Effect of Enzymatic Treatment on Polyphenolics Content in Prickly Pear *(Opuntia ficus indica)* Juice

Reham Makhdoom, Ginat El-Sherif and Maha Ahmed Hijazi

Food and Nutrition Department, Faculty of Home Economies College, King Abdul Aziz University, Jeddah, KSA
ginat.elsherif@yahoo.com, bal_gibi@windowslive.com

Abstract: Cactus fruits (*Opuntia ficus indica L.*), commonly called prickly pear or nopal cactus belongs to the dicotyledonous angiosperm *Cactaceae* family, a family that includes about 1500 species of cactus. *Opuntia ficus indica* is found in tropical and subtropical plant areas. This plant is pointed out as relevant health promoting food with a great number of potentially active nutrients, the fairly high sugar content and low acidity of the fruit give it a delicious, sweet taste. This work aimed to study the effect of enzymatic treatment on polyphenols content in prickly pear juice. The results showed that, prickly pear juice had a high amount of phenolic compounds. HPLC-analysis for prickly pear juices (before and after enzymatic treatment) were carried out for identification of phenolic compounds showed that, all phenolic compounds were increased by enzymtic treatment, except vanillic, ferulic and cinnamic, it were decreased by the treatment prickly pear juice before enzymatic treatment was contained a high amounts from pyrogallol (3.767 mg/100g), catechol (2.270 mg/100g), vanillic (1.182 mg/100g), e-vanillic (2.969 mg/100g), and benzoic (1.956 mg/100g). Also, The major flavonoid compounds in prickly pear juice before the enzymatic treatment were luteo.6-arbinose 8-glucose (3.775 mg/100g) and luteo.6-glucose 8-arbinose (1.091 mg/100g). While, the major compounds after the enzymatic treatment were luteo.6-arbinose 8-glucose (9.723 mg/100g), luteo.6-glucose 8-arbinose (1.646 mg/100g) and A pig.6-glucose 8-e-rhamnose (1.527 mg/100g). All flavonoid compounds were increased by the enzymatic treatment, except, A pig.6-rhamnose 8-glucose, narengin, quercetrin and apegenin.
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Keyword: Cactus fruits- Prickly pear- Antioxidants- Enzymatic treatment- HPLC analysis.

1. Introduction

Today the world appears to be increasingly interested in the health benefits of foods and has begun to look beyound the basic nutritional benefits of foodstuff to disease prevention. Fruits and vegetables are essential to the human diet as it is a source of energy, minerals, vitamins and antioxidants. Diets rich in such products reduce incidence and mortality by cancer, cardiovascular and chronic disease caused by oxidative stress **(Zheng and wang, 2003).**

Cactus fruits (*Opuntia ficus indica L.*), commonly called prickly pear or nopal cactus belongs to the dicotyledonous angiosperm *Cactaceae* family, a family that includes about 1500 species of cactus. *Opuntia ficus indica* is found in tropical and subtropical plant areas. This plant is pointed out as relevant health promoting food with a great number of potentially active nutrients, the fairly high sugar content and low acidity of the fruit give it a delicious, sweet taste **(Fatima, *et. al*., 2014**).

Antioxidants content

Antioxidants are compounds that can delay or prevent the oxidation of lipids or other molecules. This plant is considered one of the health promoting. This medicine is done by inhibiting the initiation or propagation of oxidative chain reactions. Plants are potential sources of natural antioxidants and certain species are particularly significant because they may be used for the production of raw materials or preparations containing photochemical with significant antioxidant capacities and health benefits (**Exarchou, *et. al*., 2002**).

The anti-oxidative effect is mainly due to phenolic compounds, such as flavonoids, phenolic acids, tannins and phenolic diterpenes (Pietta, 2000). They interfere with the oxidation process by reacting with free radicals, chelating catalytic metals and scavenging oxygen. The effect of juices on antioxidant activity could be a result of the types of polyphenolics they contained (**Cai, *et. al*., 2003**). Many natural flavonoids have considerably higher antioxidant potentials than nutrient antioxidants, such as vitamin C (ascorbic acid) and vitamin E and dietary antioxidants, such as carotenoids (**Fatima, *et. al.*, 2014).**

All parts of the cactus plant are rich in members of the polyphenol family such as various flavonoids and phenolic acids. In the fruit pulp total phenol content is 218.8 mg/ 100g **(Fernández-López, *et. al*., 2010).** Cactus pear fruits are a rich sources of flavonols. These compounds can only be found in the fruits peel, while the fruits pulp provides no flavonols **(Galati, *et. al.*, 2003)**.

