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Antioxidant and α-amylase inhibition activities of prickly pears (*Opuntia ficus indica* L.) betalains extracts and application in yoghurt as natural colorants

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Abstract

The objective of the present study was to evaluate the antioxidant and α-amylase inhibition activities of four betalain extracts of red and yellow prickly pear varieties and essay incorporate in yoghurt as a natural colorant. A highest betacyanins and indicaxanthins contents were obtained with the aqueous extracts of red and yellow varieties. The methanolic extract of red variety showed a strong ferric reducing power, likewise, the aqueous extract of yellow variety revealed an important free radical scavenging activity. Furthermore, a highest α-amylase inhibition activity was obtained with the aqueous and methanolic extracts of the yellow and red varieties, respectively. A positive correlation was observed between the betalains contents and the antioxidant, and the α-amylase inhibition activities of two varieties extracts. The free radical scavenging activity of enriched natural yoghurt was decreased significantly after 28 days of storage. However, a slight changes in physicochemical analysis of yoghurt enriched compared with control samples. The betalain enriched yoghurt can substitute a synthetic (industrial) colorants.

Keywords: Prickly pears betalains, antioxidant activity, α-amylase inhibition, enriched yoghurt, natural colorants, physicochemical characteristics.

1. Introduction

Cactus (*Opuntia ficus-indica*), commonly known as prickly pear, belongs to the family Cactaceae, contain about 1500 species widely distributed in arid and semi-arid areas including the Mediterranean basin, Middle East, South Africa, Australia and India [1]. This fruit is an good source of different phytochemicals including phenolics, ascorbic acid, and a betalain pigments (betaxanthin and betacyanin) as well as further functional properties such as antioxidant and anti-inflammatory activities [2–4].

The most apparent characteristic of cactus pear fruits and flowers is the yellow and red color. This feature is exerted by betalains, which are nitrogen-containing vacuolar pigments that replace anthocyanins in most plant families of the *Caryophyllales* [5]. In addition to color, betalains have shown antioxidant, anti-inflammatory, anti-proliferative, and vascular-protective effects [6–8].

Disorders of carbohydrate metabolism cause severe health problems such as diabetes, obesity and oral diseases worldwide. Pancreatic α-amylase is an important enzyme for dietary carbohydrate digestion. Dietary interventions with plant-derived natural inhibitors for these enzymes could delay the digestion and absorption of molecules [9].

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Colorants or color additives can be used to serve several purposes in food processing, including standardizing raw ingredient colors, providing color identities to otherwise colorless foods, and accounting for loss during processing or storage [10]. The use of Opuntia sp. as colorants has several advantages such as neutral smell and/or taste, low nitrate content, a broad color range, and represents a lower risk for microbiological carry-over [11]. Red-purple powder from *Opuntia stricta* fruit juice was successfully applied as a colorant in two food model systems: a yogurt and a soft-drink [12]. Some authors have developed studies in yoghurt enriched with natural colorant and antioxidants from plant or fruit extracts such as nonacylated anthocyanins from *Berberis boliviana* L [13], white and red dragon fruit [14], callus [15], grape seed [16], wild blackberry [17], hazelnut skins [18], *Matricaria recutita* L. (chamomile) and *Foeniculum vulgare* Mill. (fennel) [19], tocopherol of mushroom mycelia [20], cantaloupe pulp [21], *Vaccinium myrtillus* L. fruits [22], anthocyanin of strawberry fruits [23], red propolis [24], and pomegranate seeds (*Punica granatum* L.) [25] extracts.

The aim of this study was to evaluate the antioxidant (ferric reducing power and free radical scavenging activity) and the α-amylase inhibition activities of four betalain extracts (aqueous, ethanolic, methanolic, and acetonic) of red and yellow prickly pear varieties as well as we performed a assays aiming to incorporate the aqueous betalains extract in yoghurt to propose a functional food product enriched with natural colorants and antioxidants.

2. Materials and Method

2.1. Plant materials

The characterization of Red and Yellow prickly pears varieties studied in the present work was reported in our previous studies [2,3].

2.2. Extraction and determination of betalains contents

Betalains (Betacyanins and indicaxanthins) determination was carried out using the method reported by *Zin et al*. (2020) [26]. A quantity of 3g was mixed with 60 mL of different extraction solvent (water, 50% methanol (MeOH), 50% ethanol (EOH), 50%, and acetone (AC)). After homogenization, the mixtures were centrifuged at 1700 g for 5 min, and filtered through a nylon filter (0.45 µm).

