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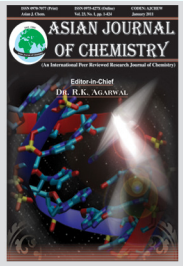

HPLC-Determination of Phenolic Composition and Antioxidant Capacity of Cactus Prickly Pears Seeds

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The aim of this investigation is to compare the total polyphenols, total tanins, total flavonoids and antioxidant activities determined by three assays (total antioxidant capacity, DPPH, reducing power) of cactus prickly seeds at two stages of ripening (in ripe fruit and over ripe fruit). It was found that their contents were highest in seeds of ripe fruit. The effects of two extracting methods (maceration and hydrolysis) on phenolic composition of cactus seeds were evaluated by RP-HPLC. In cactus seeds of ripe fruit, the major compound detectd in methanolic macerate was catechin (61.00 %) followed by rutin, (10.10 %). In contrast, the hydrolysis extract contained quercetin (37.00 %) as the most abundant component followed by dihydroxycinnamic acid (27.60 %) and epigallocatechin (10.90 %). For cactus seed of over ripe fruit, the mains compounds in macerate were gallic acid (44.70 %) and catechin, but in hydrolysis extract were dihydroxybenzoic acid (52.50 %), dihydroxyphenolic acid (17.30 %), epicatechin (11.30 %) and epigallocatechin (8.00 %).

Key Words: *Opuntia ficus indica*, Seeds, Polyphenols, Antioxidant activity, Ripening.

INTRODUCTION

Cactus (*Opuntia* spp.), a member of the cactaceae family, is an important forage crop for livestock in many arid and semi-arid regions of the world. It is widely distributed in Mexico and in all American hemispheres as well as in Africa and in the Mediterranean bassin[1]. Now-a-days cactus fruits are prevailing in daily consumption because of their health-promoting compounds, such as minerals, vitamins and fatty acids. However, less attention was paid on the composition and distribution of phenolic compounds.

The main objectives of this study are to determine the phenolic composition of cactus seeds lasting two stages of ripening (seeds of ripe fruit, seeds of overipe fruit) by RP-HPLC. Besides, this study was also designed to evaluate the antioxidant capacity of the seed methanolic extract. The usefulness of the findings of the present work in the invistegation of new source of bioactives substances especially phenolic compounds from cactus seeds which considered as by-products and their variation lasting ripening was also discussed.

EXPERIMENTAL

Butylated hydroxytoluene (BHT) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma. Folin-Ciocalteu reagent and sodium azide were purchased from Aldrich. Ammonium molybdate and authentic standards of phenolic compounds were purchased from Sigma and Fluka. Stock solutions of these compounds were prepared in HPLC-grade methanol. These solutions were wrapped in aluminium foil and stored at 4 °C. All other chemicals used were of analytical grade.

Cactus prickly pear used in this study originated from an orchard in the center of Tunisia from El ALA (Center Tunisia; latitude 35°36'57 82"(N); longitude 9°33'.34"(E), altitude 151.80 km). The prospected material was harvested at the beginning of maturity (15 June: ripe fruit) when fruit was green and at the end of maturity (15 August: overripe fruit) when fruit was red. Cactus pear seeds were obtained after juice extraction. The seeds were washed with distilled water several times, air-dried at room temperature and then ground with a blade-carbide gringing (IKA-WERK. Type: A:10).

Determination of total antioxidant capacity: The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH and determined by the method described by Dasgupta and De[2]. Aqueous extract (100 µL) was added to 1000 µL of reagent solution (0.6 M sulphuric acid, 28 mM

sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95 °C for 1.5 h. The mixture had cooled to room temperature and the absorbance of the solution was measured at 695 nm against a blank. The antioxidant activity (three replicates per treatment) was expressed as mg gallic acid equivalents (GAE) per gram of dry weight.

