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# EFFECT OF IN VITRO GASTROINTESTINAL DIGESTION ON ANTIOXIDANT POTENTIAL OF THREE PRICKLY PEAR VARIETY EXTRACTS

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The aim of this study was to evaluate the effect of in vitro gastrointestinal digestion on the phenolic amounts and their antioxidant potential of three prickly pear variety extracts. The total phenolic compounds (phenolic, flavonoid, and proanthocyanidin) contents were assessed as well as their antioxidant activities (total antioxidant capacity, ferric reducing power, and DPPH free radical scavenging activity) were evaluated before and after digestion. Our results showed that before digestion, the yellow variety possesses high phenolic and proanthocyanidin contents with values of 3176±18 mg GAE/100 g and 90.3±9.8 mg CE/100 g, respectively. However, the red variety has high flavonoids content with a value of 1638±6 mg QE/100 g. Antioxidant activities showed similar trend that phenolic compounds. During the digestion, the antioxidant potential of digested extracts decreased significantly ($P<0.001$) compared to undigested ones. Hence, this potential increased significantly ($P<0.01$) from the oral to the intestinal phases. The statistical analysis revealed a moderate correlation between phenolic compounds and antioxidant activity. Hence, IVGID affects the antioxidant potential of extracts, but pH and enzymatic changes do not affect their gut bioaccessibility.

**Keywords:** prickly pears, in vitro gastrointestinal digestion, phenolic compounds, antioxidant capacity

Cactus (*Opuntia ficus-indica*), commonly known as prickly pear, belongs to the family *Cactaceae* containing about 1500 species widely distributed in arid and semi-arid areas including the Mediterranean basin, Middle East, South Africa, Australia, and India (Griffith, 2004). In our country, the prickly pears are consumed as fresh fruit or used for preparing a traditional jam. This fruit is a good source of different phytochemicals including phenolics, ascorbic acid, and a mixture of betaxanthin and betacyanin pigments as well as further functional properties such as antioxidant and anti-inflammatory activities (Chaalal et al., 2013, 2015; Cejudo-Bastante et al., 2014).

Phenolic compounds are part of the human diet, being present in a broad range of commonly consumed fruit, vegetables, and plant-derived products. These compounds have to be released from the matrix and modified in the gastrointestinal tract to become accessible for absorption in the intestine (Carbonell-Capella et al., 2014). However, the bioavailability and stability of these compounds in digestion and absorption process affect greatly their health benefits. Hence, the in vitro gastrointestinal digestion is commonly used as an approach to obtain information on the release of phenolic compounds from the food matrix and their stability under digestive conditions (Wang et al., 2016).

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The effect of in vitro gastrointestinal digestion on phenolic compounds has been studied in numerous fruit such as apple (Bouayed et al., 2011), fig (Kamiloglu & Capanoglu, 2013), blueberry (Correa-Betanzo et al., 2014), strawberry grape (Granese et al., 2014), pomegranate (Gullon et al., 2015), and carob (Ydjedd et al., 2017). Nevertheless, to our knowledge, there is only one study regarding the intestinal bioaccessibility of polyphenols and antioxidant capacity of pulp and seeds of cactus pear, which is the investigation of Rez-Moreno and co-workers (2011). Thus, the objective of this work was to evaluate the effect of in vitro gastrointestinal digestion steps (oral, gastric, and intestinal) on phenolic compounds of extracts of three prickly pear varieties and their antioxidant capacities.

## 1. Materials and methods

### *1.1. Plant material*

The characterization of three prickly pear fruit (*Opuntia ficus-indica* L. Miller) used in the present work was reported in our previous study (Chaalal et al., 2013).

### *1.2. Extraction procedure*

The extraction procedure was done as indicated in our previous study (Chaalal et al., 2013) with slight modifications. The extraction solvent was evaporated and the remaining aqueous phase was lyophilized. The phenolic extracts were stored at 4 °C until analysis.

### *1.3. In vitro gastrointestinal digestion (IVGID)*

The in vitro gastrointestinal digestion of samples consists of a three steps procedure (oral, gastric, and intestinal). On the stock solutions of different digestion phases the procedure of digestion was performed according to the method described by Ydjedd and co-workers (2017).

### *1.4. Phenolic compounds*

Total phenolic contents (TPC) were estimated according to the method of Singleton and Rossi (1965). However, total flavonoid contents (TFC) were determined according to the method Quettier-Deleu and co-workers (2000). Likewise, condensed tannin contents (CTC) were measured by butanol–HCl assay (Maksimovic et al., 2005). The results were expressed as milligram gallic acid, quercetin, and cyanidine equivalents per 100 grams dry weight for TPC, TFC, and CTC, respectively.