This work aimed to study the effect of enzymatic treatment on polyphenols content in prickly pear juice.

2. Materials and Methods

Materials:

Cactus pear fruit (*Opuntia ficus indica Mill.*) collected during the season (September 2015) from Al-Mandah farm in Al-Taif, Kingdom of Saudi Arabia (KSA). All chemicals purchased from Faris Althaghar Establishment Co. Jeddah, Saudi Arabia. Pectinase enzyme and carboxy methyl cellulose stabiliser purchased from Acmatic for chemicals and lab. equipments. Cairo, Eygpt.

Preparation of prickly pear juice:

The cultivar used in this study was prickly pear in orange color. Only fruits without external injuries were selected, washed under tap water and the peel were removed manually after removing of uncoloured sides (top and bottom) prior to grinding. Fruit pulps (with seeds) were storage in plastic boxes in deep freezer (Hisense, type H340US) at -18°C until use. Fruit pulps were blended for 5 seconds in a blender (Braun, type JB 3010) then separated the seeds from the full pulp. Two portions of the juice were analyzed, the first portion was non-enzymatic treatment juice and the second portion was enzymatic treatment juice by pectinase enzyme in 2 mg per 1 L. of prickly pear juice for 4 h in room temperature. After enzymatic treatment, the juice was filtered twice using strainer. Both were pasteurized at 90°C for 5 min. Then, it were storage at 4°C until analysis.

Methods:

Determination of total phenolic compounds:

Total soluble phenolic content in prickly pear juice (before and after enzymatic treatment) were determined with Folin-Ciocalteu reagent according to the method of (**Slinkard and Singleton, 1977**) using gallic acid as standard phenolic compound. Briefly, 1.0 ml of extract solution containing 1.0g extracts in a volumetric flask was diluted with distilled water (46 ml). One mil Litter of Folin-Ciocalteau reagent added and the content of the flask mixed thoroughly. Three minutes later, 3 ml of Na_2CO_3 (2%) was added, and the mixture was allowed to stand for 2 hrs with intermittent shaking, the absorbance was measured at 760 nm. The concentration of total phenolic compounds in the samples extracts were determined as microgram of gallic acid equivalent using an equation obtained from the standard gallic acid graph: Absorbance=0.0028 x gallic acid (mg).

Separation of polyphenols and flavonoids by HPLC:

High performance liquid chromatography (HPLC) technique using HPLC Agilent 1100 series equipped with Quaternary pump, set at flow 1 mL/ min. Autosampler, degaser, column compartment set at 35°C and variable wavelength detector set at 330 nm for flavonoid compounds and 280 for phenolic compounds, column: Hypersil ODS 5µm, 250x4 mm was used. Pure phenolic compounds: gallic, pyrogallol, Protocatchouic, 4-Amino Benzoic acid, Chrorogenic, Catechol, Catechin, Epi-catechin, Caffeine, P-OH-Benzoic, Caffeic, Vanillic, Chicoric, Ferulic, Isoferulic, E-Vanillic, Resveratrol, Ellagic, Alpha-coumaric, Benzoic, 3,4,5 Methoxycinnamin, Salicylic, Coumerin, P-Coumaric and Cinammic and pure flavonoid compounds: Acacetin, Luteo.6-arbinose 8-glucose, Luteo.6-glucose 8-arbinose, Apig.6-arbinose 8-glactose, Apig.6-rhamnose 8-glucose, Apig.6-glucose 8-rhamnose, Luteolin, Luteo.7-glucose, Naringin, Rutin, Hespirdin, Rosmarinic, Quercetin, Quercetin-3-O-glucoside, Rosmarinic, Kaemp.3,7-dirhamnoside, Apig.7-0-neohespiroside, Apig.7-glucose, Kaemp.3-(2-p-coumaroyl) glucose, Rhamnetin, Kaempferol, Hespirdin, Apegenin and Hespirtin were used as standard obtained from El-Gomhoria-chemical company, Egypt. This work was carried out in the Food Technology Research Institute, Agric. Res. Center, Giza, Egypt. according to **Pascale, *et. al.*, (1999)** and **Mattila, *et al.*, (2000).**

Results and Discussion:

Determination of total polyphenolic compounds in prickly pear juice before and after enzymatic treatment:

The favourite fruit prickly pear has a delicious taste and characterised aroma. In order to estimate the nutritive value of such popular fruits, analyses of the major constituents were carried out.