DPPH assay: The antioxidant activity of macerates of *Opuntia ficus* indica seeds, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al.*[3]. Aqueous extract (1000 µL) was added to 250 µL of a 0.002 M DPPH in methanol. Absorbance at 517 nm was determined after 0.5 h. The antiradical activity (three replicates per treatment) was expressed as IC_{50} (mg mL^{-1}), the concentration of sample required to scavenge 50 % free radical. The percent inhibition of activity was calculated as [(Ao-Ae)/Ao] × 100 (Ao = absorbance without extract; Ae = absorbance with extract). BHT was used as a positive control.

Reducing power: The method of Oyaizu[4] was used to assess the reducing power of *Opuntia ficus* indica seed. Seed macerates (1 mL) were mixed with 2.5 mL of a 0.2 M sodium phosphate buffer (pH = 6.6) and 2.5 mL of 1 % potassium ferricyanide and incubated in a water bath at 50 ºC for 20 min. Then, 2.5 mL of 10 % trichloroacetic acid were added to the mixture that was centrifuged at 650 g for 10 min. The supernatant (2.5 mL) was then mixed with 2.5 mL distilled water and 0.5 mL of 0.1 % ferric chloride solution. The intensity of the blue-green colour was measured at 700 nm. The EC_{50} value (mg mL^{-1}) is the extract concentration at which the absorbance was 0.5 for the reducing power and was calculated from the graph of absorbance at 700 nm against extract concentration. Ascorbic acid was used as a positive control.

Polyphenol extraction: Two methods were adopted to extract total polyphenols from cactus seed powders. In the first method (maceration), powders (1 g) of ground material was extracted by stirring with 10 mL of pure methanol for 0.5 h. The extracts were then kept for 24 h at 4 °C, filtered through a Whatman No. 4 paper and evaporated under vacuum to dryness and stored at 4 °C until analyzed[5]. In the second extraction method (hydrolysis), dried samples from seeds were hydrolyzed according to the method of Proestos *et al.*[6]. 20 mL of methanol containing BHT (1 g L^{-1}) were added to 0.5 g of a dried sample. Then 10 mL of 1 M HCl were added. The mixture was stirred carefully and then sonicated for 15 min and refluxed in a water bath at 90 °C for 2 h. The obtained cactus seed extracts were injected to HPLC.

Total phenolic content: Total phenolic contents were assayed using the Folin-Ciocalteu reagent, following Singleton's method slightly modified by Dewanto *et al.*[7]. An aliquot (0.125 mL) of a suitable diluted methanolic seed extract was added to 0.5 mL of deionized water and 0.125 mL of the Folin-Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before adding 1.25 mL of 7 % Na_2CO_3 solution. The solution was then adjusted with deionized water to a final volume of 3 mL and mixed thoroughly. After incubation for 1.5 h at 23 °C, the absorbance *versus* prepared blank was read at 760 nm. Total phenolic content of seeds (three replicates per treatment) was expressed as mg gallic acid equivalents (GAE) per gram of dry weight through the calibration curve with gallic acid. The calibration curve range was 50-400 mg mL^{-1} (R^2 = 0.99).

Determination of total flavonoids: Total flavonoid contents were measured according to Dewanto *et al.*[7]. 250 µL of the methanolic seed extract appropriately diluted was mixed with 75 µL $NaNO_2$ (5 %). After 6 min, 150 µL of $AlCl_3 \cdot 6H_2O$ (10 %) was added and 5 min later, 500 µL of NaOH (1 M) were added to the mixture. Finally, the mixture was adjusted to 2.5 mL with distilled water. The absorbance *versus* prepared blank was read at 510 nm. Total flavonoid content of seeds (three replicates per treatment) was expressed as mg catechin equivalents (CE) per gram of dry weight through the calibration curve with catechin. The calibration curve range was 50-500 mg mL^{-1}.

