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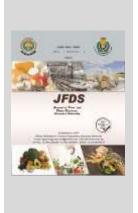
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## Utilization of Prickly Pear Peels As Antioxidants, Dietary Fiber and Functional Ingredients for Production of A Healthy Yoghurt Drink

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This study aims to evaluate the bioactive compounds content, anti-oxidant activity of prickly pear peels (PPP), which showed high levels of these components and studied the effect of adding Prickly Pear Peels (PPP) as a functional ingredient on physicochemical, total phenols, total flavonoids and sensory properties of skimmed-yoghurt drink during 14 days of refrigerated storage. Skim milk without PPP as a control was inoculated with yoghurt cultures, while PPP was used at 0.5, 1 and 1.5 % for the preparation of three trials of skimmed yoghurt drink inoculated with yoghurt culture as well. The application of PPP apparently reduced the fermentation time of inoculated skimmed milk in comparison to control. Viscosity was enhanced in yoghurt drink with PPP and developed during storage. In addition, the color and flavor intensity were improved in sense of measured sensory parameters, acetaldehyde and diacetyl contents, respectively as well as the acceptability by panelists of yoghurt drinks with added PPP when compared to control yoghurt drinks. Moreover, PPP increased the level of total phenols and flavonoids of yoghurt samples. The chemical attributes were also affected by the addition of PPP into yoghurt drinks in terms of moisture, proteins and lipids and ash contents. PPP decreased pH of yoghurt drinks in comparison to control samples. The microbial population and activity in yoghurt drinks with PPP was higher and depended on PPP level. Panelists favored yoghurt drinks with PPP and considered 1 % PPP as the best level added to yoghurt drinks.

Key words: Prickly Pear Peels, skimmed yoghurt drink, viscosity, total phenols.

### INTRODUCTION

*Prickly Pear Peels (PPP)*, the waste produced from the huge consumption of Prickly Pear fruit which represent about 40-45 % of the whole fruit; It could be an edible and functional ingredient in dairy based products due to its valuable components that promote good health such as dietary fiber, phytochemicals, biologically active compounds and functional carbohydrates (Dimou *et al.*, 2019; Gheribi *et al.*, 2019; Hernández-Carranza *et al.*, 2019).

The biologically active contents such as total phenols, total flavonoids, carotenoids, betalains and essential oils in PPP, which provide higher antioxidant capacity and coloring agents, are the main functional compounds present in PPP. They can prevent disorders and diseases associated with eating habits and might have therapeutic characteristics and contains antiviral. antibacterial, antifungal, cancer-fighting, and antiinflammatory properties (Otálora et al., 2015). In addition, PPP has several industrial applications due to its higher content of water soluble pigment (betalain) in the form of betalamic acid, which can be applied as natural colorant of foods (Abou-Elella and Ali 2014). Also, high quality polysaccharide "mucilage" which is a heteropolysaccharide found in PPP, is associated with the biologically active compounds and antioxidant activities, make PPP important for the prevention of different diseases (Otálora et al., 2015). Moreover, it is acting as a hydrocolloid with great water holding capacity, thickening agent, configuring gels

and good solubility which might contribute for improving the consistency of food products (Hernández-Carranza *et al.*, 2019; Wang *et al.*, 2020).

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Yogurt is a dairy product that is made by fermenting milk Streptococcus thermophilus and Lactobacillus delbruckii spp. bulgaricus as starter culture. It is a valuable source of proteins, minerals, vitamins and its consumption had been closely associated with the treatment of many diseases caused by pathogenic microbes, induce considerable health benefits, improving lactose digestion, lowering the acute diarrheal disorders, enhancing immune system and lowering the possibility of colon cancer (Gahruie et al., 2015; Fazilah et al., 2018). Nevertheless, mixing some natural plant components into yogurt's fortification can make it a good functional food. (Iriondo-DeHond et al., 2018). Various plant-based ingredients were evaluated and used in production of dairy fermented products such as date and orange by-products (De Toledo et al., 2018), seeds and extract of pomegranate peel (El-Said et al., 2014), waste powder of pineapple (Sah et al., 2016), herbs extract (Dabija et al., 2018), were used as source of dietary fibers, phenolic contents, antioxidants and antimutagenic agents. However, PPP powder has not been used in the production of diary functional drinks. Therefore, this study's objective was to assess the effect of PPP addition on the physicochemical, antioxidant, phenolic content and sensory properties of skimmed-yoghurt drink during its cold storage.

#### **MATERIALS AND METHODS**

#### Materials

Skimmed milk was supplied by the dairy processing unit, Faculty of agriculture, Fayoum University, Fayoum, Egypt. The starter culture, YCX-11 was supplied by dairy pilot plant, Faculty of Agriculture, Fayoum University, Fayoum, Egypt. A prickly pear (*Opuntia ficus indica*) was purchased from local market.

#### Prickly pears peels powder preparation

The fruit of prickly pears were cleaned, washed and manually peeled. The peels were cut into small pieces and steamed for 30 minutes, dried in air for 2 hours and then dried in oven at 50 °C for 72 hours as described by Habibi *et al.*, (2009). Dried peels were ground by electric blender (Kenwood ES slex, USA), then sieved to fine powder and stored at -18 °C till analysis and use.

#### **Chemical composition of PPPs**

The contents of PPP such as moisture, ash, lipids, crude protein, and crude fiber were measured in triplicate, according to the method described by (Horwitz 2010). Carbohydrate content (g/100 g DW) was calculated by different.

#### Functional properties of PPPs.

#### Oil (OHC) and water (WHC) holding capacity of PPPs.

OHC and WHC of PPPs, were measured as reported by Kahraman *et al.* (2018) with minor modifications. 24 mL of distilled water was added to 2 g of each sample, which was then stirred for 60 minutes at room temperature. After that, the samples were centrifuged for 20 min at 3000  $\times$  g, the supernatant was rejected and the WHC or OHC was determined as the amount of water or oil per g of dry sample (g/g db).

