly disliked, 5: Neither liked nor disliked, 6: Slightly liked, 7: Fairly liked, 8: Liked quite a lot, 9: Liked very much. The samples were marked with random three digit numbers and served to testers. Sensory evaluated attributes included: taste, odor, color, mouth feel, appearance and overall acceptability.

Storage stability: Storage stability experiment of guava juice samples was used to estimate the keeping capacity of total antioxidant in all guava juice samples containing EE, NEE-Alg, NEE-Chi and NEE-Alg+Chi. The guava juices were stored after pasteurization at room temperature at 23°C for 120 days period and stability was estimated every 30 days comparing to control sample.

Statistical analyses: The experiments were done in triplicate. The data were calculated as Means±SD. Analysis of variance (ANOVA) was used for the analysis of data. Significance was accepted at $p = 0.05$.

RESULTS AND DISCUSSION

Water and ethanol extract: Water and ethanol extraction was studied to screen the yield of antioxidants from prickly pears peel fruit.

The yield of ethanol extract (EE) obtained by hydrodistillation of Egyptian prickly pears peel fruit (*Opuntia stricta* fruit) as waste was lower 9.3±1.2% (w/w) dry weight basis than that found by water extraction (WE) 12.5±1.5% (w/w). The extract yield with ethanol was about 3.2% lower than the extract yield obtained by water. Possibly, this could be due to the higher polarity of water and also the elevation extraction temperature. The results obtained from the process of extraction were higher than that found by Koubaa *et al.*[33] being 0.18±0.06% (w/w) from *Opuntia ficus indica* fruit peels. Generally, ultrasound extraction, temperature and dynamic time are much influenced factors.

Total phenolic content: Results in Fig. 2 indicated that the highest total phenol content was observed in water extract (WE) of prickly pears peel fruit being 793 µg Gallic acid/g compared to the ethanol extract (EE) of prickly pears fruit peel which recorded the lowest content being 638 µg Gallic acid/g dried sample.

Total phenol content of water and solvent extracts from prickly pear fruit peel was higher than that obtained by Bouzoubaa *et al.*[40] being 289.39-337.57 µg g^{-1} for Achefri and Amouslem southern Morocco prickly pears cultivas, respectively, but lower than that found by Abou-Elella and Rehab[41] ranging from 221.3-1501.7 µg Gallic acid/100 g *Ficus indica* peel at different solvent extracts.

The antioxidant activity of phenolic compounds mainly due to their oxidation properties, which can be played an important role in neutralizing and adsorbing free radicals. From this perspective, prickly pears peel with larger bioactive compounds (%) could play a role as antioxidants in many food applications.

Antioxidant activities

DPPH radical-scavenging activity: The DPPH radical scavenging activity of water and ethanol extracts of prickly pears peels fruit compared to synthetic antioxidant (BHT) and natural antioxidant (ascorbic acid) were shown in Fig. 3.

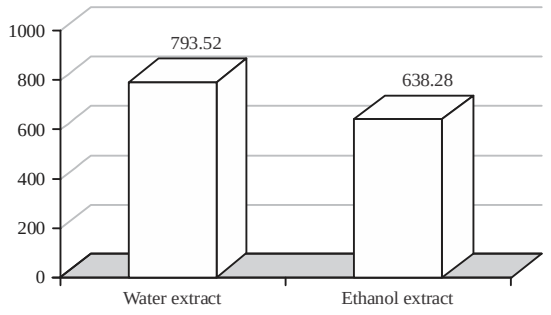

Fig. 2: Total phenolics (µg Gallic acid/g) of prickly pears peels fruit

In DPPH scavenging, the antioxidant activity was measured by the decline in absorbance during the DPPH radical received an electron or hydrogen from an antioxidant bioactive compounds extracts to be stable diamagnetic molecule. The water extract (WE) of peels from prickly pears fruit exhibited appreciable scavenging activity ranging from 10.084-56.302% for concentrate extract 5-35 mg mL^{-1}, respectively. While DPPH radical-scavenging activity for ethanol extract (EE) of prickly pears peels fruit was higher and ranging from 14.508-79.275% for the same concentrate extract. The DPPH radical scavenging activity dependent on the extracts concentration; it increased significantly and gradually with the increasing in concentration extracts.