Determination of condensed tanins: In presence of concentrated H_2SO_4, condensed tanins were transformed by the reaction with vanillin to anthocyanidols[8]. 50µL of the methanolic seed extract appropriately dilute was mixed with 3 mL of 4 % methanol vanillin solution and 1.5 mL of H_2SO_4. After 15 min, the absorbance was measured at 500 nm. Condensed tannin contents of seeds (three replicates per treatment) were expressed as mg catechin equivalents (CE) per gram of dry weight through the calibration curve with catechin. The calibration curve range was 50-600 mg mL^{-1}.

Identification of phenolic compounds using RP-HPLC: The phenolic compounds' analysis was carried out using an Agilent Technologies 1100 series liquid chromatograph (RP-HPLC) coupled with an UV-Vis multiwavelength detector. The separation was carried out on a 250 mm × 4.6 mm, 4 µm Hypersil ODS C_{18} reversed phase column at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2 % sulphuric acid (solvent B). The flow rate was kept at 0.5 mL min^{-1}. The gradient programme was as follows: 15 % A/85 % B 0-12 min, 40 % A/60 % B 12-14 min, 60 % A/40 % B 14-18 min, 80 % A/20 % B 18-20 min, 90 % A/10 % B 20-24 min, 100 % A 24-28 min The injection volume was 20 µL and peaks were monitored at 280 nm. Samples were filtered through a 0.45 µm membrane filter before injection. Peaks were identified by congruent retention times compared with standards. Analyses were performed in triplicate. Quantification of phenolic compounds was achieved while using a known quantity of *trans* hydroxy-2-cinnamic acid as an internal standard.

RESULTS AND DISCUSSION

Total phenolic, total flavonoid and total tannin contents: Phenolic compounds are widely distributed in plants[9], which have gained much attention, due to their antioxidant activities and free radical-scavenging abilities, which potentially have beneficial implications for human health[10].

Based on the absorbance values of extract solutions reacted with the Folin-Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents, total phenolic content in cactus seeds are given in Fig. 1. Phenolic contents varied significantly during maturity. In fact, cactus seeds of ripe fruit presented higher amount of total polyphenol (1.72 mg GAE g^{-1} DW) than that of cactus seeds of over ripe fruit (0.98 mg GAE g^{-1} DW). Total flavonoid content estimated by vanillin-H_2SO_4 assay showed that cactus seeds of ripe fruit

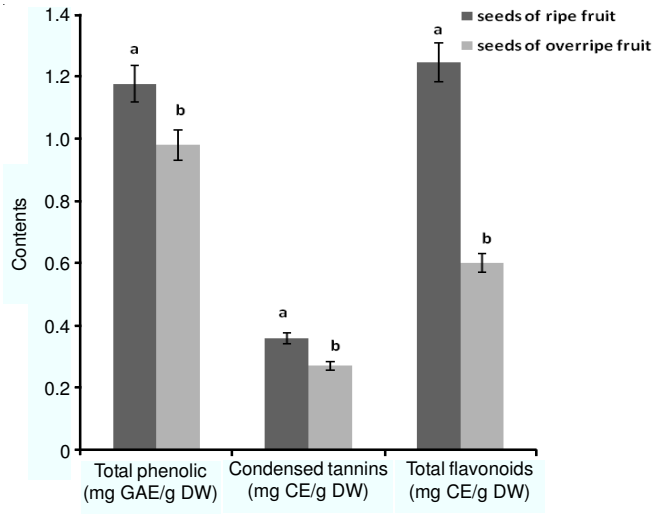

Fig. 1. Total phenolic, flavonoid and tannin contents in cactus seeds during ripening

had the important quantity (1.25 mg EC g^{-1} DW) than cactus seeds of overripe fruit (0.6 mg EC g^{-1} DW).

