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Antioxidant activity and physico-chemical characterstics during development of *Prosopis cineraria* pods

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Abstract

Prosopis cineraria commonly called 'khejri' is highly versatile tree supporting wildlife, human beings and livestock in Indian desert. It is used as food, fodder, fixes atmospheric nitrogen, stabilizes sand dunes and its fruits are consumed fresh as well as after drying. Pods were sampled at 5 developmental stages *viz*., S1(3.5 cm), S2 (5.7 cm), S3 (9.2 cm), S4 (15.3 cm), S5 (17.4 cm, naturally dried pods on tree) to evaluate the changes in phytonutrients (total sugars, reducing sugars, protein and total phenol) and antioxidant activity in terms of DPPH radical scavenging activity, nitric oxide scavenging and ferric reducing power assay during fruit development from S1 to S5. Total soluble sugar content increased from S1 stage (47.1 mg^{-1}f.wt.) to S5 stage (64.28 mg^{-1}f.wt.), reducing sugar and sucrose content also increased from 20.65 mg^{-1}f.wt to 29.45 mg^{-1}f.wt and 18.75 to 33.84 mg^{-1}f.wt, respectively. Free amino acid and protein content increased during the development of the pods from very young to ripened stage. Whereas, total phenol content (TPC) was high at S1 stage (229.59 mg^{-1}f.wt.) as compared to S5 stage (55.24 mg^{-1} g.f.wt.). DPPH radical scavenging activity decreased from 78.46 to 22.72 % during fruit development but ferric reducing activity increased with maturity of the pods. The study is significant in evaluating phytochemical and antioxidant activities of the fruits that are consumed in every season by the local rural people. This study may help in prioritization of these local underutilised fruits as they are rich in some of the phytonutrients and antioxidants.

Key words: *Prosopis cineraria,* DPPH, antioxidant activity, phytonutrients, underutilised fruits

Introduction

Phytochemicals are well known plant based chemicals or nutrients beneficial for human health. Healthy diet includes food rich in phytochemical/phytonutrients and antioxidants. About 200,000 phytochemicals are known, of which 20,000 are derived from fruits, vegetables and grains (Oz and Kafkas, 2017). Phytochemicals play role as anticancer, anti-inflammatory agents and antioxidant regulates specific signalling pathways and molecular markers to inhibit the occurrence of cancer. Dietary phytochemicals or antioxidants have been reported to have a role in the reduction of platelets aggregation, modulation of cholesterol synthesis and absorption and lipid profiles, reduction of blood pressure and anti-inflammation (Yin *et al.,* 2016).

Phytochemicals protect the skin by reducing inflammation and quenching free radicals. Sugars, acids and polysaccharides are important sources of phytochemicals, secondary plant metabolites also known for their antioxidant activity and other properties (Escobedo-Avellaneda *et al.,* 2014). In addition, phytochemicals are some of the most important natural preservation structures to reduce and inhibit pathogenic microorganism growth and preserve the overall quality of food products (Tajkarimi *et al*., 2012). The term phytochemical actually refers to the plant-derived medicines, which local people have used for treatment of diseases for hundreds of years. Recent revival of this nature-based drug industry has brought a new ray of hope to local ethnic people who find it difficult to access and afford the expensive allopathic medications.

The arid zone of Rajasthan and Gujarat in India is comprised of vast stretches of sandy soil and saline tracts in form of sand dunes and salt basins, respectively, where evapotranspiration exceeds the precipitation. Most commonly found tree species which are adapted to such harsh environmental conditions of arid zone are *Prosopis cineraria*, *Acacia nilotica*, *Acacia senegal, Tecomella undulata etc.* Most of the tree species found in arid zone are multipurpose and fulfil needs of local residents. *P. cineraria* (Fabaceae), locally called khejri is the state tree of Rajasthan. It is also known as "Kalapavriksh" (Burkat, 1976) and "King of Indian desert" (Rani *et al.,* 2013) as every part of this plant from root to fruits is useful for humans. Roots fixes nitrogen and helps in sand dunes stabilization (Toky and Harris, 2004), bark has abortifacient and laxative properties, leaves (locally called 'loonk') have antibacterial, antihyperglycemic, antihyperlipidemic and antioxidative activities (Malik *et al*., 2013; Velmurugan *et al*., 2011). Unripe pods locally called 'sangri' and ripened pods called 'khokha', fresh or dry areb used as food and fodder for the local habitants and animals, respectively (Chogem, 2007).

The nutraceutical value and the antioxidant activity of wild, semi-cultivated or neglected vegetables is regarded worldwide as an important area of the nutritional and medicinal research. *Prosopis cineraria* is also an underutilised multipurpose tree and has high phytochemical, medicinal and antioxidative values. The present investigation is focussed on the change in phytonutrients and antioxidant activity at different developmental stages during pod growth and maturity.