### *1.5. Antioxidant activities*

The total antioxidant capacity (TAC) of the extracts was evaluated by the phosphor-molybdenum method as described by Ramalakshim and co-workers (2008). The ferric reducing power (FRP) was measured according to the method of Oyaizu (1986). DPPH free radical scavenging activity (FRSA) was measured according to the procedure described by Brand-Williams and co-workers (1995). The results of all activities tested were expressed as milligrams of ascorbic acid equivalents per 100 grams (mg AAE/100 g).

### *1.6. 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 *: $P<0.05$, **: $P<0.01$, or ***: $P<0.001$ were considered statistically significant.

## 2. Results and discussion

### *2.1. Phenolic compounds*

Total phenolic, flavonoid, and condensed tannin contents of extracts of three prickly pear varieties before and after in vitro gastrointestinal digestion (IVGID) are showed in Figure 1A, B, and C, respectively. The results showed that the TPC, TFC, and CTC decreased significantly ($P<0.001$) after the in vitro digestion for the three extracts in comparison to the undigested ones. These results are in agreement with those reported by Granese and co-workers (2014) in their study on the variation of polyphenol content in strawberries after simulated gastrointestinal transit. In addition, Bouayed and co-workers (2011) showed that the flavonoid contents during digestion of four apple varieties were lower than in undigested extracts. In addition, the phenolic compounds (TPC, TFC, and CTC) showed significant differences between the digestion phases (oral, gastric, and intestinal).

Before digestion, the three extracts of prickly pear varieties studied revealed high phenolic contents with values of 3175±18 mg GAE/100 g for the yellow variety followed by red and red-yellow ones with values of 2821±18 and 2139±4 mg GAE/100 g, respectively. Likewise, during digestion, the phenolic contents increased significantly ($P<0.01$) from the oral (405.70±3.76 mg GAE/ 100 g for red-yellow variety) to intestinal phases (907.8±10.7 mg GAE/100 g for yellow variety).

Regarding the total flavonoid contents, the results showed that before digestion, the TFC varied between 1415±13 (red-yellow variety) and 1638±6 mg QE/100 g (red variety). Significant difference ($P<0.05$) was observed between the flavonoid amounts during digestion phases for the three varieties studied. Indeed, the values varied between 110.1±5.7 mg QE/100 g in the oral phase for the red variety and 449.9±5.5 mg QE/100 g in the intestinal phase for the red yellow one.

Concerning the condensed tannin contents, the results showed the same pattern as phenolic and flavonoid contents. Before digestion, the condensed tannin contents varied between 77.48±2.51 mg CE/100 g (red variety) and 90.28±9.83 mg CE/100 g (yellow variety). During gastro-intestinal digestion phases, the CTC also increased significantly ($P<0.01$) from the oral to intestinal phases.

Low values of phenolic compounds in the oral phase (after 2 min of digestion) can be explained by the low solubility of these compounds in salivary fluid and the short period of this step. However, the high values could be due to more contact between the gastric, intestinal mediums and phenolic extracts (2 hours of digestion in each phase). In addition, the intestinal environment includes enzymes that hydrolyze the bonds between phenolic compounds and micronutrients that leads to their release. Ydjedd and co-workers (2017) found increase in phenolic and flavonoid contents of carob pulp in the gastric phase. The low pH of the gastric phase (pH=3) influenced the release of condensed tannins, which led to an increase in their content. The pancreatic environment of the intestinal phase does not influence the amount of condensed tannins but it has an effect on their structure.