The betalains contents (BC) were expressed as mg/100g and calculated using the following equation:

$$BC = \frac{A \times DF \times MW \times 1000}{\varepsilon \times L}$$

Where *A* is the absorption at 532 (betacyanins) and 482 nm (indicaxanthins), *DF* is the dilution factor, *L*: path-length (1cm), *MW*: molecular weight (550 and 308 g/mol for Betacyanin and indicaxanthin, respectively), and *ε*: molar extinction coefficients (60,000 l/mol.cm for betacyanins and 48,000 l/mol.cm for indicaxanthins).

2.3. Antioxidant activities

The ferric reducing power (FRP) was measured according to the method of *Oyaizu* (1986) [27] and the DPPH free radical scavenging activity (FRSA) was measured according to the procedure described by *Brand-Williams et al.* (1995) [28]. The results were expressed as mg AAE/100g FW for FRP and as a percentage (%) for FRSA.

2.4. α-amylase inhibition activity

The α-amylase inhibition activity was evaluated according to the method reported by *Boudjelthia et al.* (2017) [29]. The betalains extracts were concentrated and reconstructed in buffer solution (0.02M, pH=6). A volume of 200 µl of each extracts was mixed with 500 µl a-amylase solution (1%). After incubation for 10 min at 25 °C, 200 µl of substrate solution (potato starch mixed with phosphate buffer solution 0.02M, pH=6) was added to the mixture. The reaction was stopped by adding 600 µl of DNSA (1g/40ml) and boiling at 95 °C for 5 min. The absorbance was measured 540 nm. The mixture of substrate solutions and extracts without α- amylase was used as control. The results were expressed as a percentage and calculated using the following equation:

$$\text{inhibition } (\%) = \frac{(Ac - Ae)}{Ac} \times 100$$

Where: Ac: Absorbance of control; Ae: Absorbance of extract

2.5. Incorporation of betalains extract in yoghurt

The aqueous betalain extract of red variety was added immediately in yoghurt formulation in order to improve their aspect.

Different concentration (2%, 4%, 6%, and 8%) of betalain extracts were incorporated in natural stirred yoghurt without synthetic additives. The manual homogenisation was performed during few seconds (45 s) until we obtained a homogenous structure of yoghurt with red color.

2.6. Enriched yoghurt analysis

An aliquot of 2 g of yogurt was mixed with 20 mL of distilled water. The agitation was carried out by magnetic stirring (Agimatic-S, P-Selecta, Spain) at 800 rpm for 5 min. After centrifugation (Sigma 2-16 P Centrifuge, Germany) at 24869g for 5 min, the extract was subsequently filtered (13 µm, Grade F1001, Chem,Barcelona, Spain). Physico-chemical characteristics including pH, acidity, viscosity, syneresis degree, and density and free radical scavenging activity (DPPH) of yoghurt samples were determined immediately after baking and also after 8, 14, 21 and 28 days of storage at 4°C.

2.7. Physico-chemical analysis of enriched yoghurt

Hydrogen potential (pH) measurements were performed using pH meter (Metrohm model 692, Herisau, Switzerland) at 20°C. The titrable acidity was determined through titration with 0.1 N standardized NaOH in the presence of phenolphthalein. Briefly, 9 g of yogurt sample was put into a 100 mL of distilled water. This solution was titrated until end point (pH = 8.2 ± 0.1) Yogurt viscosity has been determined by viscometric REOTEST RV. Synereses index of yogurt included the separation and dosing the quantity of the exudate whey. A quantities of 10g yogurt samples were placed in centrifuge tubes and centrifuged for 5 minutes at the speed of 1000 revs/min. The density was the ratio of the density of a substance to the reference substance density. A volume of 10 ml of yogurt is put into a 10 ml graduated cylinder and the volume is weighed using an electronic balance.

2.8. Statistical analysis

All analyses were carried-out in triplicate and the experimental data were expressed as means± standard deviation. The software STATISTICA® 5.5 was used to compare the different results by the analysis of variance (ANOVA). Differences between the means at *$P<0.05$ were considered statistically significant.