Materials and methods

Fresh pods from the tree of *P. cineraria* were harvested at New Campus, Jai Narain Vyas University, Jodhpur in the month of

March when the maximum to minimum temperature ranges from 38 to 25 ^oC, respectively. Pods were selected at five different developmental stages S_1(3.5 cm), S_2 (5.7 cm), S_3 (9.2 cm), S_4 (15.3 cm), S_5 (17.4 cm, naturally dried pods on tree, locally called '*khokha*'). After harvesting fresh pods were used for analysis. Plant material was weighed and utilized to prepare extracts with measured volume of solvents like acetone, alcohol, methanol or solutions like phosphate buffer, oxalic acid according to the method used for analysis.

Total sugars: Total soluble sugar was estimated from fresh samples as described by Sadasivam and Manickam (1992).

Reducing sugar: Reducing sugar was estimated by the method suggested by Nelson (1944) and Somogyi (1952).

Sucrose: Sucrose was estimated using the method suggested by Sadasivam and Manickam (1992).

Soluble proteins: Soluble proteins were estimated by dye binding method (Bradford, 1976). Protein content measured was expressed as $mg^{-1}g$ fresh weight.

Free amino acids: Free amino acid was estimated using method suggested by Hirs *et al*. (1954). Standard curve was made using 100 ppm of glycine.

Total phenols: Total phenol content was estimated using method illustrated by Singleton and Lamuela-Raventos (1955) in fresh plant material. The quantity of phenolic content was expressed as gallic acid equivalent in mg/g of ethanol extract.

DPPH radical scavenging activity or hydrogen-donating activity: Scavenging activity against DPPH radicals was assessed according to the method of Krings and Berger (2001) with some modifications.

DPPH-scavenging activity (%) = [1-(absorbance of sample - absorbance of blank)/absorbance of control] x 100.

Nitric oxide scavenging activity: Nitric oxide scavenging activity was measured spectrophotometrically (Balakrishnan *et al.,* 2009). Ascorbic acid was used as control.

% inhibition or scavenging $=\{(A_{blank} - A_{sample})/ A_{blank}\} \times 100$

Ferric reducing power: The ferric reducing power of the fruit extracts was determined by using the potassium ferricyanide-ferric chloride method (Oyaizu, 1986). FRAP of a sample was estimated in terms of gallic acid equivalents (GAE) in mg GAE/g of fresh sample.

% inhibition or scavenging $= \{(A_{blank} - A_{sample})/ A_{blank}\} \times 100$

Statistical analysis: Statistical analysis was performed using SPSS (Version 16.00). DMRT was used for mean seperation.

Results and discussion

Total soluble sugars: Total soluble sugar increased during fruit development in *Prosopis cineraria*. TSS content was 47.1 mg g^{-1}.f.wt and increased up to 64.28 mg g^{-1}.f.wt at S_5 stage (Fig. 1A). The increase in total sugar content was also observed in guava during ripening (Bashir *et al.,* 2003). Similar findings were reported for banana (Ibrahim *et al*., 1994), mango (Abu-Goukh and Abu-Sarra, 1993). The increase in the total sugar content is attributed to the increase in the activity of enzymes responsible for the starch hydrolysis and for decline in the rate of sugar breakdown by respiration (Bashir *et al*., 2003).

Reducing sugar and sucrose: Reducing sugar level also increased from S_1 to S_4 stage and then remained constant up to S_5 stage in fresh fruits of *P. cineraria.* Reducing sugar content at S_1 stage was 20.65 mg g^{-1}.f.wt and rose to 29.45 mg g^{-1}.f.wt at S_5 stage (Fig. 1A). Sucrose level increased from 18.75±1.06 mg g^{-1}.f.wt to 33.84±1.04 mg g^{-1}.f.wt from S_1 to S_5 stage (Fig. 1A). According to Abu-Goukh *et al.* (2010) the amount of reducing sugar decreased till physiological maturity in the cultivars of papaya and then sharply increased. Both reducing sugars and non reducing sugars increased up to maturity (Bal *et al*., 1982) in fruits of *Z. mauritiana* fruits. Bal *et al*. (1979) reported that in ber cv. Umran, sucrose and fructose continued to increase whereas glucose decreased slightly with the advancement of ripening. Similar pattern of reducing sugar change was observed in mango (Abu-Goukh *et al.,* 2005) and guava (Bashir and Abu-Goukh, 2003). During fruit ripening process starch and sucrose are hydrolysed to glucose, the main substrate utilised in respiration (Wills *et al.,* 2000).