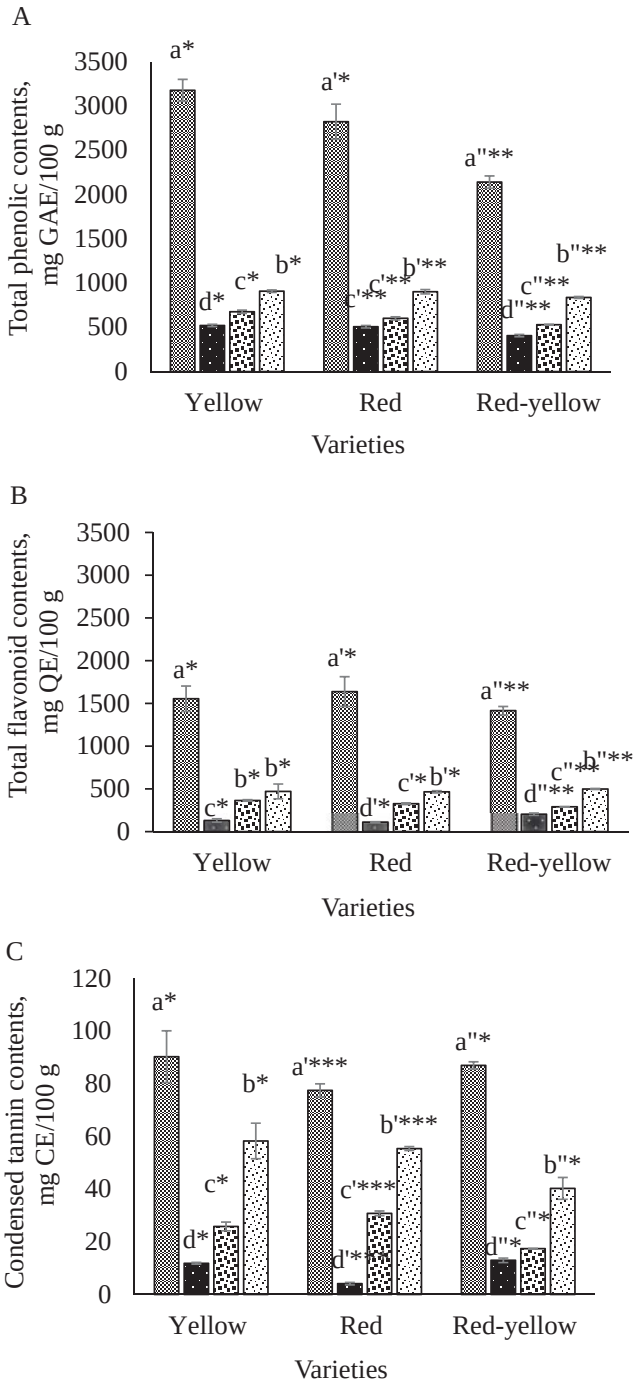


*Fig. 1.* Changes in the total phenolic (A), flavonoid (B), and condensed tannin (C) contents of extracts of three prickly pear varieties before and after IVGID.
▨: Before digestion; ▩: oral phase; ▦: gastric phase; □: intestinal phase
Columns marked with the same letter do not differ significantly at *: P<0.05, **: P<0.01, or ***: P<0.001

### *2.2. Antioxidant activities*

Changes in the antioxidant activities of three prickly pear extracts before and after in vitro gastrointestinal digestion are showed in Table 1. The antioxidant activities tested decreased significantly ($P<0.001$) after the digestion process in comparison to the values of undigested extracts. Before digestion, a high total antioxidant capacity (TAC) was observed for the yellow variety (795.2±49.1 mg AAE/100 g) followed by the red and red-yellow varieties with values of 653.6±29.6 and 559.9±12.1 mg AAE/100 g, respectively. However, during digestion phases, this activity increased significantly ($P<0.05$) from oral to intestinal phase for the three varieties.

*Table 1.* Changes in the antioxidant activities of extracts of three prickly pear varieties before and after IVGID

| | Before IVGID | Oral | Gastric | Intestinal |
|---|---|---|---|---|
| | | Yellow variety | | |
| TAC (mg GAE/100 g) | $795.2^{a}$±49.1 | $82.72^{c}$±3.0 | $113.6^{c}$±7.7 | $218.3^{b}$±6.8 |
| FRP (mg AAE/100 g) | $3545^{a}$±48 | $735.8^{c}$±5.8 | $871.9^{b}$±6.8 | $921.2^{b}$±15.1 |
| FRSA (mg GAE/100 g) | $668.8^{a}$±16.2 | $44.65^{d}$±0.6 | $82.9^{c}$±1.4 | $149.4^{b}$ ± 2.3 |
| | | Red variety | | |
| TAC (mg GAE/100 g) | $653.6^{a}$±29.6 | $74.01^{c}$±6.3 | $104.4^{c}$±2.9 | $215.5^{b}$±11.3 |
| FRP (mg AAE/100 g) | $2801^{a}$±95 | $294.3^{c}$±24.7 | $603.3^{b}$±11.4 | $670.0^{b}$ ±17.7 |
| FRSA (mg GAE/100 g) | $669.4^{a}$±3.8 | $43.91^{d}$±0.8 | $86.64^{c}$±2.9 | $152.1^{b}$±1.3 |
| | | Red-yellow variety | | |
| TAC (mg GAE/100 g) | $569.6^{a}$±50.7 | $64.0^{c}$±5.0 | $104.2^{b,c}$±3.6 | $172.6^{b}$±9.4 |
| FRP (mg AAE/100 g) | $3427^{a}$±39 | $416.3^{c}$±8.9 | $709.0^{b}$±21.9 | $779.0^{b}$ ±14.8 |
| FRSA (mg GAE/100 g) | $639.8^{a}$±17.5 | $41.2^{d}$±0.2 | $81.4^{c}$±3.8 | $152.0^{b}$±0.1 |