Like total phenolic and total flavonoid, total tannin content in cactus seeds of ripe fruit (0.36 mg EC g^{-1} DW) was more important than this in cactus seeds of over ripe fruit (0.27 mg EC g^{-1} DW). Indeed, these results showed that cactus seeds were richer in polyphenols at the beginning of maturity. This decrease of polyphenol contents lasting maturity was confirmed by Macheïx[11] who mentionned that phenolic contents are important in young organs and decrease lasting grouth. Morever, Ding *et al.*[12] showed that, in young fruit (cv. Mogi), total phenolics and individual compounds decreased steadily during growth between 2 and 4 weeks prior to harvest. Such decreases have been reported in apple[13], peach[14] and grapes[15]. In addition, Harris and Brannan[16] reported that total phenolics were affected by ripeness, so the concentration of total phenolics in pawpaw pulp were in the order: under ripe = ripe > over ripe, while the concentration of flavonoids was in the order: ripe < under ripe < overripe.

The change in the amount of polyphenols during ripening was reported also in three apricot cultivars[17]. The content of individual polyphenols during ripening was quite similar, whereas their amounts differed significantly. Immature fruits showed the highest level of polyphenols, which decreased at semi-mature fruits and did not change remarkably in mature fruits. Furthermore, grape seed polyphenols decrease dramatically during ripening with a 90 % decrease in flavan-3-ol monomers and a 60 % decrease in procyanidins[18]. Present results correspond with these references which reported that total phenolics, flavonoids and tannins were affected by ripeness, so it was indicated that cactus seeds contained moderate amount of polyphenol and flavonoid constitued the major class. Concerning the variation of phenolic compounds in cactus seeds during ripening, RP-HPLC analysis of methanolic extracts of studied samples had been acheived.

Identification and quantification of phenolic compounds by HPLC: The RP- HPLC analysis of cactus seed extracts (macerate and hydrolysis) revealed the presence of phenolic compounds. By this means, in the four analyzed extracts (macerate cactus seed of ripe fruit, macerate cactus seed of over ripe fruit, hydrolysis extract cactus seed of ripe fruit and hydrolysis extract cactus seed or ripe fruit, it was possible to identify different phenolic compounds (Table-1).

For cactus seed of ripe fruit, catechin was the major compound detected in macerate (61 %) followed by rutin, (10.1 %). In contrast, the hydrolysis extract contained different phenolic compounds and quercetin (37 %) was the most abundant component followed by dihydroxycinnamic acid (27.6 %) and epigallocatechin (10.9 %). In addition, it was noticed that catechin was present at low rate (2.6 %) and rutin was missing in hydrolysis extract. Moreover, dihydroxycinnamic acid rate was important in hydrolysis extract but lower (0.2 %) in macerate. In addition, amentoflavone and flavone were detected only in hydrolysis extract.

For cactus seed of over ripe fruit, the mains compounds in macerate were gallic acid (44.7 %) and catechin, but in hydrolysis extract were dihydroxybenzoic acid (52.5 %), dihydroxyphenolic acid (17.3 %), epicatechin (11.3 %) and epigallocatechin (8 %).

In other hand, macerate of cactus seed of overripe fruit contained gallic acid as the most predominant phenolic compounds (44.7 %). It is noted an increase of its rate compared to this in macerate of cactus seeds of ripe fruit (0.8 %). However, the amount of catechin (19 %) of cactus seeds of over ripe fruit decrease lasting cactus maturity. This result was confirmed by Hatzidimitriou *et al.*[19] that gallic acid formation compensates for catechin loss lasting grape maturity. Quercetin was identified in macerate of cactus seed of over ripe fruit (3.5 %) but not present in macerate of cactus seed of ripe fruit. In addition, Table-1 showed that quercetrin, *p*-coumaric acid, amentoflavone and flavone were detected only in cactus seed of ripe fruit and apigenin was present only in cactus seed of over ripe fruit.