Total phenol content (TPC): Phenolic compounds are essential antioxidant that protect against oxidative damage, as these act as scavengers of reactive oxygen species. Phenols can retard or inhibit lipid autoxidation. *P. cineraria* had very high amount of TPC at S_1 stage as compared to other stages of development. At S_1 stage, it had 229.58 mg g^{-1}.f.wt of TPC and at S_5 it decreased to 61.54 mg g^{-1}.f.wt (Fig. 1B). Similar decrease in the level of total phenolic content was observed in the fruits of banana (Goldstien and Swain, 1963) and guava fruits (Bashir and Abu-Goukh, 2003). There was nearly 73.90 % of reduction in total phenolic content in 20-140 days of fruit development of pomegranate (Kulkarni and Aradhya, 2005).

Extractable tannins: Tannins are polyphenolic compounds and form complex with protein and carbohydrates (Ammar *et al*., 2004). Tannin contents decreased in fruits of *P. cineraria* from mature green to ripened stage (Fig. 1B). Similarly, highest accumulation of tannins was recorded in grapes at the time of fruit set and declined towards ripening (Hanlin and Downey, 2009).

Soluble protein: *P. cineraria* fruits have very high protein content as compared to other desert fruits. Soluble protein content increased from S_1 to S_5 stages of fruit development. At S_5 stage soluble protein content in fruit was 25.76 mg g^{-1}.f.wt and at S_1 stage it was recorded as 16.25 mg g^{-1}.f.wt (Fig. 1D). Systematic increase in the protein content was observed in the fruits of guava up to full ripe stage (Bashir and Abu-Goukh, 2003). Similar increase in protein content up to full ripened stage was observed in the pulp and peel of different cultivars of mango and then the value decreased at the over-ripe stage. This decline was explained as the breakdown of protein during senescence.

Free amino acids: Free amino acid content also increased as fruit attained maturity. The amount of FAA at S_1 stage was 5.4±0.83 mg g^{-1}.f.wt and it increased up to 13.26±0.18 mg g^{-1}.f.wt at S_5 stage (Fig. 1D). of fruit development. Kliewer (1968) reported increase in total free amino acids during fruit development and ripening in grape berries.

DPPH radical scavenging activity: DPPH radical scavenging activity measures the total antioxidant capacity of the fruits and vegetables. *P. cineraria* fruits had very high DPPH radical scavenging activity at S_1 stage (78.26±0.44 %) and decreased up to S_5 (29.54±0.55 %) stage (Fig. 1C). High antioxidant