Table (1) and fig. (1) show the results of total polyphenolic compounds, total phenols and total flavonoids content. As seen, prickly pear juice after enzymatic treatment has a high amounts from total phenols (2.97 mg/ml of juice), total flavonoids (0.31 mg/ml of juice) and total polyphenols (3.28 mg/ml of juice). While the same juice before enzymatic treatment recorded (2.66, 0.29 and 2.95 mg/ml of juice) as total phenols, total flavonoids and total polyphenols, respectively. These results were agreement with **El-Sayed, *et. al.*, (2014).** They reported that the juice of red cactus contain high total phenolic contents equivalent to 1152.97 to 1065.15 mg gallic acid equivalent /100 ml Juice.

The total flavonoids of red cactus had 0.29 mg/ml before the enzymatic treatment. These result is not agreement with Yeddes, *et. al.,* (2013). The result difference may be due to the differences in climate and soil factors. While, the total flavonoids of red cactus was 0.31 mg/ml.

Identification of phenolic compounds in prickly pear juice before and after enzymatic treatment by HPLC:

HPLC-analysis for prickly pear juices (before and after enzymatic treatment) were carried out for identification of phenolic compounds. Figures (2 and

3) shows that chromatograms of the two extracts of prickly pear juice and table (2) show the amount of the identified phenolic compounds and its retention time. As seen, all phenolic compounds were increased by enzymtic treatment, except vanillic, ferulic and cinnamic, it were decreased by the treatment prickly pear juice before enzymatic treatment was contained a high amounts from pyrogallol (3.767 mg/100g), catechol (2.270 mg/100g), vanillic (1.182 mg/100g), e-vanillic (2.969 mg/100g), and benzoic (1.956 mg/100g). While, the same juice after the enzymatic treatment was contained a high amounts from 4-Amino-benzoic (13.36 mg/100g), catechol (4.072 mg/100g), epicatachin (1.229 mg/100g), ellagic (1.126 mg/100g), e-vanillic (5.117 mg/100g), and benzoic (3.135 mg/100g). From the same table, there are some compounds in the prickly pear juice befor the enzymatic treatment with low amounts such as epicatachin (0.775 mg/100g), and elllagic (0.250 mg/100g), which increased highly by the enzymatic treatment. It became (1.229 and 1.126 mg/100g) for epicatachin and ellagic compounds, respectively. On the other hand, some phenolic compounds had slightly high amount in the juice before the enzymatic treatment such as vanillic (1.182 mg/100g), Ferulic (0.284 mg/100g), and cinnamic (0.081 mg/100g), it were decreased by the enzymatic treatment. It were recorded (0.242, 0.117 and 0.015 mg/100g) for the three compounds, respectively.

Table (1): Determination of total polyphenolic compounds in prickly pear juice before and after enzymatic treatment

Components (mg/ml)	Juice before enzymatic treatment	Juice after enzymatic treatment
Total phenols	2.66	2.97
Total flavonoids	0.29	0.31
Total Polyphenols	2.95	3.28

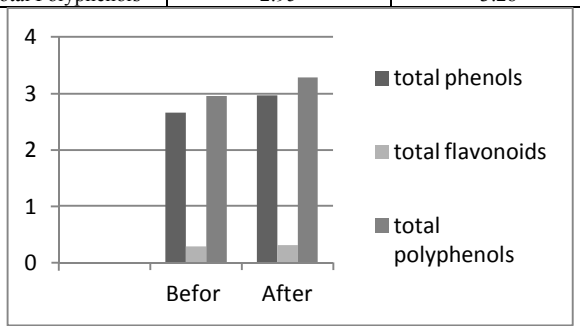

Figure (1): Determination of total polyphenolic compounds in prickly pear juice before and after enzymatic treatment

Table (2): Identification of phenolic compounds in prickly pear juice before and after enzymatic treatment by HPLC

Phenolic compounds	Prickly pear pulp juice without enzymatic treatment mg/100g	Prickly pear pulp juice with enzymatic treatment mg/100g
Gallic	12.62	0.428
Pyrogallol	3.767	13.36
4- Amino-benzoic	0.101	0.130
Protocatechuic	0.093	0.111
Catechein	0.333	0.530
Chlorogenic	0.604	0.927
Catechol	2.270	4.072
Epicatachin	0.775	1.229
Caffiene	0.284	0.329
P.oH.benzoic	0.196	0.939
Caffeic	0.141	0.206
Vanillic	1.182	0.242
P-Coumaric	0.360	0.538
Ferulic	0.284	0.117
Iso-ferulic	0.080	0.781
Reversetrol	0.046	0.084
Ellagic	0.250	1.126
e-vanillic	2.969	5.117
α-Coumaric	0.078	0.139
Benzoic	1.956	3.135
3.4.5.methoxy cinnamic	0.040	0.068
Coumarin	0.067	0.095
Salycilic	0.450	0.697
Cinnamic	0.081	0.015