#### Foaming and stability's foam of PPPs:

Foaming and stability's foam of PPPs were evaluated as Lamghari El Kossori *et al.* (2000). Mcllvaine buffer are used at different pH degrees (2, 5.6 and 8).

Vol. After whipping - Vol. before whipping

#### Vol. before whipping

After 30 minutes, the foam height was measured to determine the stability of the foam.

#### Bioactive component of ppps.

#### Total polyphenols:

Volume increase (f.c) =

Total polyphenol contents (TPC) in prickly pear peels and yoghurt drinks were measured according to Folin-Ciocalteau method at 765 nm (UV-Vis spectrophotometer) (Meda *et al.*, 2005). The results were presented as mg gallic acid equivalents/100g (mg GAE/100g).

#### Flavonoid contents:

Total flavonoids were determined spectrophotometrically (Meda et al., 2005) Absorbance at 510 nm. was read, and flavonoid compounds in triplicate was estimated and expressed as mg quercetin equivalents/100g (mg QE/100g).

#### Isolation and identification of phenolic fractions:

Phenolic and flavonoid fractions were determined by HPLC instrument used 100  $\mu$ g of evaporator methanolic 80:20 v/v extract dissolved in 1ml HPLC grade methanol 50%, filtered through PTFE filter with pore size 0.2  $\mu$ m.

#### Determination of antioxidant activity:

The free radical scavenging assay of different extracts of PPP was estimated using DPPH assay (Juntachote and Berghofer 2005). At 517 nm, the samples' absorbance was measured. The formula below was used to compute the radical-scavenging activity, which was given as a percentage of inhibition:

#### % inhibition = [(A control – A sample) / (A control)] × 100. Where, A is absorbance at 517nm.

The concentration of the test sample required to inhibit 50% of the DPPH is known as the IC50, and it was determined by the use of a linear regression analysis.

#### Anti-radical power ARB= 1/ IC50

#### **Determination of carotenoids:**

In a dark bottle, 10 gram of powdered prickly pear peels are combined with 30 ml of 85% acetone and let to stand for 15 hours at room temperature. The sample is then purified through filter paper into a 100 ml volumetric flask, and complement to volume with 85% acetone solution. The absorbance was measured by spectrophotometer (at 440, 644 and 662 nm). The following formulas were used to spectrophotometrically estimate the carotenoids pigment:-

#### Chlorophyll a = $(9.784 \times E \ 662)$ - $(0.99 \times E644)$ Chlorophyll b = $(21.426 \times E644)$ - $(4.65 \times E662)$ Carotenoids = $(4.695 \times E440) - 0.268$ (chl. a + chl. b)

The carotenoids content was calculated using the aforementioned equation described by Askar and Treptow (2013). Then calculated as mg/100g.

#### **Betalains Content:**

Betalain content of prickly pear peel powder was measured at a wavelength of 535 nm after being diluted with distilled water, and the results were represented as mg betalains/100g (Javanmardi *et al.*, 2003).

#### Ascorbic acid:

Ascorbic acid was determination the method described by Askar and Treptow (2013).

#### Mineral analysis:

%x100

Minerals (Na, K, Ca, Mg, Cu, and Fe) were determination using MPAE spectrometer (Agilent 4100 MPAES, USA) after the digestion in an  $H_2$  SO<sub>4</sub>, HNO<sub>3</sub> and HClO<sub>4</sub> mixture. Results were represented as mg/100g of dry weight (DW) (Stintzing *et al.*, 2001).

#### **Production of yogurt drink**

Yoghurt was manufactured according to Wang et al. (2020). Skimmed milk was heat treated at 85 °C for 15 min, then cooled to 42 °C and inoculated with 1.5 % yoghurt starter culture. Milk was divided into four equal portions. The first one left as control without PPP and three other portions were mixed with 0.5, 1 and 1.5 % (w/w) of PPP respectively. Yoghurt samples were incubated at 42 °C. Samples were stored at refrigerator for further analysis at 1, 7 and 14 days intervals.

#### Physico-chemical properties of yogurt

pH of yoghurts were measured using pH meter (model Kent EIL 7020, UK).

#### Apparent Viscosity:

Viscosity of yogurts was measured at  $25 \pm 2$  °C using Brookfield digital viscometer (Middleboro, MA 0236, USA) with a spindle No, 4 with 50 rpm. Triplicates were recorded/ sample and expressed as (Cp.s) centipoise according to Srisuvor *et al.* (2013).

pH:

#### Acetaldehyde and diacetyl:

Acetaldehyde and diacetyl were determined using a spectrophotometer according to the method used by Lees and Jago (1969). A reaction between semicarbozide and acetaldehyde in yoghurt samples was present; Semicarbozone was measured at 224 nm. The reaction between creatinine and  $\alpha$ - naphthol, the color intensity was determined at 550 nm.

#### **Chemical composition of Yoghurt**

Yoghurt samples were gently stirred to obtain a homogenous mixture. Then samples analyzed for their moisture, fat, protein, pH and ash according to AOAC (2019).

#### Sensory properties of Yoghurt

Different yoghurt drinks after, 1, 7, and 14 days of storage were evaluated as described by Zaki and Naeem (2021) for flavor (10 pints), color (10 points), odor (10 points), viscosity (10 points), shape (10points) and overall acceptability (10 points). The evaluation made by 25 members of Food and Dairy Science Departments, Fac. of Agric., Fayoum University, Egypt.

#### Statistical analysis

Statistical analysis was done using SPSS program (24, IBM, USA). Statistical significance at  $\rho < 0.05$  was measured using univariate analysis of variance and tested with a Tukey test.