On other hand, the increased values of DPPH radical scavenging activity were observed for BHT and ascorbic acid. There are significant differences in radical scavenging activity between ascorbic acid as natural antioxidant and ethanol extract (EE) of peels from prickly pears fruit at 35 mg mL^{-1} concentration, but lower than butylated hydroxytoluene (BHT) at all concentrations. This result was in agreement with that found by Koubaa *et al.*[33].

The positive control (35 mg mL^{-1} of BHT or ascorbic acid) produced an inhibition of 96.95 or 86.42%, respectively. With respect to the WE or EE of peels from prickly pears fruit, it found that varieties of extracts peels from prickly pears fruit showed a concentration dependent antioxidant capacity 56.302 or 79.275%, respectively.

Reducing power capacity: Reducing power capacity of WE and EE extract from peels of prickly pears fruit in comparison to synthetic antioxidant (BHT) and natural antioxidant (Ascorbic acid) were shown in Fig. 4.

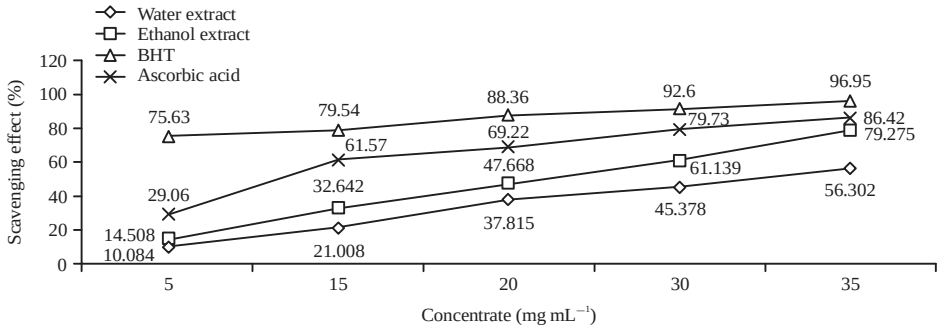

Fig. 3: DPPH radical scavenging activities of water and solvent extract from prickly pears peel compared with (BHT), ascorbic acid

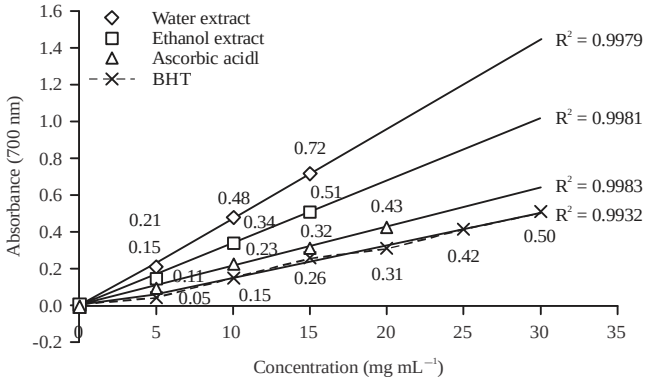

Fig. 4: Reducing power capacity of water and ethanol extract from prickly pears peel fruit