3. Results and discussion

3.1. Betalains contents

The betalains pigments are divided into two groups: the violet betacyanins and the yellow betaxanthins. Hence, the absence or presence of indicaxanthin and betacyanin derivatives govern the fruit color [6]. The results showed that the high betalains (betacyanins and indicaxanthins) contents were obtained in the aqueous extracts for the both varieties studied (figure 1). Indeed, the aqueous extract of red variety showed a high betacyanins contents with value of 829.7±2.33 mg/100g FW followed by ethanol, acetone, and methanol extracts. However, the aqueous extract of yellow variety present a high indicaxanthins contents with value of 904.8±3.55 mg/100g FW followed by methanol, acetone, and ethanol extracts. A significant difference was observed between the betalains contents of four-extraction solvent used for the both prickly pears varieties. These obtained results agreed with the study of *Castellar et al*. (2006) [30] which reported that the water extracts showed a higher levels of prickly pears pigments than other solvents. Betalains are water-soluble which facilitates their incorporation into aqueous food system [11]. Furthermore, the aqueous extraction promoted better the stability of the pigments. The indicaxanthins contents of the yellow variety studied were higher to those previously reported by *El-Gharras et al.* (2006) [31] and *Stintzing* et al. (2001) [32] with values of 723.8 and 483.0 mg/100g for Moroccan and Italian yellow prickly pear juice, respectively. In addition, ours results were higher than those obtained by Koss-*Mikolajczyk et al.* (2019) [33] who reported that the indicaxanthins contents of orange prickly pear variety were 490.44 mg/100g DW and the betacyanins contents of red prickly pears variety was 287.55 mg/100g DW. *Likewise, Dehbi et al.* (2013) [34] reported that indicaxanthins and betanins contents of Moroccan prickly pear juices were 51.33 and 52.04 mg/l, respectively. Furthermore, *Gómez-Maqueo et al.* (2019) [35] reported that betacyanins contents in purple-skinned prickly pears (Morada and Pelota varieties) were 274.4 and 435.3 µg/mL, respectively, in whole-fruit extracts, while, the Yellow and orange-skinned prickly pear extracts presented higher betaxanthins contents with rates of 72.4% to 89.1% regarding total betalains.

Likewise, Hernández-Carranza et al. (2019) [36] reported that the betalains contents of red prickly pear peel (*Opuntia ficus-indica* L.) cultivated in Mexico were ranged from 1.12 to 1.48 mg/g.

3.2. Antioxidant activities (FRP and FRSA)

For the antioxidant activity, the results showed that the ethanol extracts of red variety and the methanol extract of yellow variety present a strong ferric reducing power with values of 1209±5.55 and 1043±4.62 mg AAE/100g FW, respectively. Likewise, the aqueous extracts of red and yellow varieties present a strong free radical scavenging activity with values of 67.85±2.45 and 61.57±2.32 %, respectively, followed by methanol, ethanol, and acetone (figure 2). In addition, the aqueous extract of red and yellow varieties showed also a noticeable ferric reducing power. A significant difference ($P \leq 0.05$) was observed between the antioxidant activities of different extractions solvent used.

A strong free radical scavenging activity of aqueous extracts of both varieties studied can be explained by their higher betalains contents that contributed significantly in this activity. Likewise, a strong ferric reducing power showed by methanol and ethanol extracts can be explained by the presence of the others molecules in these extracts that exhibit an antioxidant activity. *Cai et al*. (2003) [37] reported that the betalains from plants of the Amaranthaceae presented a high antioxidant activity (DPPH) which was four times higher than ascorbic acid and rutin. In addition, *Koss-Mikolajczyk et al.* (2019) [33] reported that betalains contents of prickly pears varieties studied presented a highest antioxidant potential. Furthermore, *Gómez-Maqueo et al.* (2019) [35] reported that betanins of prickly pears from Spain variety presented a highest antioxidant activity with a value of 33.9 µmol L^{-1} Trolox equivalents.

3.3. α-amylase inhibition activity

In order to determine the effect of betalains extracts of two prickly pear varieties on the activity of α-amylase *in vitro*, we tested the effect of different extracts on the activity of the enzyme with fixing of substrate concentration constant. As showed in the results illustrated in figure 3, the aqueous extracts of yellow variety presented a strong α-amylase inhibition activity with value of 57.28% followed by methanol, ethanol, and acetone extracts, while the methanolic extract of red variety showed a strong inhibition activity with value of 43,52%. A significant difference ($P<0.05$) was observed between the α-amylase inhibition activity of the four extraction solvent. de *Souza et al.* (2015) [38] reported that prickly pear water extracts were able to inhibit a-amylase activity with rate of 37% in comparison to control samples. Furthermore, the study of *Koss-Mikolajczyk et al*. (2019) [33] confirmed that all three prickly pear varieties exhibit a good inhibitors of a-amylase enzyme with rates of 32% for the orange and red prickly pears varieties and 36% for yellow ones. Likewise, *Gómez-Maqueo et al.* (2019) [35] reported that indicaxanthins and betanins presented a 98.0 and 10.5% *α*-amylase inhibiting activity, respectively.