#### **RESULTS AND DISCUSSION**

# Proximate composition, and phytochemicals characteristics of prickly pear peels (PPPs) and skimmed milk.

#### Proximate composition prickly pear peels (PPPs):

The peels of prickly pears make up about half of the fruit and are typically thrown away, creating an environmental problem. Prickly pear peels could easily be employed as a nutraceutical and functional component in several food preparations, like dairy products, because of their high bioactive components. The chemical characteristics of skim milk and prickly pear peels were determined and the results are presented in Table 1. The results showed obviously that the moisture content of fresh and dried PPPs were 74.62 % and 8.29 % respectively, protein 4.16 %, lipid 2.66%, ash 10.27 %, and ascorbic acid 74.59 mg / 100gm . Dried Prickly pear peels had a moisture content of  $8.29 \pm 0.29\%$ , (Larrauri 1999). PPPs had a good protein and fat content of  $4.16 \pm 0.17$ % and 2.88  $\pm$  0.16 %, respectively. Moreover, PPPs demonstrated a high level of dietary fiber, value attainment of  $33.39 \pm 0.13$  %. The high ash and fiber content of PPPs offered the most pertinent finding. The mechanical properties of gluten for interactions with amino acid side groups are improved by the presence of minerals like magnesium and calcium in ash. Dietary fiber is crucial for maintaining human health. High dietary fiber diets are linked to the treatment, lowering of the risks, and prevention of various diseases. (Sehn et al., 2015). These results agreed with those reported by Anwar and Sallam (2016), they cleared that the chemical composition of prickly pear peels (on dry matter) composed of protein 8.30%, ash 12.13%, and total dietary fiber 40.80%. Higher concentrations of polysaccharides (25%) and cellulose (29%) as well as hemicellulose (8.5%) were found in prickly pear peels.

Table	1.	Proximate	composition,	and	phytochemicals
		characteris	stics of prickly	pear	peels (PPPs) and
		skimmed r	nilk.		

	Samples			
Components	Prickly pear peels	Skimmed		
	Skimmed milk	milk		
Moisture %( Fresh PPPS).		$90.11{\pm}0.02$		
Moisture % (Dry PPPS).	$8.29 \pm 0.29$	-		
Lipids % *(DW)	$2.88\pm0.16$	$0.86 \pm 0.02$		
Protein % (DW)	$4.16\pm0.17$	$2.92 \pm 0.03$		
Ash % (DW)	$10.27\pm14$	$0.81{\pm}0.01$		
Dithery Fiber % (DW)	$33.39\pm0.13$	-		
Carbohydrates % (DW)	$41.01 \pm 0.14$	-		
Ascorbic acid mg/ 100g *(WW)	$74.59 \pm 0.94$	-		
Carotenoids mg/100g	$11.05\pm0.11$	-		
Betaline mg/100g	$29.27 \pm 0.98$	-		
Lactose %	-	$5.30 \pm 0.07$		
Minerals: mg / 100g DW				
Mg	1020	-		
Ca	922	-		
Na	893	-		
К	312	-		
Fe	132	-		
Cu	42	-		

# Phytochemicals characteristics of prickly pear peels (PPPs)

#### **Total Carotenoids and total betaline:**

Carotenoids represented the major insoluble water pigments in ppps and the total carotenoid content in ppps was 11.05  $\pm$  0.11 mg /100 g of DM Table1. Pro-vitamin A, commonly known as  $\beta$ -carotene, is crucial for maintaining the health of cells. The content of Betalains found in the tested powder of PPPs was 29.27  $\pm$  0.98 mg/100 g of DM. This results agreed with Bourhia *et al.* (2020). In the other hand content of total betalain in (Aakria, Derbana, and Mles) peels ranged from  $2\pm0.69$  to 37.66  $\pm$  2.65 mg/100 g of dry matter. **Ascorbic acid** 

Opuntia spp., have a high levels of ascorbic acid ranging from 10 - 41 mg /100g dry weight, (Piga 2004). However, according to our findings observed that PPPs contained significant amount of ascorbic acid, (74.59  $\pm$  0.94 mg/100g) based on DW Table 1. These findings concur with those recognized by Stintzing *et al.* (2001), who mentioned that, the cactus pear fruit has content vitamin C about (18-23 mg/100 g fresh weight) and with the results reported by Feugang *et al.* (2006), who confirmed that vitamin C, is the third major vitamin in prickly pears fruits and was found in fruit pulp ranged from 12-81 mg/100 g fresh weight. **Minerals** 

The mineral components of PPPs are shown in Table1. Mg, Ca, and Na was found in high concentrations in the mineral analysis of PPPs were 1020, 922 and 893 mg/100 g, respectively, followed by K was (312.0 mg/100g). While Fe and Cu levels were lower recording 132.0 and 42.0 mg/100 g, respectively. As a result, prickly pear peel can contribute to covering part of the recommended dietary allowances (RDA) of these minerals. These results are agree with those obtained by Feugang *et al.* (2006).

# Physical and Functional characteristics of prickly pear peels.

Water holding capacity (WHC) and oil holding capacity (OHC): Physical and functional characteristics of PPPs

illustrated in Table 2 and Figure 1. shows that water holding capacity (WHC) and oil holding capacity(OHC) of prickly pear peels were  $3.61\pm0.19$  g H<sub>2</sub>O/ g and  $0.85\pm0.01$  g oil /g respectively. The larger concentration of hydrophilic components, such as soluble fiber that contains lignin that absorbs oil and water is likely what caused the increase in OHC and WHC in PPP. (Akubor and Badifu 2004).

Table 2. Functional properties, of prickly pear peels (PPP).

(111).	
Properties	PPP
WHC g water/ g DW	3.61±0.19
OHC g oil / g DW	$0.85 \pm 0.01$
Water activity (aw)	$0.32 \pm 0.02$
Foam power %	
pH2	$33.49\pm0.66^b$
pH5.6	$21.56\pm0.61^{\rm c}$
pH8	$41.59 \pm 0.58^{a}$

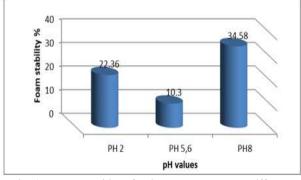


Fig. 1. Foam stability of prickly pear peels at different pH degrees.