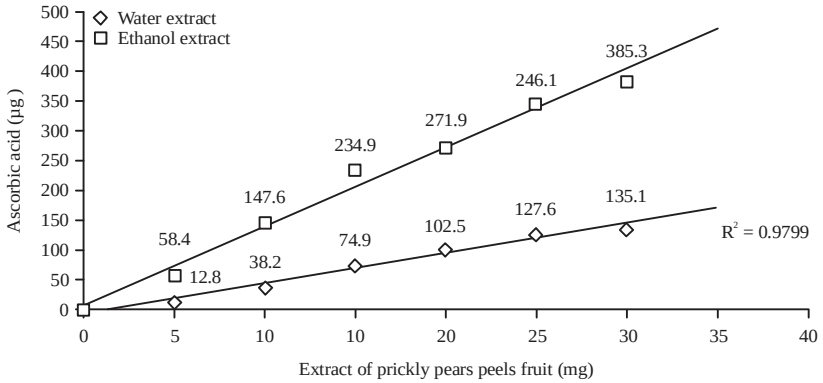

Fig. 5: Total antioxidant activity of solvent and water extract from prickly pears peels fruit, expressed as µg of ascorbic acid equivalent

The reducing power capacity reflects the presence of an antioxidant for the reduction of ferricyanide ions $[Fe(CN)_6]^3$ to ferrocyanide ions $[Fe(CN)_6]^4$ form depending on the presence of antioxidants. This reducing property is generally associated with the presence of a reducer exercising an antioxidant action by breaking the free radical chains; yielding a hydrogen atom.

The reducing power of extracts was increasing proportionally with sample's concentration and was even higher than that observed for ascorbic acid and followed by BHT. In fact, at 10 mg mL^{-1}, the measured values at 700 nm were 0.15, 0.23, 0.34 and 0.48 for BHT, ascorbic acid, EE and WE, respectively. The correlation coefficients R^2 were 0.99 about all samples. These results revealed the high reducing power capacity of EE.

The obtained results agreed with those occurred by Yeddes *et al.*[42], Obon *et al.*[43], Butera *et al.*[44] and Cha *et al.*[45].

Results in Fig. 4 indicated that the best reducing power was obtained with EE reaching (0.51) when tested at 15 mg mL^{-1} concentrate extract and even less than that obtained with ascorbic acid being (0.31) for the same concentration. Other values were 0.72 and 0.32 for WE and BHT, respectively. The ethanol extract from prickly pears peels fruit showed high reducing power capacity than water extract due to the conversion of free radicals to more stable products in preventing oxidative damage.

Total antioxidant activity: The total antioxidant activity was followed by the reduction of the phosphomolybdate by WE or EE from prickly pears fruit peels and was expressed as ascorbic acid equivalent (Fig. 5). The obtained results showed a linear behavior between concentrate extracts and ascorbic acid, with correlation coefficient R^2 of 0.987 and 0.9799 for EE and WE, respectively. In fact, the antioxidant activity increases proportionally with the extracts concentration. At 30 mg the total antioxidant activity were equivalent to 385.3 and 135.1 µg for EE and WE, respectively.