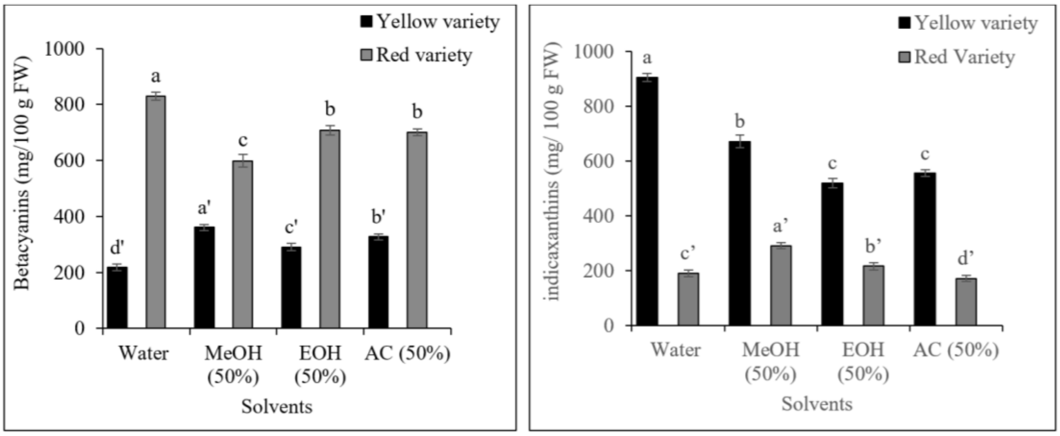


Figure 1. Betalains (betacyanins and indicaxanthins) contents of red and yellow prickly pears varieties

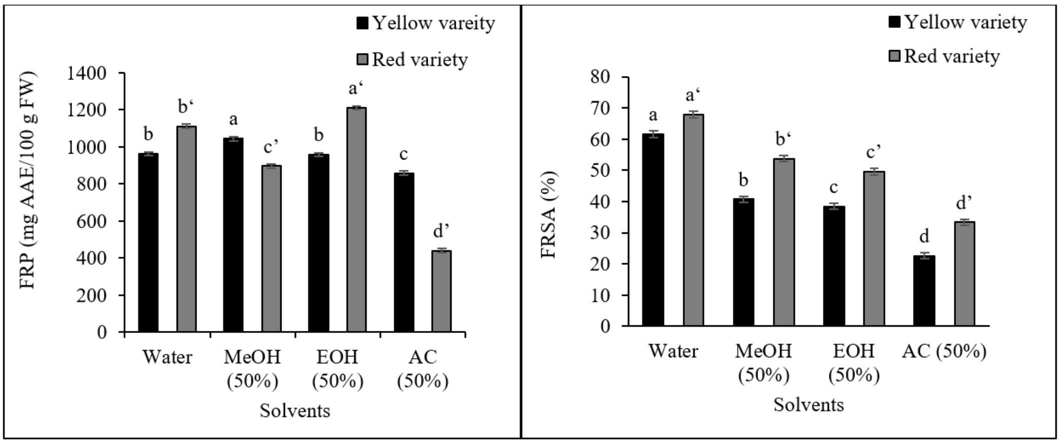


Figure 2. Antioxidant activity (FRP and FRSA) of red and yellow prickly pears betalains extracts

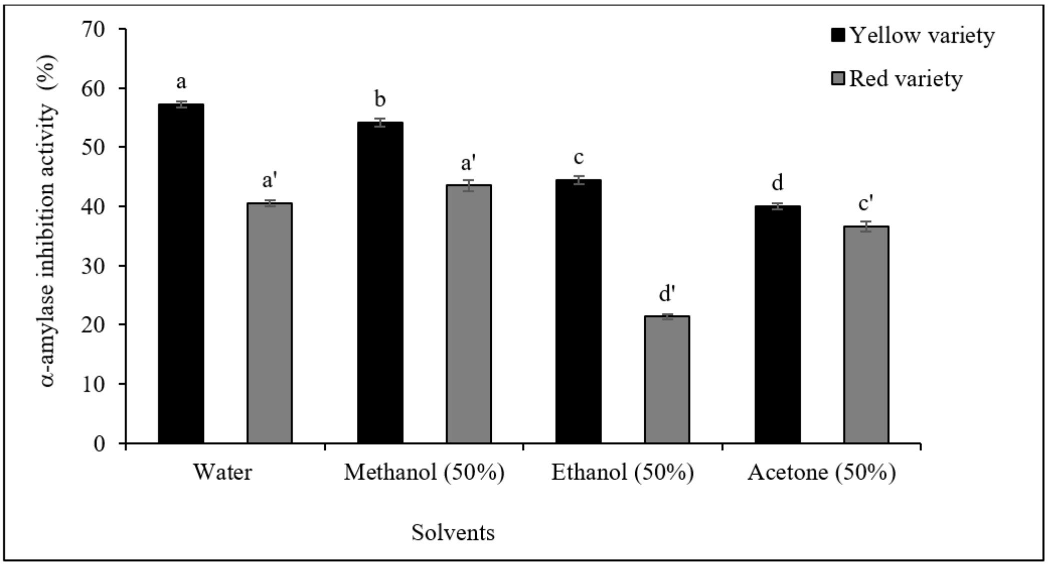


Figure 3. α-amylase inhibition activity of red and yellow prickly pears betalains extracts