The present result showed that phenolic component variations depend on the method of extraction and the maturity stage. This finding was in agreement with Hayouni *et al.*[20] who found that the technique of extraction, as well as the extracting solvent, significantly affected extraction yield, total polyphenol and biological activities (antioxidant and antibacterial) of several extracts from *Juniperus phoenicea* L. and *Quercus coccifera* L. fruits.

The importance of present study is the useful imformation given on phenolic composition to found the desired compound. In fact, to found catechin we must chose macerate of cactus seed at the start of maturity. In contrast, to found quercetin, hydrolysis acid of cactus seed of ripe fruit was needed. However, hydroxybenzoic acid was detected in hydrolysis extract of cactus seed at the end of maturity. In addition, it is noted that cactus seed extracts (macerate and hydrolysis extracts) at the start of maturity (of ripe fruit) are rich in quercetin and catechin.

Quercetin and catechin are among the most widely consumed flavonoids but this consumption may vary according to the individual food habits. Quercetin is one of the most abundant of the flavonoids and occurs in food as aglycone (attached to a sugar molecule). It is found in many common foods including apple, tea, onion, nuts and berries. Quercetin has many health promoting effects, including antiinflammatory and antiallergic effects as well as improvement of cardiovascular health and

TABLE-1
PERCENTAGE OF PHENOLIC COMPOUNDS IN CACTUS SEEDS DURING RIPENING

Phenolic compounds	Seeds of ripe fruit (%)		Seeds of over ripe fruit (%)	
	Hydrolysis	Maceration	Hydrolysis	Maceration
Gallic acid	4.36 ± 0.02$^{aa'}$	0.85 ± 0.05$^{bb'}$	3.00 ± 0.16$^{ab'}$	44.7 ± 0.09$^{aa'}$
Cafeic acid	2.1 ± 0.19$^{aa'}$	0.9 ± 0.08$^{ab'}$	1.8 ± 0.1$^{aa'}$	1.37 ± 0.23$^{aa'}$
Chlorogenic acid	–	1.20 ± 0.14$^{aa'}$	4.20 ± 0.04$^{aa'}$	2.00 ± 0.11$^{ab'}$
Cinnamic acid	27.70 ± 0.24$^{aa'}$	0.6 ± 0.03$^{bb'}$	1.1 ± 0.07$^{bb'}$	4.50 ± 0.06$^{aa'}$
Dihydroxy benzoic acid	–	3.00 ± 0.19$^{aa'}$	52.5 ± 0.17$^{aa'}$	1.2$^{bb'}$ ± 0.15
Dihydroxyphenolic acid	–	0.4 ± 0.02$^{ba'}$	17.3 ± 0.05$^{aa'}$	5.00 ± 0.22$^{aa'}$
p-Coumaric acid	–	2.15 ± 0.61$^{aa'}$	–	–
Syringic acid	6.00 ± 0.31$^{ab'}$	8.40 ± 0.12$^{aa'}$	0.86 ± 0.10$^{bb'}$	2.10 ± 0.18$^{ba'}$
Vanillic acid	–	3.30 ± 0.5$^{aa'}$	–	1.50 ± 0.37$^{ba'}$
Amenthoflavone	1.40 ± 0.09$^{aa'}$	–	–	–
Apigenin	–	–	0.70 ± 0.29$^{aa'}$	–
Catechin	2.6 ± 0.15$^{ab'}$	61.00 ± 0.11$^{aa'}$	2.60 ± 0.16$^{ab'}$	19.00$^{ba'}$ ± 0.15
Epicatechin	–	1.00 ± 0.16$^{ba'}$	11.26 ± 0.13$^{aa'}$	5.00 ± 0.23$^{ab'}$
Epigallocatechin	13.30 ± 0.18$^{aa'}$	–	8.10 ± 0.02$^{ba'}$	–
Flavone	1.16 ± 0.01$^{aa'}$	–	–	–
Quercitrin	–	1.10 ± 0.36$^{aa'}$	–	–
Quercetin	37.05 ± 0.02$^{aa'}$	–	2.20 ± 0.31$^{bb'}$	3.44$^{aa'}$ ± 0.12
Quercetin-3-galactoside	–	3.80 ± 0.02$^{aa'}$	0.76 ± 0.08$^{bb'}$	2.00 ± 0.16$^{ba'}$
Quercetin-3-glucoside	0.83 ± 0.25$^{bb'}$	3.50 ± 0.31$^{aa'}$	2.10 ± 0.27$^{aa'}$	2.70 ± 0.12$^{aa'}$
Quercetin-mannoside	0.89$^{bb'}$ ± 0.07	4.10 ± 0.17$^{aa'}$	3.28 ± 0.13$^{aa'}$	2.6 ± 0.09$^{ba'}$
Rutin	–	10.00 ± 0.20$^{aa'}$	0.80 ± 0.11$^{ab'}$	3.00 ± 0.11$^{ba'}$