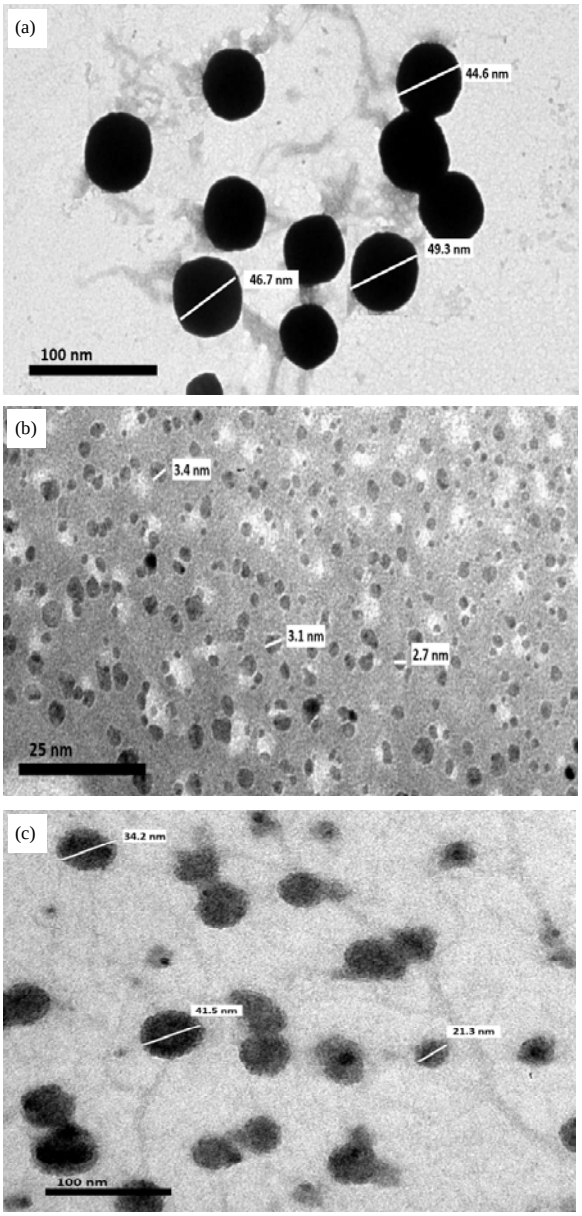

Fig. 6: Transmission electron microscopy images of bioactive compounds extract from prickly pears peels encapsulated with (a) Alginate, (b) Chitosan and (c) Alginate+chitosan

Characterization of encapsulated peels extract

Morphology evaluation by TEM: The morphology size, shape and structure of the nanoparticles of three capsule formulas were evaluated by TEM (Fig. 6a-c). TEM image in Fig. 6a showed that the nanoparticles of bioactive compounds encapsulated with sodium alginate maintain the spherical shape with a solid dense structure and size ranged from 44.6-49.3 nm.

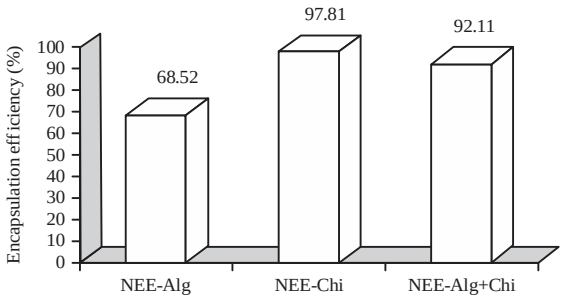

Fig. 7: Encapsulation efficiency (%) of encapsulate peels extract formulas

The results in Fig. 6b showed that the nano-particles of bioactive compounds encapsulated with chitosan are composed of a core shape entrapped in a shell material of a fairly constant thickness. Nano-capsules appear to be made up of spherical particles of about 2.7-3.4 nm in diameter. The external surface of each particle is almost regular and smooth, showing that chitosan polymer formulas continuous film surrounding the bioactive compounds droplets.

When bioactive compounds extract from prickly pears peel was encapsulated in chitosan nanoparticles fluorescence was detected confirming the presence of those compounds extract inside the nanoparticles structures. Round shaped and apparently compact structures could be observed, together with the bioactive compounds extract are distributed into chitosan nanoparticles.

The morphological studies by TEM showed that the bioactive compounds nanoencapsulated in sodium alginate+chitosan was nearly spherical in shape with smooth surfaces (Fig. 6c). The result indicated that using sodium alginate with chitosan for encapsulation decrease the particle size of prickly pears peels fruit. The difference in diameter of particles size observed by TEM might be a result of aggregation and swelling of nanoparticles during dispersion in aqueous solution[46].

Encapsulation efficiency (%): The encapsulation efficiency of NEE-Alg, NEE-Chi and NEE-Alg+Chi capsules were evaluated as illustrated in Fig. 7.