3.4. Correlation

A strong correlation was observed between betalains contents of red variety and FRSA (r= 0.9263), however, a moderate correlation was noted between betalains contents and FRP and α-amylase inhibition activity (r= 0.4621 and 0.3858, respectively). For the yellow variety, a strong correlation was observed between their betalains contents and antioxidant (FRP and FRSA) and α-amylase inhibition activities with a correlation coefficient values of r=0.71, r=0.96, and r=0.86, respectively (table 1). This relationship suggested that the indicaxanthins contents might be contributors significantly in the different activities evaluated. However, betacyanins contents were contributed moderately in these activities as well as the presence of other molecules besides betalains, which contributed also in these activities. *Koss-Mikolajczyk et al.* (2019) [33] reported the betalains contents of different prickly pears verities studied were strongly correlated with the antioxidant activity tested. *Likewise, Stintzing et al.* (2005) [6] found that the betacyanins content of in prickly pears correlated better with antioxidant activity than content of betaxanthins and vitamin C.

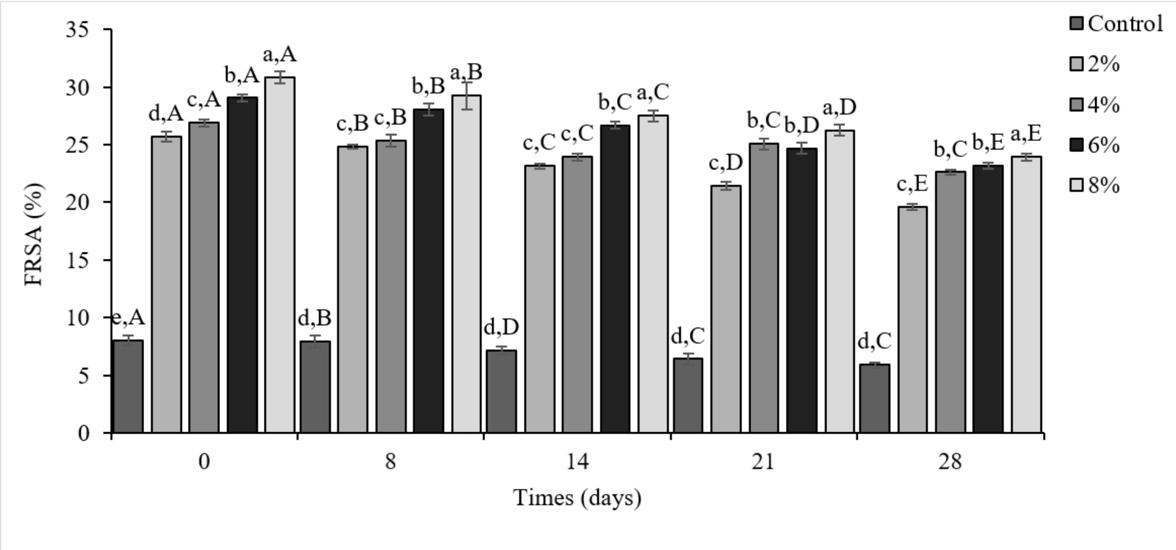
3.5. Antioxidant activity of enriched yoghurt

The free radical scavenging activity of yoghurt after incorporation with different concentration of betalains extracts during storage was illustrated in Figure 4. After baking (1 to 8 days), the results showed that the activity of yoghurt enriched with prickly pear extracts was higher (25.70 to 30.86%) than the control yogurt samples (8.69%).

However, this activity was decreased significantly (*P* < 0.05) during storage time until values of 5.90% for the control and varied between 22.61% and 23.94% for the different concentration of betalains added. The incorporation of betalain extracts was greatly improved the antioxidant activity of fortified yogurt. Furthermore, the yogurts enriched with different concentration of betalains extracts exhibited higher (*P* < 0.05) DPPH-radical scavenging rate than the controls during the whole storage time. This results was in accordance with the those obtained by many researchers for yogurt added with 8–16% sour cherry pulp [39], 40–100 mg of dry grape seed extracts [16], 40% pomegranate juice [40] and 0.25–1.0% Pleurotus ostreatus aqueous extract [41], and 1%–5% pomegranate juice powder [42]. Moreover, the significant (*P* < 0.05) decrease in DPPH-radical scavenging activity was observed during storage contrasts with results obtained by *Bertolino et al.* (2015) [18] who added hazelnut skins into yogurts, but it is in accordance with the results obtained by many other researchers [16,39,41,42]. This result is probably associated with the formation of a diffkoerent complex between different phenolic compounds and milk proteins, which might affect the total phenolics recovery and their activities [41]. Several studies describe that the antioxidant activity of yogurts was enhanced by the presence of natural extracts, for example, in studies with yogurts fortified with white and red dragon fruit [14], callus [15], grape seed [16] or with wild blackberry [17] extracts.