Foam Capacity and Stability: Gas bubbles scattered in an aqueous phase are what produce foam. In comparison to other polysaccharides such flax mucilage, xanthan, and guar gums, fruit peels demonstrated good foaming power. (Kaewmanee *et al.*, 2014). The foam power (FP) of PPPS shown in Table 2. The lowest FP ( $21.56 \pm 0.61\%$ ) was produced at pH 5.6 (protein's isoelectric point), where the molecules are in a more compact state than at other pH values. FC significantly increased, especially at pH 8 reaching  $41.59\pm 0.58$ . Fig. 1

illustrated the effect of pH and time on the foam stability of the PPPS. Regardless of the mixture pH, FS progressively diminishing with time. At pH 5.6 the foam stability progressively diminishing and reached to  $21.56 \pm 0.61\%$ when the foam remained for 30 min, while at pH 2 and pH 8 the foam stability was increased ( $33.49 \pm 0.66$  and  $41.59\pm$ 0.58), respectively. This increase is due to the density of the proteins' negative charges have increased. it's would promote the unfolding of the protein molecules into more flexible structures, which form foams better than the more compact molecules that exist at pH 2 and 5.6.

#### **Bioactive Compounds of Prickly Pear Peels (PPPS)** Total Phenolic compounds

Fruits and vegetables have a large distribution of phenolic components (Li et al., 2006), which have gained significant attention because of their possible antioxidant activities and free radical-scavenging capacities, both of which are likely to have positive effects on human health. (Govindarajan et al., 2007). The potentially antioxidant characteristics of polyphenols, which play a role in health advantages such as reduction of inflammation, dysregulation of the cardiovascular system, and neurodegenerative illnesses, have sparked an increase in interest. Additionally, polyphenols have shown antitumor properties (Osorio-Esquivel et al., 2011). Tables 3 illustrates that total phenolic, flavonoids, and antioxidants activity of different solvent extracts of PPPS. Total phenolic of different extracts of Prickly pear peels ranged from 525.43 to 1441.36 mg gallic /100g DW. The highest levels of total phenolic content were significantly (p 0.05) present in methanolic and ethanolic extracts.  $(1441.36 \pm 9.05 \text{ and } 768.23 \pm 2.36 \text{ mg} (GAE) / 100$ g DW, respectively). The lowest phenolic compounds values found in water extract was 525.43± 16.83mg GAE/ 100 g DW. These results indicated that a solvent with a high polarity received a higher amount of phenol compounds. These results may be due to the fact that phenolic are often extracted in higher levels in more polar solvents such as aqueous methanol /ethanol as compared with absolute methanol/ ethanol (Sultana et al., 2009).

Table 3. Total phenolic, total flavonoid compounds and antioxidant activity of prickly pear peel flour (PPF).

Solvents	TDC mg Callia/100g	TEC ma OnE /100a	Antioxidants activity		
Solvents	TPC mg Gallic/100g	TFC mg QuE. /100g —	IC50	ARP*	
Methanol 80 %	$1441.36 \pm 9.05^{a}$	$172.09 \pm 2.82^{a}$	$3.09 \pm 0.03^{d}$	0.32±0.02 <sup>a</sup>	
Ethanol	$768.23\pm2.36^b$	$85.14 \pm 3.41^{b}$	4.83±0.19°	$0.22 \pm 0.01^{b}$	
Hexane	$656.94 \pm 4.71$ °	$64.48 \pm 2.21^{\circ}$	6.97±0.23 <sup>b</sup>	$0.14\pm0.02^{\circ}$	
Water	$525.43 \pm 16.83^{d}$	$48.04 \pm 2.16^{d}$	$9.07 \pm 0.25^{a}$	0.11±0.02 <sup>d</sup>	

# \*ARP = Anti radical power

#### Total flavonoid contents

The data in Table 3 shows the total flavonoid contents of prickly pear peels extracts. In the four extracts flavonoid concentrations varied ranging from  $48.04\pm 2.16$  to  $172.09\pm 2.82$  mg QuE. /100g. Methanol extract was observed significantly ( $\rho < 0.05$ ) the highest amounts of total flavonoid content ( $172.09 \pm 2.82$  mg QuE. /100g). This finding could be explained by the fact that highly polar methanol-water combinations exhibit increased efficacy in the extraction of polar phytochemicals such phenolic and flavonoids. (Shabir *et al.*, 2011). However, hexane and water extracts of prickly pear peels have the smallest levels of flavonoids being ( $64.48\pm 2.21$  and  $48.04\pm 2.16$  mg/100g), respectively.

#### Antioxidant activity

Antioxidant activities (% inhibition, IC50 and ARP) of different extracts of PPPs are given in Table 3 and Figure 2 (A, B, C and D). The reduction in absorbance resulting from the DPPH radical receiving an electron or hydrogen radical from an antioxidant agent and converting to a stable diamagnetic compound served as a measurement for the antioxidant activity in the DPPH scavenging assay. The capability of DPPH reduction was determined by the decrease in its absorbance at 517 nm, which is increased by antioxidants.

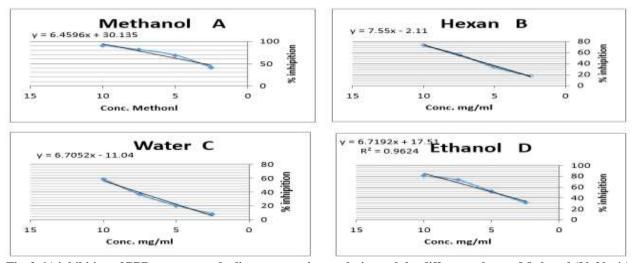


Fig. 2. % inhibition of PPPs extracts and a linear regression analysis result by different solvents, Methanol (80:20 v/v) (A), Hexane ( B), Water (C) and Ethanol (D).