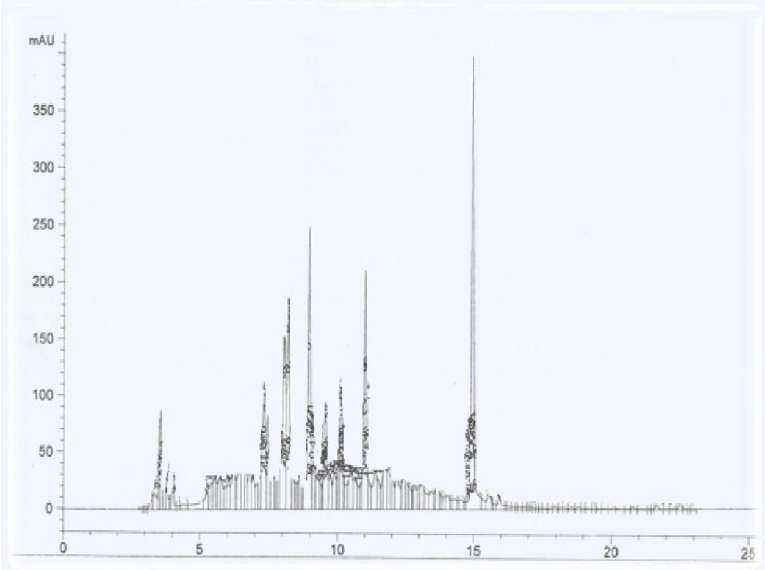

Figure (2): Identification of phenolic compounds in prickly pear juice before enzymatic treatment by HPLC

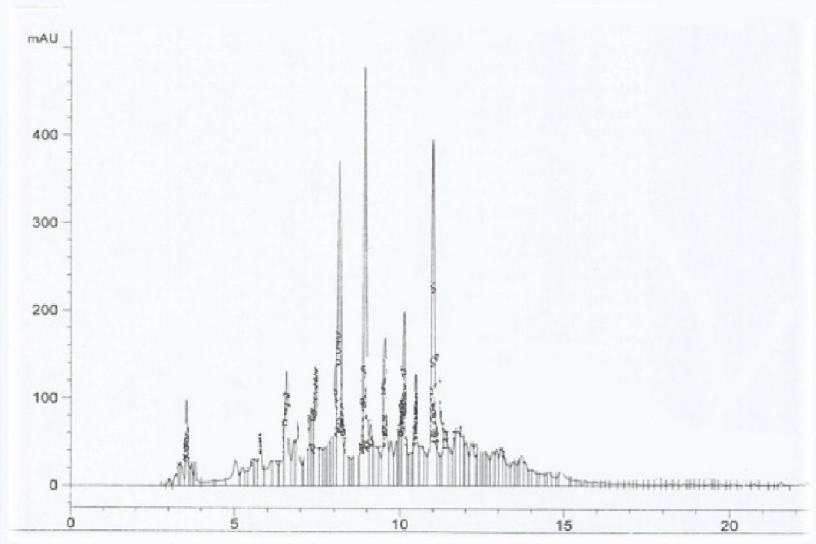

Figure (3): Identification of phenolic compounds in prickly pear juice after enzymatic treatment by HPLC

HPLC- identification of flavonoid compounds in prickly pear juice before and after enzymatic treatment:

Data illustrated in figures (4 and 5) and presented in table (3) show the HPLC-chromatograms for the two prickly pear juice (before and after enzymatic treatment) and representing the identified flavonoid compounds monitored at 330 nm. prickly pear juice after treatment were contained 23 flavonoid compounds. According to data in table (3), only A pig.6-glucose 8-e-rhamnose (1.527 mg/100g) was found in prickly pear juice after the enzymatic treatment. The major flavonoid compounds in prickly pear juice before the enzymatic treatment were luteo.6-arbinose 8-glucose (3.775 mg/100g) and luteo.6-glucose 8-arbinose (1.091 mg/100g). While, the major compounds after the enzymatic treatment were luteo.6-arbinose 8-glucose (9.723 mg/100g), luteo.6-glucose 8-arbinose (1.646 mg/100g) and A pig.6-glucose 8-e-rhamnose (1.527 mg/100g). All flavonoid compounds were increased by the enzymatic treatment, except, A pig.6-rhamnose 8-glucose, narengin, quercetrin and apegenin.