Table 1. Correlation between betalains contents and antioxidant activities and α-amylase inhibition red and yellow prickly pear varieties

	Yellow variety		Red variety	
	Correlation coeficient (r)	Equation	Correlation coeficient (r)	Equation
Betalains-FRP	0.7101	Y= 0.0007x + 0.4629	0.4621	Y= 0.0012x + 0.019
Betalains-FRSA	0.9658	Y= 0.1041x – 31.188	0.9263	Y= 0.0886x – 10.165
Betalains-α-amylase	0.8574	Y= 0.4668x + 16.611	0.3858	Y= 0.0255x + 17.817



a, b, c, d, and e: Significant difference (*P* ≤ 0.05) between each parameters under effect of extracts concentration; A, B, C, D and E: Significant difference (P ≤ 0.05) between each parameters under effect of storage times.

Figure 4. Free radical scavenging activity (DPPH) of yogurt incorporated by different concentration of betalains extracts during storage.

Table 2. Physico-chemical parameters of yogurt incorporated by different concentration of betalains extract during storage.

Time (days)	Parameter	Concentration				
		Control	2%	4%	6%	8%
0	pH	$4.58\pm0.01^{a,A}$	$4.54\pm0.02^{a,A}$	$4.52\pm0.09^{a,A}$	$4.51\pm0.02^{a,A}$	$4.50\pm0.08^{a,A}$
	Titrable acidity	$56.4\pm1.44^{d,C}$	$56.1\pm1.33^{d,E}$	$57.9\pm1.33^{c,E}$	$62.7\pm1.56^{b,E}$	$66.9\pm1.21^{a,E}$
	Viscosity	$2858.4\pm5.65^{a,A}$	$2166.22\pm7.34^{b,A}$	$1770.32\pm4.23^{c,A}$	$656.22\pm5.11^{d,A}$	$84.44\pm4.45^{e,A}$
	Synereses	$39.21\pm2.45^{c,B}$	$42.22\pm3.45^{b,B}$	$44.33\pm3.56^{a,B}$	$45.12\pm2.55^{a,C}$	$46.33\pm3.56^{a,B}$
	Density	$1.11\pm0.01^{a,A}$	$1.07\pm0.01^{a,A}$	$1.11\pm0.02^{a,A}$	$1.14\pm0.01^{a,A}$	$1.14\pm0.02^{a,A}$
8	pH	$4.58\pm0.02^{a,A}$	$4.52\pm0.03^{a,A}$	$4.49\pm0.01^{a,A}$	$4.51\pm0.02^{a,A}$	$4.49\pm0.02^{a,A}$
	Titrable acidity	$56.10\pm2.34^{d,C}$	$63.01\pm2.44^{c,D}$	$65.11\pm1.99^{b,D}$	$66.91\pm1.92^{b,D}$	$71.71\pm3.22^{a,D}$
	Viscosity	$2797.8\pm4.34^{a,B}$	$2042.2\pm3.54^{b,B}$	$1706.4\pm4.56^{c,B}$	$661.41\pm4.77^{d,B}$	$78.82\pm2.43^{e,B}$
	Synereses	$39.13\pm2.43^{c,B}$	$43.67\pm2.46^{b,B}$	$43.67\pm3.21^{a,B}$	$44.33\pm3.11^{a,C}$	$45.33\pm2.43^{a,B}$
	Density	$1.07\pm0.01^{a,A}$	$1.08\pm0.01^{a,A}$	$1.15\pm0.02^{a,A}$	$1.17\pm0.02^{a,A}$	$1.21\pm0.03^{a,A}$
14	pH	$4.57\pm0.01^{a,A}$	$4.51\pm0.02^{a,A}$	$4.49\pm0.03^{a,A}$	$4.48\pm0.03^{a,A}$	$4.47\pm0.04^{a,A}$
	Titrable acidity	$57.93\pm2.05^{d,C}$	$67.82\pm2.45^{c,C}$	$69.12\pm3.02^{b,C}$	$72.9\pm3.21^{a,C}$	$73.8\pm2.05^{a,C}$
	Viscosity	$2668.9\pm6.65^{a,C}$	$2001.5\pm8.23^{b,C}$	$1451.4\pm5.34^{c,C}$	$532.8\pm4.56^{d,C}$	$42.6\pm2.43^{e,C}$
	Synereses	$40.21\pm2.45^{b,B}$	$44.33\pm3.54^{b,B}$	$44.33\pm3.24^{b,B}$	$46.34\pm2.54^{a,B}$	$47.45\pm2.34^{a,A}$
	Density	$1.08\pm0.01^{a,A}$	$1.10\pm0.01^{a,A}$	$1.19\pm0.02^{a,A}$	$1.20\pm0.03^{a,A}$	$1.20\pm0.02^{a,A}$
21	pH	$4.53\pm0.03^{a,A}$	$4.44\pm0.04^{a,B}$	$4.43\pm0.02^{a,A}$	$4.42\pm0.04^{a,A}$	$4.40\pm0.02^{a,A}$
	Titrable acidity	$60.22\pm2.33^{d,B}$	$71.11\pm3.24^{c,B}$	$73.51\pm3.12^{b,B}$	$75.33\pm3.45^{a,B}$	$76.54\pm3.42^{a,B}$