All prickly pear peel extracts showed notable antioxidant activity, with values of inhibitions percentage ranging from 8.22 % to 90.97 %. The capacity of antioxidant compounds to lose hydrogen and their structural shape are both necessary for the free radical scavenging properties of extracts. DPPH radical-scavenging activity was concentration-dependent; when extract concentration increased, it did so gradually and dramatically increase. Methanol/water (80:20 v/v), extract had significantly ( $\rho <$ 0.05) the highest inhibitions % at different extract concentration ranging from 41.22 to 90.97 %. The amount of phenolic compounds present or the degree of hydroxylation of those phenolic compounds can improve antioxidant activity (Javanmardi et al., 2003). On the other hand, the lowest inhibitions % values were found for hexane and water extracts at different concentrations ranging from 8.22 to 72.92 %. As extract concentration increased, the percentage of DPPH inhibition increased from 2.5, 5, 7.5 and 10 mg /mL (Table 4and figure 2, A, B, C and d).

The concentration of the test sample required to inhibit 50% of the free radicals is given by the  $IC_{50}$  values. The  $IC_{50}$  value is a parameter exceedingly used to measure the free radical scavenging activity; a lower IC<sub>50</sub> value denotes a higher level of antioxidant (Shahidi et al., 1992). The primary flavonoids in prickly pear peels are what give them their antioxidant properties. By generating stable radicals, phenolic compounds can prevent pro-oxidative effects in proteins, DNA, and lipids. Flavonoids are more effective antioxidants than vitamins (Aires et al., 2004). The methanolic and ethanol extracts of prickly pear peels showed low IC<sub>50</sub> (3.09± 0.03and 4.83±0.19 mg /ml), respectively than hexane and water extracts (Table 3). In order to divert free radicals into more stable products and end the free radical chain reactions. the methanolic extracts of PPPS may function as electron donors and interact with the free radicals.

#### Phenolic and flavonoids constituents of PPPs

Prickly pear is an active medicinal plant and a natural cure due to its amazing chemical polymorphism, the extract examined by HPLC that represents a significant quantification of the phenolic components. Data in Table (4) showed that the methanolic extract of PPPs have shown the presence of different ingredients and compounds. The main phenolic components in PPPs extract is Benzoic acid (1482.46 µg/mg DW ) followed by Ellagic acid (1329.43µg/mg DW), p-Hydroxy benzoic acid, Vanillic acid and Ferulic acid. Gallic acid is the least phenolic compounds (9.01 µg/mg DW). In addition, the methanolic 80:20 v/v extract of prickly pear peel contains a number of flavonoids components such as myricetin, Kaempferol and Quercitin. Quercetin possesses antiproliferative, anticarcinogenic and antioxidant activities (Lou *et al.*, 2012).

Table 4. HPLC analysis of phenolic and flavonoid components of methanolic extract of Prickly near neel

pear peer.				
Commonweater	Concentration µg /100mg DW			
Components -	Phenolic	Flavonoids		
Gallic acid	9.01	-		
Catechol	31.92	-		
Quinol	64.53	-		
P-hydroxy benzoic acid	478	-		
Benzoic acid	1482.46	-		
Vanillic acid	340.24	-		
Ferulic acid	243.98	-		
Ellagic acid	1329.43	-		
Salicyalic acid	12.95	-		
Cinanmic acid	15.98	-		
Myricetin	-	395.43		
Kaempferol	-	52.85		
Quercitin	-	101.62		
Querentin		101.02		

#### Physicochemical Properties of yoghurt drinks

Table (5) represents the physicochemical properties of yoghurt drinks, like moisture, protein, fat, pH and ash. There was a significant difference ( $\rho < 0.05$ ) in the average composition of yoghurt drinks with added PPP. The moisture content of yoghurt samples varied from 88.83% to 90.06 %, while protein and fat were ranged from 2.95 to 3.19% and from 0.87 to 1.07% respectively. In addition, ash was in range 0.82 to 1.15%. The addition of PPP into yoghurt significantly ( $\rho < 0.05$ ) affected the pH values of samples during storage. There were an increase in protein, fat and ash contents of yoghurt samples enriched with PPP with a decrease in moisture content. These values also increase as the level of PPP increase due to the high level of total solid in PPP and its valuable content of protein, fat and ash. However, there were significant (p<0.05) differences between control and other yoghurt samples with added PPP.

Comple				Item		
Sample	Storage (days)	Moisture %	Fat %	Protein %	Ash %	pН
	1	90.06 <sup>a</sup> ±0.19	0.87 <sup>a</sup> ±0.03	2.95 <sup>b</sup> ±0.02	$0.82^{c}\pm0.01$	4.62 <sup>a</sup> ±0.03
С	7	90.51 <sup>a</sup> ±0.02	0.90 <sup>a</sup> ±0.03	3.05 <sup>b</sup> ±0.01	0.85°±0.02	4.51 <sup>a</sup> ±0.03
	14	90.29 <sup>a</sup> ±0.02	0.93 <sup>a</sup> ±0.05	3.08 <sup>b</sup> ±0.01	$0.88^{d}\pm0.01$	4.45 <sup>a</sup> ±0.03
	1	90.00 <sup>ab</sup> ±0.19	0.87 <sup>a</sup> ±0.03	2.97 <sup>ab</sup> ±0.02	0.87 <sup>b</sup> ±0.01	4.60 <sup>b</sup> ±0.03
T1	7	89.83 <sup>b</sup> ±0.02	$0.90^{a}\pm0.03$	3.10 <sup>a</sup> ±0.01	0.91 <sup>b</sup> ±0.02	4.40 <sup>b</sup> ±0.03
	14	89.65 <sup>b</sup> ±0.02	0.97 <sup>a</sup> ±0.05	$3.16^{a}\pm0.01$	0.97°±0.01	4.12 <sup>b</sup> ±0.03
	1	89.51 <sup>bc</sup> ±0.19	0.90 <sup>a</sup> ±0.03	2.99 <sup>ab</sup> ±0.02	0.92 <sup>a</sup> ±0.01	4.61 <sup>ab</sup> ±0.03
T2	7	89.39°±0.02	0.93 <sup>a</sup> ±0.03	3.12 <sup>a</sup> ±0.01	0.96 <sup>b</sup> ±0.02	4.35°±0.03
	14	89.15°±0.02	$1.00^{a}\pm0.05$	3.17 <sup>a</sup> ±0.01	1.03 <sup>b</sup> ±0.01	4.05°±0.03
	1	89.15 <sup>c</sup> ±0.19	0.90 <sup>a</sup> ±0.03	3.04 <sup>a</sup> ±0.02	0.97 <sup>a</sup> ±0.01	4.60 <sup>b</sup> ±0.03
T3	7	89.02 <sup>d</sup> ±0.02	0.97 <sup>a</sup> ±0.03	3.12 <sup>a</sup> ±0.01	1.02 <sup>a</sup> ±0.02	4.31 <sup>d</sup> ±0.03
	14	88.83 <sup>d</sup> ±0.02	1.07 <sup>a</sup> ±0.05	3.19 <sup>a</sup> ±0.01	1.15 <sup>a</sup> ±0.01	3.99 <sup>d</sup> ±0.03