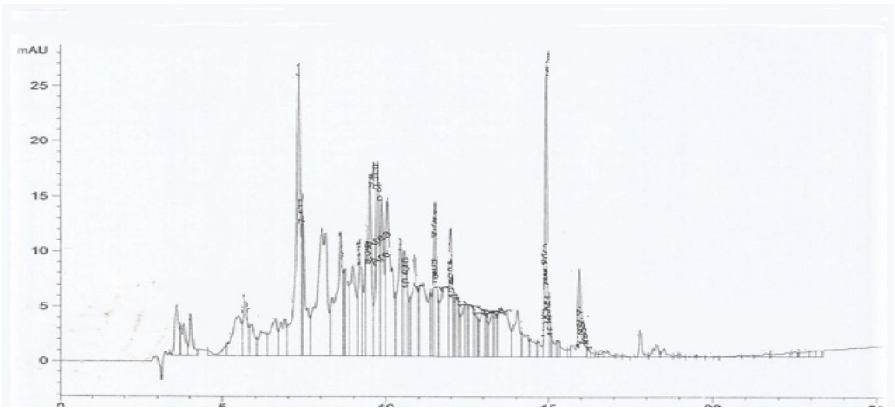

Figure (4): Identification of flavonoids compounds in prickly pear juice before enzymatic treatment by HPLC

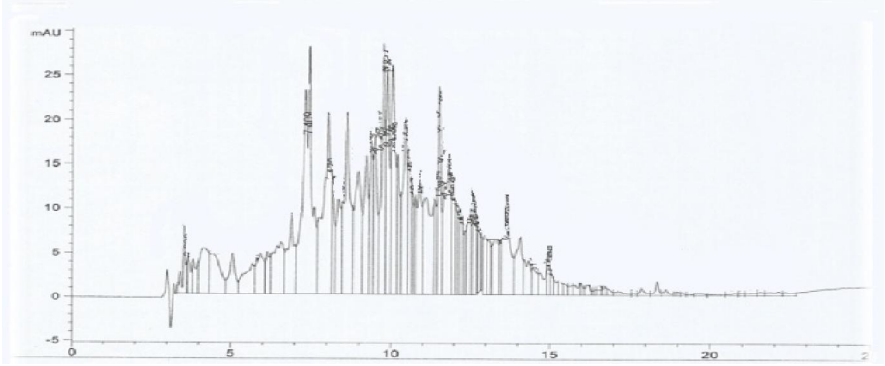

Figure (5): Identification of flavonoids compounds in prickly pear juice after enzymatic treatment by HPLC

Table (3): Identification of flavonoid compounds in prickly pear juice before and after enzymatic treatment by HPLC

Flavonoids compounds	Prickly pear juice without enzymatic treatment mg/100g	Prickly pear pulp juice with enzymatic treatment mg/100g
Luteo. 6-arbinose 8-glucose	3.775	9.723
Luteo. 6-glucose 8-arbinose	1.091	1.646
A Pig. 6-arbinose 8-glactose	0.329	0.537
A Pig. 6-rhamnose 8-glucose	0.415	0.375
A Pig.6-glucose 8-e rhamnose	-	1.527
Luteolin	0.229	0.277
Luteol. 7-glucose	0.058	0.127
Narengin	0.381	0.289
Rutin	0.080	0.211
Quercetrin -3-O-neohespiroside	0.045	0.079
Hisperidin	0.449	0.782
Rosmarinic	0.022	0.053
Kacmp. 3,7-dirhmoside	0.061	0.209
A Pig. 7-O-neohespiroside	0.031	0.118
A Pig. 7-glucose	0.040	0.106
Quercetrin	0.035	0.038
Quercetin	0.324	0.047
Kamp. 3,(2-p-coumaroyl)glucose	0.157	0.302
Hespertin	0.024	0.054
Kampferol	0.005	0.003.
Rhamnetin	0.003	8.03
Apegenin	0.004	3.12
Acacetin	0.056	142.41

Conclusion

The juice from red prickly pear has potent antioxidant activity. It showed the highest amount of phenolic compounds. From our data, prickly pear juice could be used in various industrial forms such as juice, powder (dry juice), jam, jelly, ice cream ...etc. as a good food supplement. Prickly pear juice could be used as a powerful natural antioxidants to prevent human health against degenerative disease and could be an important additives for functional foods.

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