Results are expressed as means ± standard deviation of three measurements.

reducing risk for cancer. All these activities are caused by the strong antioxidant action of quercetin. It helps to combat free radicals, which can damage cells. As many other flavonoids, quercetin prevents the oxidation of LDL cholesterol[21]. Catechins exist in both the monomer and the polymer form. These forms are found in many types of fruits and red wine (*i.e.* apricots: 25 mg/100 g fresh weight), but green tea and chocolate are by far the richest sources (chocolate: 46-61 mg/100 g product)[22]. The daily intake of catechin monomers was estimated to be 50 mg/d in a Dutch cohort, with tea, chocolate, apples and pears as the main sources[23]. Moreover, previous studies reported that flavonoids could interact together and that these interactions could exhibit several properties, hence synergistic antioxidative properties or efficient competitions against drugs for metabolic enzymes[24]. In addition, Silberberg[25] signaled that no effect of the co-administration of quercetin and catechin on their metabolism has been observed. According to these data and present results, it is noted that cactus seed of ripe fruit is an important source of these desired flavonoids known by their antioxydant activity. Recent interest in these substances has been stimulated by the potential health benefits arising from the antioxidative activity, free radical scavenging capacity, coronary heart disease prevention and anticancer activity, whilst some flavonoids exhibit potential for anti-human immunodeficiency virus functions[26]. To evaluate present samples, we investigated the antioxidant activity of the methanolic extract of cactus seeds of ripe fruit and overripe fruit by three *in vitro* systems of assay.

Antioxidant activity of seed macerates: Several methods have been used to determine antioxidant activity of plants. Present study therefore involved three various established methods to evaluate antioxidative activity of cactus seed grounds, namely, DPPH radical-scavenging activity, reducing power assay and total antioxidant capacity (Fig. 2).

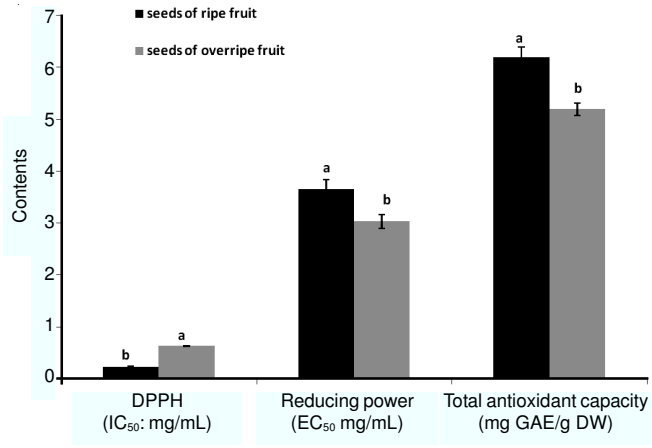

Fig. 2. Antiradical, reducing activity and total antioxidant capacity of cactus seeds during ripening

Total antioxidant capacity of methanol cactus seed extracts is expressed as number of equivalents of gallic acid. The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. For total antioxidant capacity, there is a significant difference among maturity stage. It varies from 6.19 mg AGE/g DW in seeds of ripe fruit to 4.19 mg AGE/g DW in seeds of over ripe fruit. Present results were compared with Kubola and Siriamornpun[27] where green fruit had a higher capacity than had the ripe fruit of bitter gourd (*Momordica charantia* L.).