Table 5. Some physicochemical characteristics of yoghurt drink samples during cold storage periods.

Means  $\pm$  SD for the same storage period with different lowercase letters are significantly different ( $\rho$ < 0.05). C = control yogurt drink without PPP, T1 = yogurt drink enriched with 0.5% PPP, T2 = yogurt drink enriched with 1% PPP, T3 = yogurt drink enriched with 1.5% PPP.

pH values of yoghurt samples with added PPP were lower than control yoghurts, and there was a significant difference ( $\rho$ <0.05) between control yoghurt and other yoghurt samples. The results were in line with do Espírito Santo *et al.*(2012), who found that yoghurt enriched with passion fruit had the lowest pH values and the highest was found in control. In addition, Mada *et al.*(2022) found similar results. Otherwise, pH values decreased in all yoghurt samples during storage. These might be due to the stimulation effect of PPP on the microbial growth and the production of lactic acid during storage.

#### Microbiological Properties of yogurt drinks

The microbiological properties of yogurt drinks are presented in Table 6. The bacterial growth and viability was affected significantly ( $\rho <0.05$ ) by the addition of PPP and the bacterial count was influenced by the level of added PPP when compared with control yoghurt drinks. The bacterial count at the beginning of storage period varied between  $35x10^3$  CFU/g and  $169x10^3$  CFU/g, while it was between  $59.67x10^3$  CFU/g and  $766x10^3$  CFU/g at the end of storage period. The level of bacterial growth reached its highest vale at the 14<sup>th</sup> day of storage in added-PPP yoghurt drinks when compared to control samples.

Table 6. Microbiological properties of	yoghurt drink samples during cold storage.
	Itom

Communa	Item					
Sample	Storage (days) Total viable count (TVC) CFU/g		Yeast and molds CFU/g	Coliform groups CFU/g		
	1	$35.00 \times 10^{3d} \pm 0.28$	ND	ND		
С	7	49.91x10 <sup>3b</sup> ±2.49	ND	ND		
	14	59.67x10 <sup>3d</sup> ±2.30	24x10 <sup>a</sup> ±0.89	ND		
	1	60.00x10 <sup>3c</sup> ±0.28	ND	ND		
T1	7	89.87x10 <sup>3b</sup> ±2.49	ND	ND		
	14	$350 \times 10^{3c} \pm 2.30$	ND ND 24x10ª±0.89 ND	ND		
	1	150x10 <sup>3b</sup> ±0.28	ND	ND		
T2	7	230x10 <sup>3ab</sup> ±2.49	ND	ND		
	14	499.67x10 <sup>3b</sup> ±2.30	17.00x10 <sup>b</sup> ±0.89	ND		
	1	169x10 <sup>3a</sup> ±0.28	ND	ND		
T3	7	480x10 <sup>3a</sup> ±2.49	ND	ND		
	14	766x10 <sup>3a</sup> ±2.30	12.33x10 <sup>c</sup> ±0.89	ND		

The highest viable counts of bacteria was detected in yoghurt drinks with higher level of PPP which might be due to that PPP improved the growth of bacteria in yoghurt samples. These improvements might be explained by the valuable content of PPP from amino acids, dietary fibers, carbohydrates and other nutrients, which could stimulate the bacterial growth of yoghurt samples and enhance its prebiotic effect. Our results were in line with Hamdy *et al.*(2021). Other works reported that the bacterial growth showed a significant viability increase due to the presence of dietary fiber in yoghurt samples due to its enrichment with lemon and orange fibers (Aportela-Palacios *et al.*, 2005). During the storage period, the highest viable bacterial count was determined in 1.5 % PPP yoghurt samples at the end of cold storage.

#### Apparent viscosity

The apparent viscosity of yoghurt drinks was shown in Figure 3. The lowest values of viscosity were measured in control samples, while the highest were found in yoghurt drinks enriched with 1.5 % PPP at the end of cold storage.

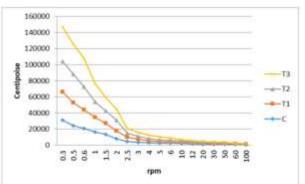


Fig. 3. Viscosity values of different yoghurt drink samples.

The higher the level of PPP, the higher viscosity value was noted. The addition of PPP % in yoghurt drink samples increased viscosity due to its content of dietary fibers, which enhance viscosity of the product as reported by Isanga and Zhang (2009).