The encapsulation efficiency of three capsulate formulas of prickly pears peel fruit extracts was 68.52, 97.81 and 92.11% for NEE-Alg, NEE-Chi and NEE-Alg+Chi, respectively. The results showed that at the same concentration the encapsulation efficiency of peels extract formula NEE-Chi was the higher and NEE-Alg was the lowest. The difference in encapsulation efficiency was due to the well dispersion characteristics in formulas and homogeneous distribution of

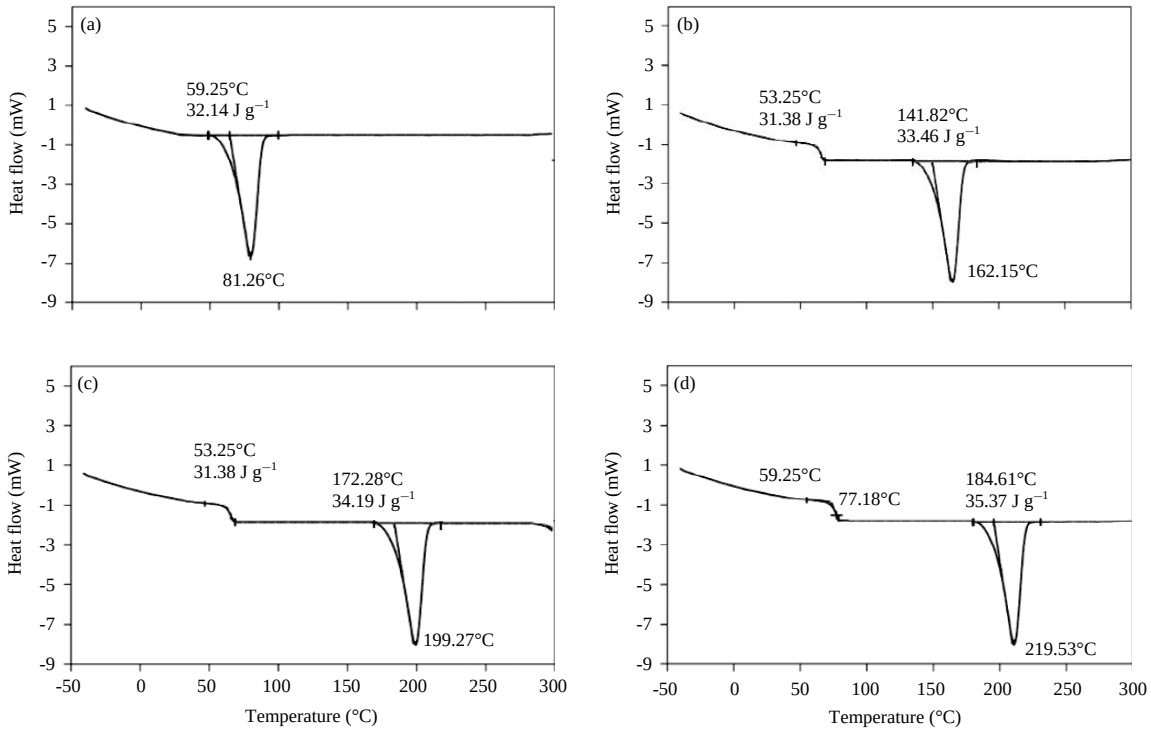

Fig. 8(a-d): Thermograms obtained by DSC for EE and its capsules formulas. Where, (a) Ethanol extract (EE), (b) Nanoencapsulate extract on sodium alginate (NEE-Alg), (c) Nanoencapsulate extract on chitosan (NEE-Chi) and (d) Nanoencapsulate extract on Alginate+Chitosan (NEE-Alg+Chi)

peels extracts into the matrix. The particle size of matrix used as encapsulation play an important factor in encapsulation efficiency as it increase the surface area or adhered it to the outer shape causing an efficiency reduction.

On the other hand, increasing the encapsulation efficiency of NEE-Chi formula might be due to the stability of emulsion droplets caused by higher amounts of surfactant.

The results showed that the prickly pears peels fruit ethanol extract was very effective in extracted the bioactive compounds than water extract. So, the researchers choose it and their encapsulate formulas to continues the experiment and application.