DPPH is a free radical compound and has been widely used to test the free radical-scavenging ability of various samples[28-30]. It is a stable free radical with a characteristic absorption at 517 nm, was used to study the radical-scavenging effects of extracts. As antioxidants donate protons to this radical, the absorption decreases. To evaluate the scavenging effects of DPPH of cactus seed extracts, Fig. 2 shows that

activity of seeds of ripe fruit (IC_{50} = 250 µg/mL) was the highest followed by seeds of overripe fruit (IC_{50} = 650 µg/mL). These results are shown as relative activities against BHT and ascorbic acid.

The reducing capacity of seed extracts may serve as indicator of its potential antioxidant activity[31]. The presence of reducers (*i.e.* antioxidants) causes the conversion of the Fe^{3+}/ferricyanide complex to the ferrous form. The EC_{50} value of reducing power ability of ascorbic acid (38 µg mL^{-1}) was lower than that of methanol seed extracts (3.033 mg mL^{-1} for cactus seeds of overripe fruit and 3.666 mg mL^{-1} for cactus seeds of ripe fruit). Morever, cactus seeds of over ripe fruit exhibited superior reducing capacity than cactus seeds of ripe fruit. In addition, the finding showed that reducing power ability varied with maturity stages.

The present finding confirmed that the antioxidant activity of some fruits at different stages of ripening depends on the scavenging methods used for their determination[32]. For example the oxygen-scavenging capacity of mature meifruit (*Prunus mume Seibu. et Zucc*) in water fraction was lower than that of Trolox. However, both oxygen- and hydroxyl-scavenging capacities of immature fruit and hydroxyl scavenging capacities of mature fruit showed no significant difference from Trolox.

Conclusion

The results of this work indicate the presence of compounds possessing high antioxidant activity in cactus prickly seeds. In addition, the finding showed large differences were found among the ripening stages in relation to the polyphenol, flavonoid and tannin contents. However, the technique of the extraction, significantly affected phenolic composition and yield of studied seeds. In fact, it was found that quercetin detected in hydrolysis extract and catechin identified in methanolic macerate was the main compounds in ripe fruit cactus seeds (of ripe fruit). Meanwhile, the two extracting method, showed that methanolic extract had stronger yield of gallic acid and hydrolysis extract allowed higher yields of dihydroxybenzoic acid in over ripe fruit cactus seeds. In addition, the antioxidant activity of cactus seeds in ripe and overripe fruits depends on the scavenging methods used for their determination. In the face of this study, cactus seeds can be considered as new source of agro-food co-products.