#### Total phenols and flavonoids of yoghurt drink samples.

Table 7 showed total phenols and flavonoids of yoghurt drinks as affected by different levels of PPP. It shown

that the addition of PPP in yoghurt drinks affected the total phenolic and flavonoids contents of different yoghurt drinks. The increase of phenolic and flavonoid contents was proportional to the level of added PPP with significant differences ( $\rho$ <0.05) between control yoghurt and other treatments. This might be due to the high content of phenolics and flavonoids with its antioxidant activities. The results showed that the addition of PPP into yoghurt drinks increased

the level of phenols from 9.25 mg /100g in control yoghurt into 106.96 mg /100g in 1.5 % added-PPP yoghurt drinks. These results were in consistent with Zaki and Naeem (2021) who reported that the addition of Citrus Peels in yoghurt increased the level of phenols. In addition, these results were in line with Alqahtani *et al.* (2021) and Hamdy *et al.*(2021), who reported a relation between phenolic content and antioxidant activities of the dairy product.

Table 7. Total phenols and flavonoids of yoghurt drink samples during cold storage.

14	Treatments				
Item	С	T1	T2	T3	
Total phenols (mg gallic acid/100 g)	9.25 <sup>d</sup> ±0.94	35.83°±0.94	70.99 <sup>b</sup> ±0.94	106.96 <sup>a</sup> ±0.94	
Total flavonoids (mg QuE/100 g)	5.86 <sup>b</sup> ±3.20	15.15 <sup>b</sup> ±3.20	29.39 <sup>a</sup> ±3.20	37.93 <sup>a</sup> ±3.20	
As in table 5.					

#### Acetaldehyde and diacetyl:

It was shown in Fig 4 and Fig 5 that diacetyl and acetaldehyde were affected by the addition of PPP, which might enhance the flavour of yoghurt drink. The amount of diacetyl was higher in control sample than other samples at zero time. While after 7 days of storage, 1.5 % PPP-added yoghurt had a comparable value for control yogurt.

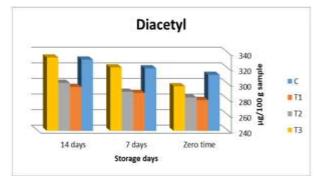


Fig. 4. Diacetyl of yoghurt drink samples during cold storage time.

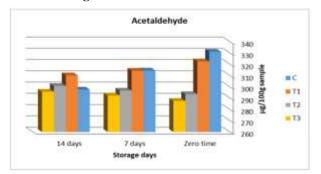


Fig. 5. Acetaldehyde of yoghurt drink samples during cold storage time.

Table 8. Sensory evaluation of yoghurt drink samples during cold storage.

This may explain the effect of PPP on the production of yoghurt flavour. For acetaldehyde, at zero time, control yoghurt had the highest value over other samples. While on the seventh day of storage, 0.5 % PPP-added yoghurt had a comparable value of acetaldehyde. At the end of storage time, control sample had the lowest value of acetaldehyde, which means that PPP improved the production of acetaldehyde in yoghurt drink samples. It was shown that the storage time had an effect on the development of acetaldehyde and diacetyl in all samples.

At the end of storage time, 1.5 % PPP-added yoghurt

drinks had higher diacetyl than other samples and control.

development of acetaldehyde and diacetyl in all samples. Acetaldehyde decreased during cold storage time in some yoghurt samples due to the conversion of acetaldehyde to ethanol by alcohol dehydrogenase (Ertekin and Guzel-Seydim 2010). Diacetyl decreased on the 7<sup>th</sup> day of storage in some yoghurt samples and increased again at the 14<sup>th</sup> day of storage. Such an effect was observed by Hussein *et al.*(2011). These results were in line with Hamdy *et al.*(2021). The development of diacetyl and acetaldehyde levels was due to the metabolic activity of starter cultures in the presence of PPP (Aljewicz *et al.*, 2020).

#### Sensory evaluation of yogurt drink samples.

The sensory scores of control and yogurt drink samples treated with different levels of PPP during storage for 14 days are presented in Table 8. The results showed that all scored improved during storage. There were no significant differences ( $\rho < 0.05$ ) between all sensory attributes in all yoghurt drink samples at zero time of storage. During storage, different sensory attributes were enhanced due to the effect of PPP on such attributes. At the end of storage time, 1 % PPP-added yoghurt sample had the highest overall score.

				0	0				
Sampla				Item					
Sample	Storage (days)	Flavor (10)	Color (10)	Odor (10)	Viscosity (10)	Shape (10)	Overall (10)		
	1	7.10 <sup>ab</sup> ±0.29	7.50 <sup>a</sup> ±0.31	8.10 <sup>ab</sup> ±0.19	7.00 <sup>a</sup> ±0.35	7.10 <sup>a</sup> ±0.30	7.00 <sup>ab</sup> ±0.34		
С	7	7.30 <sup>a</sup> ±0.23	7.40 <sup>a</sup> ±0.29	6.80 <sup>b</sup> ±0.26	7.00 <sup>b</sup> ±0.24	7.00 <sup>a</sup> ±0.22	7.20 <sup>b</sup> ±0.24		
	14	7.60 <sup>a</sup> ±0.29	7.60 <sup>a</sup> ±0.20	7.30 <sup>a</sup> ±0.27	6.80 <sup>b</sup> ±0.28	$7.40^{a}\pm0.17$	7.40 <sup>ab</sup> ±0.20		
	1	7.60 <sup>a</sup> ±0.29	7.60 <sup>a</sup> ±0.31	8.30 <sup>a</sup> ±0.19	7.30 <sup>a</sup> ±0.35	7.00 <sup>a</sup> ±0.30	7.00 <sup>ab</sup> ±0.34		
T1	7	7.50 <sup>a</sup> ±0.23	7.50 <sup>a</sup> ±0.29	7.80 <sup>a</sup> ±0.26	7.40 <sup>b</sup> ±0.24	7.50 <sup>a</sup> ±0.22	7.90 <sup>ab</sup> ±0.24		
	14	6.80 <sup>a</sup> ±0.29	7.30 <sup>a</sup> ±0.20	7.40 <sup>a</sup> ±0.27	7.40 <sup>ab</sup> ±0.28	7.10 <sup>a</sup> ±0.17	7.50 <sup>ab</sup> ±0.20		
	1	7.70 <sup>a</sup> ±0.29	7.70 <sup>a</sup> ±0.31	8.30 <sup>a</sup> ±0.19	7.30 <sup>a</sup> ±0.35	7.00 <sup>a</sup> ±0.30	7.50 <sup>a</sup> ±0.34		
T2	7	7.60 <sup>a</sup> ±0.23	7.50 <sup>a</sup> ±0.29	8.00 <sup>a</sup> ±0.26	7.60 <sup>b</sup> ±0.24	7.40 <sup>a</sup> ±0.22	8.00 <sup>a</sup> ±0.24		
	14	7.60 <sup>a</sup> ±0.29	7.30 <sup>a</sup> ±0.20	7.00 <sup>a</sup> ±0.27	7.60 <sup>ab</sup> ±0.28	$7.40^{a}\pm0.17$	7.70 <sup>a</sup> ±0.20		
T3	1	6.40 <sup>b</sup> ±0.29	7.10 <sup>a</sup> ±0.31	7.70 <sup>b</sup> ±0.19	7.60 <sup>a</sup> ±0.35	7.20ª±0.30	6.20 <sup>b</sup> ±0.34		
	7	7.80 <sup>a</sup> ±0.23	7.50 <sup>a</sup> ±0.29	8.10 <sup>a</sup> ±0.26	8.30 <sup>a</sup> ±0.24	7.60 <sup>a</sup> ±0.22	7.20 <sup>b</sup> ±0.24		
	14	7.10 <sup>a</sup> ±0.29	7.30 <sup>a</sup> ±0.20	7.40 <sup>a</sup> ±0.27	7.70 <sup>a</sup> ±0.28	7.10 <sup>a</sup> ±0.17	7.10 <sup>b</sup> ±0.20		