Thermal analysis (DSC): Thermal analysis measuring the stability of the bioactive compounds of prickly pears peels fruit ethanol extract (EE) and its nanoencapsulate formulas were studied by Differential Scanning Calorimetry (DSC).

Ethanol extracted and its nanoencapsulate formulas are established endothermic process, and the thermal analysis methods are valuable for studying themoxidation and thermostability. The induction temperature of the start oxidation process is useful in order to assess the antioxidant activity for prickly pears peel fruit extracts and nanoencapsulated formulas.

Results indicated that the temperature program was from (0-300°C) and that the point degradation for EE was 81.26°C (Fig. 8a). The encapsulation showed the withstand of bioactive compounds to high temperatures and the preserving of antioxidant activity for those compounds during their application in foods.

Results in Fig. 8b-d also showed that the point degradation for the encapsulated formulas of NEE-Alg, NEE-Chi and NEE-Alg+Chi were 162.15, 199.27 and 219.53°C, respectively. It was observed from the results that the encapsulated formula sodium alginate+chitosan gave high endothermic peak 219°C and remained stable at it. It could be concluded that using sodium alginate+chitosan matrix for encapsulated of bioactive compounds extracted from prickly pears peels fruit is the best way for preserving their activity during food application processes.

Antioxidant activity of peel extract encapsulate formulas: The DPPH test is based on the ability of stable free radical 2,2-diphenyl-1-picrylhydrazyl to interact with hydrogen donors including phenols. The phytochemical properties (DPPH radical, reducing power and total phenolic) of ethanol extract in three formulas nano-encapsulated NEE-Alg, NEE-Chi and NEE-Alg+Chi with EE were showed in Table 1. Results in

Table 1: Phytochemical properties for three formulas of nano-encapsulated NEE-Alg, NEE-Chi and NEE-Alg+Chi

Ethanol extract formulas	DPPH radical scavenging (%)	Reducing power (μg GAE/100 g DW)	Total phenolic content (μg gallic acid equivalent/g DW)
EE	79.28±3.51[a]	458.51±11.03[a]	793.52±13.24[a]
NEE-Alg	62.18±2.18[b]	394.40±10.02[b]	758.14±11.82[b]
NEE-Chi	84.04±5.54[a]	481.76±12.02[a]	781.45±13.05[a]
NEE-Alg+Chi	80.31±3.37[a]	462.49±12.02[a]	785.02±12.84[a]

a,b: Values are significant at p = 0.05

Table 2: Sensory Evaluation of guava juice with or without additions of bioactive compounds extract encapsulate formulas of prickly pears peels fruit

Guava juice samples	Taste	Odor	Color	Mouth feel	Appearance	Overall acceptability
Guava juice with addition of NEE-Alg	7.8±0.918	7.7±1.494	8.4±1.075	7.6±1.075	7.8±0.788	7.88±0.953
Guava juice with addition of NEE-Chi	6.9±1.101	7.4±1.074	7.8±0.919	6.9±1.370	7.3±1.059	7.26±0.869
Guava juice with addition of NEE-Alg+Chi	6.9±1.449	6.7±1.337	6.8±0.919	6.8±1.686	6.8±1.316	6.70±1.124
Guava juice with addition of EE	7.4±1.075	7.4±1.074	7.6±1.429	7.2±1.229	7.7±1.251	7.44±1.153
Guava juice without any additions	7.2±1.686	7.5±1.433	7.9±1.595	7.6±1.265	8.1±1.197	7.66±1.369

Table 1 showed the DPPH radical scavenging increased from 79.28% in EE to 84.04% NEE-Chi formula after nano-encapsulation process, followed by NEE-Alg+Chi formula being 80.31% and the least was in NEE-Alg formula being 62.18% depending on the extracts concentration. The NEE-Chi formula showed differences significantly ($p<0.05$) with the DPPH radical, which may be due to their ability for donating the electron.