REFERENCES

1. A. Acevedo, E. Fonseca, J.M. Viguer and F. Contreras, *Med. Cutan Ibero Lat. Am.*, **13**, 237 (1985).
2. N. Dasgupta and B. De, *Food Chem.*, **101**, 471 (2006).
3. A. Braca, D.B.N. Tommasi, D. Lorenzo, C. Pizza, P. Mateo and M. Ivano, *J. Nat. Prod.*, **64**, 892 (2001).
4. M. Oyaizu, *J. Clin. Biochem. Nutr.*, **44**, 307 (1986).
5. J.L. Mau, G.R. Chao and K.T. Wu, *J. Agric. Food Chem.*, **49**, 5461 (2001).
6. C. Proestos, I. Boziaris, S.G.J.E. Nychas and M. Komaitis, *Food Chem.*, **95**, 664 (2006).
7. V. Dewanto, X. Wu, K.K. Adom and R.H. Liu, *J. Agric. Food Chem.*, **50**, 3010 (2002).
8. B. Sun, J.M. Richardo-da-Silvia and I. Spranger, *J. Agric. Food Chem.*, **46**, 4267 (1998).
9. B.B. Li, B. Smith and Md.M. Hossain, *Sep. Purif. Technol.*, **48**, 182 (2006).
10. R. Govindarajan, D.P. Singh and A.K.S. Rawat, *J. Pharm. Biomed. Anal.*, **43**, 527 (2007).
11. J.J. Macheïx, A. Fleuriet and J. Billot, Fruit Phenolic, CRC Press, Boca Raton, p. 378 (1990).
12. C.K. Ding, K. Chachin, Y. Ueda, Y. Imahori and C.Y. Wang, *J. Agric. Food Chem.*, **49**, 2883 (2001).
13. S. Burda, W. Oleszek and C.Y. Lee, *J. Agric. Food Chem.*, **38**, 945 (1990).
14. C.Y. Lee, V. Kagan, W. Jaworski and S.K. Brown, *J. Agric. Food Chem.*, **38**, 99 (1990).
15. F.M. Romeyer, J.J. Macheix, J.P. Goiffon, C.C. Reminiac and J.C. Sapis, *J. Agric. Food Chem.*, **31**, 346 (1983).
16. G.G. Harris and R.G. Brannan, *LWT*, **42**, 275 (2009).
17. V. Dragovic-Uzelac, B. Levaj, V. Mrkic, D. Bursac and M. Boras, *Food. Chem.*, **102**, 966 (2007).
18. J.A. Kennedy, M.A. Matthews and A.L. Waterhouse, *Phytochemistry*, **55**, 77 (2000).
19. E. Hatzidimitriou, N. Nenadis and M.Z. Tsimidou, *Food Chem.*, **105**, 1504 (2007).
20. E. Hayouni, M. Abedrabba, M.. Bouix and M. Hamdi, *Food Chem.*, **105**, 1126 (2007).
21. J. Kroll, H. Rawel and S. Rohn, *Food Sci. Technol. Bull.*, **9**, 205 (2003).
22. C. Manach, A. Scalbert, C. Morand, C. Rémésy and L. Jiménez, *Am. J. Clin. Nutr.*, **79**, 727 (2004).
23. I.C. Arts and P.C. Hollman, *Eur. J. Clin. Nutr.*, **55**, 76 (2001).
24. C. Malien-Aubert, O. Dangles and M.J. Amiot, *J. Agric. Food Chem.*, **49**, 170 (2001).
25. T.M. Silberberg, C. Morand, C. Manach, A. Scalbert and C. Remesy, *Life Sci.*, **77**, 3156 (2005).
26. L.H. Yao, Y.M. Jiang, J. Shi, F.A. Tomas-baeberan, N. Datta, R. Singanusong and S.S. Chen, *Plant Foods Hum. Nutr.*, **59**, 113 (2004).
27. J. Kubola and S. Siriamornpun, *Food Chem.*, **110**, 881 (2008).
28. T. Hatano, M. Takagi, H. Ito and T. Yoshida, *Chem. Pharm. Bull.*, **45**, 1485 (1997).
29. S. Sakanaka, Y. Tachibana and Y. Okada, *Food Chem.*, **89**, 569 (2005).
30. Y. Shimoji, Y. Tamura, Y. Nakamura, K. Nanda, S. Nishidai and Y. Nishikawa, *J. Agric. Food Chem.*, **50**, 6501 (2002).
31. S.J. Meir, B. Kanner, S. Akiri and P. Hadas, *J. Agric. Food Chem.*, **43**, 1813 (1995).
32. S.B. Lin, C.A. Wu, M.J. Tsai, H.T Yang and R.Y. Chen, *Taiwan nong ye hua xue yu shi pin ke xue*, **45**, 46 (2007).