As in table 5.

#### CONCLUSION

In conclusion, this study of prickly pear peels indicated the presence of bioactive compounds include polyphenols, flavonoids, minerals, dietary fiber and betalains, all of which have anticancer and antioxidant activities. Therefore, it could have commercial and nutritional applications such as yoghurt drinks. The addition of PPP in yoghurt drink samples decreased the fermentation time and shortens the coagulation period. The physicochemical, sensorial properties, diacetyl and acetaldehyde were enhanced upon using PPP in the production of yoghurt drinks in comparison to control. Yoghurt drinks with PPP contained higher values of phenols and flavonoids enhancing its functionality when compared to controls. It could be concluded that the addition of 1.00 % PPP can improve the physicochemical, sensorial and functional properties of yogurt drinks.

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# إستخدام قشور التين الشوكى كمضادات أكسدة، مصدر للألياف الغذائية وكمكون وظيفى فى إنتاج مشروب زبادى صحى

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#### الملخص

الهدف الرئيسى من هذه الدراسة هوتقييم محتوى مطحون قشور التين الشوكي من المركبات النشطة بيولوجيا و مصدات الأكسدة وكذلك الخصائص الوظيفية بالأضافة الى در اسة تأثير إضافة مطحون قشور التين كمكون وظيفي على الخصائص الفيز وكميائية، محتوى الفينولات و الفلافونويدات الكلية، والخصائص الحسية لمشروب الزبادي منز وع الدسم والمخزن تحت ظروف التبريد لمدة 14 يومًا. حيث تم إضافة قشور التين بنسب 0, 0.5 , 1, 5.1 % الى اللبن الخالي الدسم . أشارت النتائج إلى تقليل ز من التخمر وتحسين لزوجة مشروب الزبادي مقارنة بالكنترول. بالأضافة الى ذلك أدت الأضافة الى تحسين الخصائص الحسية لمشروب الزبادي من حيث اللون والنكهة وكذلك حدث تحسن في محتوى مشروب الزبادي من وين الإمتار ولي بالأضافة الى ذلك أدت الأضافة الى تعسين الخصائص الحسية لمشروب الزبادي من حيث اللون والنكهة وكذلك حدث تحسن في محتوى مشروب الزبادي من حيث الأستالدهيد والداي استيل وتطور خلال فترة التخزين. بالأضافة الى القبول العالم لمشروب الزبادي واعتبروا نسبة 1 ٪ كافضل مستوى مضاف إلى مشروبات الزبادي . وأظهرت النتائج أيضا زيادة محتوى مشروب الزبادي من وطور القائر ويدات الكلية بزيادة نسب الأضافة من مطحون قشور التين. كما تترش الصفات الكيميانية بإضافة مطحون القشور إلى مشروب أيضا زيادة محتوى مشروب الزبادي من الفينولات والفلافونيدات الكلية بزيادة نسب الأضافة من مطحون قشور التين. كما تترش الصفات الكيمانية بإضافة مطحون القشور إلى مشروب الزبادي من حيث الرطوبة والبروتينات والمدن ومحتويات الرمد. وخفض الرقم الهيدروجيني لمشروبات الأربادي مقار ول. كذلك وحد أن إضافة مسحوق قشور التين الى مشروب الزبادي من حيث الرطوبة والبروتينات والمدي ومحتويا المره و التشاط بزيادة نسب الأضافة من مطحون بالأضافة الى دين ال الزبادي أدى الى زيادة مسلوبة عاليات الدها ومحتويات المولية وزاد والنشاط بزيادة من المطحون والزولي ولى نشاط القاون والخرون والذمين ول خلك الخصافي الوبادي من وول والزمان والزبادي الزبادي أدى الى زيادة نشاط الكانيات الحية ألمسئولة عن التفر وزاد هذا النشاط بزيادة نسب الأضافة الى والصفات الحسائ المسرو النون والخمال الزبادي أدى الى زيادة بي التحسن الحاد في الخواص الفرو وزاد والفافغونوية والأونية والموات الى مناط الربادي ألمن الزبادي من وول التزرين وبناءا على التحس الدائ أم مسؤول النشاط بزيلة نسب الأضافة ولى والمفات ا