No significant differences were observed in radical-scavenging activity between ethanol extract (EE) and nanoencapsulate formula NEE-Alg+Chi of prickly pears peels fruit at 15 mg mL^{-1} concentration. The DPPH radical scavenging activity of NEE-Alg of prickly pears fruit peels at the same concentration was also significantly less than other formulas.

Results of reducing power activity of various extract formulas of prickly pears peels fruit were shown in Table 1. Results showed that the reducing power for all encapsulated formulas ranged from 394.40-481.76 μg GAE/100 g DW and that the higher was in NEE-Chi and the lower was in NEE-Alg encapsulated formula with different significantly. It has been reported that the reducing power of bioactive compounds ethanol extract of prickly pears peels fruit and their encapsulation formulas (NEE-Chi and NEE-Algi+Chi) is probably due to their hydrogen donating ability and which might explain its higher amount of chitosan than in other formulas. Total phenolic compounds content in prickly pears peel fruit extract may contribute directly to the antioxidant action; therefore, it is necessary to investigate total phenolic content after encapsulation formulas.

Results in Table 1 showed the effect of different encapsulation formulas on total phenolic contents of prickly pears peels fruit ethanol extract. The initial level of total phenolic in EE was 793.52 mg/100 g (on dry weight basis). Nanoencapsulation of peel extract in sodium alginate (NEE-Alg) losses 5.01% of total phenolic compounds.

Application of peel extracts nanoencapsulate formulas in guava juice: The addition of bioactive compounds extracted of prickly pears peel fruit and nanoencapsulated formulas to guava juice to enhance antioxidant activity were studied.

Guava fruit was exposed to elevated temperatures during the preparation and manufacture of juices (especially during pasteurization and sterilization), and different conditions of light and oxidation, which affect their natural antioxidants.

So, this study aims to add natural antioxidants in the encapsulation formulas extracted from prickly pears peel fruit to guava juice and subjected to pasteurization process, to protect it from high temperatures during manufacturing.

Actually, the major of bioactive compounds in guava juice are carotenoids, especially β-cartene and lycopene in pink varieties[47,48] was affected by thermal coefficients processing and decreased by 40% after pasteurization at 87°C for 90 sec[49].

So, it was necessary to application in guava juice by the addition of natural antioxidants of prickly pears peel nanoencapsulated formula to protection from oxidation and increases the shelf life for guava juice.

Sensory evaluation of guava juice: The addition of bioactive compounds extracted from prickly pears peel fruit and nanoencapsulated formulas to guava juice to enhance its antioxidant activity were tested by sensory evaluation (Table 2).

Sensory evaluation attributes of variance samples showed no significant differences in color, taste, odor, mouth feel and overall acceptability between guava juice and guava juice supplemented with peel extract and both guava juices supplemented with NEE-Alg and NEE-Chi. But, addition of NEE-Alg+Chi to guava juice was significantly affected the taste, odor, color, mouth feel, appearance and overall acceptability being 6.9, 6.7, 6.8, 6.8, 6.8 and 6.7, respectively, compared to other samples of guava juice.

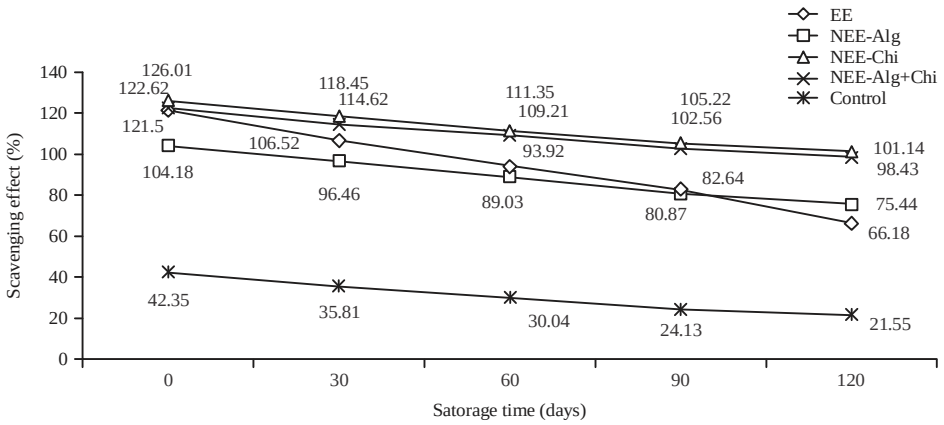

Fig. 9: Effect the storage stability of DPPH radical scavenging activity in guava juice contain peels ethanol extract nanoformulas