IVGID: In vitro gastrointestinal digestion; TAC: total antioxidant capacity; FRP: ferric reducing power; FRSA: DPPH free radical scavenging activity; values marked with same letters in a row ate not significantly different at ($P<0.05$)

Regarding ferric reducing power (FRP), the results showed that the FRP varied between 2801±139 (red-yellow) and 3545±48 (yellow variety) mg AAE/100 g. Nevertheless, during digestion, this activity increased in the following order: oral phase > gastric phase > intestinal phase. In addition, FRP can reach up to 921.2±15.2 mg AAE/100 g (yellow variety) in the intestinal phase.

Concerning DPPH free radical scavenging activity (FRSA), no significant differences were recorded between the extracts of the three varieties before and after digestion. However, during digestion, the FRSA increased significantly ($P<0.05$) from oral phase (41.18±0.20 mg AAE/100 g) to the intestinal phase (152.1±1.4 mg AAE/100 g).

The antioxidant properties might change due to the contents and chemical transformations of the phenolic compounds after and during the gastrointestinal digestion. According to Moran and co-workers (1997), the effect of the pH could be different for various polyphenols; hence, at neutral pH, some phenolics have displayed pro-oxidant activities, whereas at lower pH others have exhibited antioxidant activities. The study of Chen and co-workers (2016) on nutraceutical potential and antioxidant benefits of several fruit seeds in an in vitro digestion reported that FRP values decreased after the duodenal phase. Correa-Betanzo and co-

workers (2014) mentioned that FRSA of blueberry extracts increased significantly ($P<0.05$) after gastric phase. Furthermore, the increment in antioxidant activity could be attributed to higher release of bioactive compounds with scavenging properties (Gullon et al., 2015). Likewise, Rice-Evans and co-workers (1996) reported that the chemical structure of phenolics plays a pivotal role in the free radical-scavenging activity.

Correlation analysis was carried out to explore the relationships between different phenolic compounds and the antioxidant activities measured in extracts of three prickly pear varieties (Table 2). A moderate correlation was observed between TPC, TFC, and CTC and antioxidant capacity. This relationship indicates that phenolic compounds contribute to antioxidant activity. The flavonoids alone, with some structures, can act as donors of protons or electrons, which explains the good correlation (Rice-Evans et al., 1996). Chaalal and co-workers (2013) also showed a strong correlation between the phenolic compounds and antioxidant activity. However, a low or a negative relationship indicates that the extracts contained other compounds, such as E and C vitamins, which exhibit this activity stronger.

*Table 2.* Correlation between total phenolic compound contents and antioxidant activities

| | Before IVGID | Oral | Gastric | Intestinal |
|---|---|---|---|---|
| TPC-TAC | –0.99 | 0.93* | 0.89 | 0.99* |
| TPC-FRP | 0.99* | 0.36 | 0.61 | 0.16 |
| TPC-FRSA | –0.86 | 0.99* | 0.27 | –0.53 |
| TFC-TAC | –0.99 | –0.78 | 0.89 | –0.98 |
| TFC-FRP | 0.99 | –0.05 | 0.61 | 0.05 |
| TFC-FRSA | –0.86 | –0.92* | 0.27 | 0.34 |
| CTC-TAC | 0.39 | –0.16 | 0.17 | 0.99* |
| CTC-FRP | –0.25 | 0.62 | –0.25 | 0.22 |
| CTC-FRSA | –0.28 | –0.43 | 0.93* | –0.58 |

IVGID: In vitro gastrointestinal digestion; TAC: total antioxidant capacity; FRP: ferric reducing power; FRSA: DPPH free radical scavenging activity; CTC: condensed tannin content
*: significantly different

## 3. Conclusions

The present study revealed that the phenolic compounds and the antioxidant activities of three prickly pears varieties extracts studied were significantly ($P<0.001$) decreased after IVGID. While, a high concentration of phenolic compounds and a strong antioxidant activity were noted before digestion. However, after digestion, the phenolics and the antioxidant activities increased significantly ($P<0.01$) from the oral phase to intestinal phases. A moderate correlation was observed between phenolic compounds and antioxidant activities tested. Hence, IVGID affects phenolic amounts and their antioxidant capacity, but pH and enzymatic changes do not affect their intestinal bioaccessibility.

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