	Viscosity	$2493.61\pm9.22^{a,D}$	$1869.11\pm6.44^{b,D}$	$1357.83\pm6.32^{c,D}$	$522.83\pm5.34^{d,D}$	$30.34\pm2.33^{e,D}$
	Synereses	$40.33\pm1.23^{c,B}$	$44.33\pm3.32^{b,B}$	$44.67\pm3.45^{b,B}$	$47.33\pm2.35^{a,A}$	$48.44\pm3.54^{a,A}$
	Density	$1.19\pm0.02^{a,A}$	$1.13\pm0.02^{a,A}$	$1.23\pm0.05^{a,A}$	$1.24\pm0.04^{a,A}$	$1.20\pm0.06^{a,A}$
28	pH	$4.51\pm0.02^{a,A}$	$4.39\pm0.01^{a,B}$	$4.34\pm0.02^{a,A}$	$4.29\pm0.04^{a,B}$	$4.27\pm0.02^{a,A}$
	Titrable acidity	$62.76\pm1.03^{d,A}$	75.35 ± 2.32^{cA}	$78.33\pm2.12^{b,A}$	$78.62\pm1.05^{b,A}$	$81.91\pm3.02^{a,A}$
	Viscosity	$2471.63\pm8.454^{a,D}$	$1671.51\pm7.45^{b,E}$	$1312.91\pm4.56^{c,E}$	$255.64\pm3.56^{d,E}$	$12.83\pm2.12^{e,E}$
	Synereses	$43.33\pm1.23^{c,A}$	$46.34\pm2.32^{b,A}$	$46.67\pm3.23^{b,A}$	$48.44\pm2.32^{a,A}$	$48.33\pm2.34^{a,A}$
	Density	$1.14\pm0.01^{a,A}$	$1.65\pm0.02^{a,A}$	$1.26\pm0.03^{a,A}$	$1.28\pm0.02^{a,A}$	$1.32\pm0.05^{a,A}$

3.6. Physical-chemical of enriched yoghurt

The physical-chemical of enriched yoghurt during storage was showed in the table 2. The result showed that the pH all products dropped slightly during storage independent of the betalains extract addition. Indeed, the pH values of the samples analysed were decreased from 4.5 (t= 1 day) to 4.33 (t=28 days). However, the pH of control was decreased from 4.58±0.03 to 4.5±0.01 during the storage. Generally, the yogurt products fortified by all concentration betalain extracts showed the lowest pH reduction during storage including control samples. None of the fortifications changed the parameters, which is quite important to maintain the yogurts' stability during the storage time. The pH values are also within the acceptable range and in accordance with previously published studies with the same foodstuffs [18,21,25,43]. The decrease in pH of yogurt enriched with different concentration of prickly pear during storage can be explained by the effect of lactic acid bacteria (favored by the availability of nutrients) which acidify the environment by the production of lactic acid hence the drop in pH. This can be also explained by microbial activity in fruit-added yoghurts. Yeasts also use sugar and organic acids; therefore, pH value can decrease [39].

The acidity in yoghurts enriched with betalains extracts was found higher than those of control. There were significant differences ($P < 0.05$) in titratable acidity between the control yoghurt and the yoghurts containing betalains extracts concentration of 4% and 16%. This variation can be probably due to the nature of acidity produced by betalains of prickly pear fruit added to the yoghurts. Parallel to the changes in pH, the titratable acidity of control and yoghurts enriched increased significantly during the storage time ($P < 0.05$) by increasing concentration of betalains extracts added.