In general, consumer perception accepted the addition of 0.1% for both peel extract concentrate and nanocapsuleted formulas to guava juices.

Application of prickly pears peels fruit extracts and its nanoencapsulated formulas in guava juice to improve the ability of antioxidants was not demolished during pasteurization and sterilization operations and therefore continues to retain its activity for a long period up to high temperatures. Guava juice supplemented with NEE-Alg formula was the most acceptable by testers, receiving the largest number of indications for the responses 'liked quite a lot' and "liked very much". While, guava juice supplemented with NEE-Alg+Chi formula was receiving the largest number of responses 'disliked very much', for a product to be considered acceptable.

The results focused on the beneficial effects of prickly pears peels fruit extract and of its nanoencapsulated formulas. The data suggested that these phytochemicals could be effective in improving human health, can decrease the body oxidative stress in healthy individuals[50,51] and considered as functional food.

Storage stability of guava juice: The results of storage stability (120 days) of guava juice supplemented with bioactive compounds extract formulas are shown in Fig. 9. Radical scavenging activity of prickly pears peel fruit extracts was expressed as total antioxidant capacity and compared with guava juice without any addition as a control. Using the DPPH assay, the results of total antioxidant capacity of both guava juice samples contained EE and NEE-Alg stored at room temperature (23°C) decreased over 120 days storage period from 121.5-66.18 and 104.18-75.44%, respectively.

The highest content of total antioxidant in guava juice samples was obtained with NEE-Chi followed by that with NEE-Alg+Chi formula, but there was no difference significant between them during the storage period.

So, the nanoencapsulation have the ability to protect the bioactive compounds extracted from prickly pears peel fruit during different storage period (120 days). The best results were noticed in guava juice with NEE-Chi formula.

CONCLUSION

This study confirmed that the traditional methods for prickly pears peel fruit extracts as waste and their nanoencapsulation formulas contained precious amount of antioxidant potential and could be replaced the synthetic ones. These results undertaken together showed that the extract of prickly pears peels fruit are promising feedstock for phytochemical compounds, and extracting these bioactive compounds constitutes a way among others for its valorization. The nano-encapsulation process of bioactive ingredients extracted from prickly pears peels fruit was successful to maintain their activity. The supplementing these nanoencapsulates in guava juice explained the ability of these of packaging materials to withstand the high temperatures and pasteurization especially NEE-Alg+Chi formula. This suggested that the use of encapsulating materials chitosan with sodium alginate was more able to protect those bioactive ingredients other than each material separately.

SIGNIFICANCE STATEMENT

This study aimed to investigate the effect of nanoencapsulation delivery systems on the antioxidant of Egyptian prickly pears peel fruit (*Opuntia stricta* fruit) as by-products by using different polymers such as sodium alginate (Alg), chitosan (Chi) and combination between sodium alginate and chitosan (Alg+Chi) using ultrasonic homogenization method. The impact of this study indicated that nanoencapsulation affects positively the bioactive compounds extracted from prickly pears peels fruit, improves

the antioxidant activity, enhancing the quality and stability of bioactive compounds. This suggested that the use of encapsulating materials chitosan with sodium alginate was more able to protect those biactive ingredients other than each material separately. This study indicated that prickly pear peel fruit are promising feedstock for phytochemical compounds and their protection by nanoencapsulation are successful in maintaining their activity in guava juice.

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