These values were varied between 56.4 and 66.1 °D at t = 0 days up to 71.3 and 80 °D at t = 28 days. These values were significantly higher compared to the control yogurt which was varied between 55.8 ± 0.09 and 59.7 ± 0.2 °D. Our results are in accordance with earlier report who showed that the titratable acidity of fruit-flavoured yoghurts increased during storage [25,39]. The results were explained by the presence of organic acids (main products of lactic acid bacteria metabolism). This production of organic acids, lactic acid bacteria lowered the pH as well as increased the acidity of the medium.

The viscosity is an important factor that affects the final product quality. The obtained results showed that the viscosity of the all enriched with betalain extracts and the control were decreased significantly ($P < 0.05$) during storage. The viscosity values were decreased from 2176.3 ± 10.7 (t = 0) to 396.5 ± 8.4 (t = 28). The viscosity of the yogurts enriched was lower than the control sample it was decreased from 2867.2 ± 14.3 to 2431.1 ± 10.2 during storage time. The viscosities of the yoghurts enriched with betalains extracts were influenced by the rates of prickle pear extracts fruit addition. The increasing betalains extracts to yoghurt reduced the viscosity values of all yoghurt samples (Table 1), and it was also found to be a concentration dependent. Similar results were reported by *Celik et al.* (2006) [44] and *Tarakci* (2010) [45]. *Likewise*, *Bakirci* and *Kavaz* (2008) [46] and *Şengül et al*. (2012) [39] are reported different results compared with our results.

The syneresis of the yoghurt samples ranged from 39.6±0.05% and 43.9±0.2%. In general, during storage time, a slight increase in the syneresis values was recorded by the yogurt enriched with aqueous betalains extract of prickly pear. Likewise, the rate of syneresis of the yogurts enriched with high concentration (8%) of betalains was increased gradually over time with values varied between 42.5±0.2 and 49.1±0.08%. furthermore, the level of syneresis of control yogurt samples was increases during storage time. This increase in the syneresis of fruit-added yoghurts during storage was accordance with earlier reports [25,44]. Likewise, the syneresis values reported in this study were different than the results of previous reports [39,45,46].

The syneresis of yoghurts enriched was influenced by the rates of fruit extracts added.

The increased fruit addition also increased the syneresis values of all yoghurt samples. The acidity and syneresis increase of yoghurts when betalains extracts added could be result of high acidity content of prickly pears fruits. Fruit-added yoghurts are in general characterised by lower viscosity and higher syneresis. The addition of concentrated fruit decreases water-holding capacity of protein. Therefore, fruit flavoured yoghurt decreases the viscosity and increases the syneresis [47]. Increased separation of whey (syneresis) might be due to the breaking of yoghurt gel. *Vareltzis et al*. (2016) [48] showed that the gel matrix of the yoghurt supplemented with cucumber is broken and cannot hold the extra water, resulting in increased syneresis.

According to the results showed in the table 2, the density of yogurt was gradually increased with increasing rate of fortification and with storage time. Indeed, the density of yoghurts enriched with prickly pear extract increased from 1.08 ± 0.001D until a maximum value of 1.3±0.001 D at the end of the storage time. However, the control yogurt samples was characterized by the lower density which varied from 1.08±0.002 to 1.1±0.003 during storage time. The increase in density cab be explained by the increase in the total dry extract of the yogurt which results from the incorporation of the food matrix. This enrichment leads to an increase in the concentration of phenolic compounds which forged bonds with the proteins of the mixture, thus creating a network of large particles while also causing an increase in the density of the enriched yogurts.

4. Conclusion

The present study revealed that all extract of two varieties showed a high betalains contents with remarkable antioxidant potential. Indeed, a high betacyanin and betaxanthins contents recorded for the aqueous extract of red and yellow varieties, respectively. For the antioxidant activity, the ethanolic extract of red variety and the methanolic extract of yellow variety reveled a strong ferric reducing power. However, a strong free radical scavenging activity was obtained with the aqueous extracts of two varieties studied. Furthermore, the results showed that the aqueous and methanolic extract of yellow and red varieties presented a high α-amylase inhibition activity, respectively. A positive correlation was observed between the betalains contents and various activities evaluated, which indicated the contribution of these pigments on these activities.

The incorporation of betalains extract in yogurt was performed to substitute synthetic colorants used in the food industry and a new functional yogurt with orange-yellow natural colorants was purposed. A significant decrease of FRSA was obtained under effect of storage time and extract concentration. However, a slight changes in physicochemical analysis (pH, titrable acidity, viscosity, synereses, and density) was observed.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

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