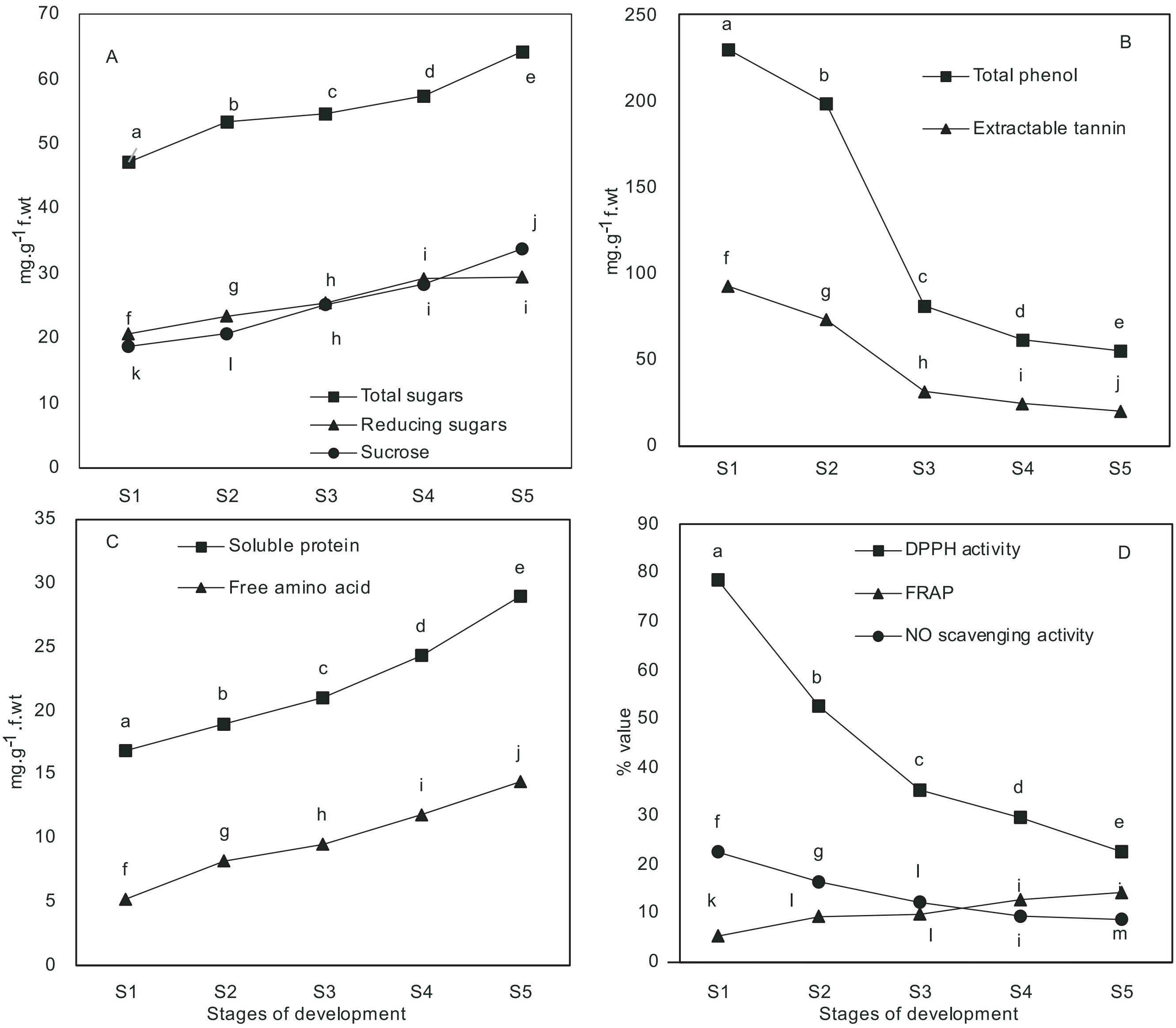

Fig 1. Sucrose, total sugars and reducing sugars (A), Total phenols and tannins (B), Protein and free amino acid (C), Antioxidant activity assay (D). Each value is the mean of five replicates. Mean value with the different letter are significantly different from each other and with same letter are not significantly different at $P \leq 0.05$ by Duncan's Multiple range test (DMRT).

activity was also recorded in arils of pomegranate at 20 days old fruit and decreased significantly up to 60 days, concomitantly with a decrease in ascorbic acid and total phenolics (Kulkarni and Aradhya, 2005). As observed by Cristina *et al.* (2017), an increase in the of total phenolic content, bioactive compounds and antioxidant activity was observed to be increasing with the ripening of fruits of nine commercial fig (*Ficus carica* L.) varieties. Present investigation results also coincides with the investigation of Kulkarni and Aradhya (2005) and Zozio *et al.* (2014) in pomegranate.

Nitric oxide (NO) radical scavenging activity: In present investigation nitric oxide scavenging activity of pods of *P. cineraria* decreased as it matured. NO radical scavenging activity was found to be 22.65% at S_1 stage and decreased upto 8.84 % in S_5 stage (Fig. 1C). Similar trend was observed by Raffo *et al.* (2002) in cherry tomatoes.

Ferric reducing antioxidant power: The ferric reducing antioxidant power assay is based on the reducing power of the compound. It measures the ability of the plant extract to donate electron to Fe^{3+} and reduce it to Fe^{2+} ion. The higher the FRAP value, the greater is the antioxidant activity (Yan *et al.,* 2006).

In the pods of *P. cineraria,* FRAP value (Fig. 1C) increased from S_1 to S_5 stage of fruit development which showed a contrasting trend with the DPPH activity. Similar trend was observed in the developing fruits of *Cordia myxa* and *Carrisa carandus* (Printal and Gehlot, 2015). The trends in the present investigation are similar to the mango as preorted by Palafox-Carlosa *et al.* (2012).

Antioxidant activity and polyphenolic content decreased during the development of fruit growth of unripe fuji apples (Zheng *et al.,* 2012). Highest amount of DPPH scavenging activity and phenolic concentration was found to be present at the start of the fruit development but decreased as fruit matured as evaluated in different cultivars of kiwifruit. Free radical scavenging assay has high values at the immature stages and decreased during maturation. Similar results in different kiwi cultivars was shown by Hui-Na-Chou *et al.* (2008) and Ming-Wei Sherry (2006). Polyphenols and vitamin C contributes a large portion of the free radical scavenging activity because these are the very strong antioxidants present in the fruits and vegetables (Pal *et al.,* 2015). Total antioxidant activity, total phenolic content and total soluble sugar content increased in the later stages of fruit development (Tosun *et al.,* 2008). Rapid increase in the total sugar was observed from the immature to the early half-ripe stage probably due to intense fruit expansion during the maturity stages in the fruits of pomegranate cultivars. This was followed by a relatively slower but significant accumulation of sugars until the full-ripe stage. The accumulation of simple sugars is one of the processes occurring during the final developmental stages of fruit, resulting in increase in sweetness as fruit approach ripeness (Shwartz *et al*., 2009; Zarei *et al.,* 2011). Ding and Syazwani (2016) reported that TPC varied significantly during fruit ripening in the fruits of MD-2 pineapple cultivar, which is in line with other researchers (Chiniros *et al.*, 2010; Pineli *et al.*, 2011; Tlili *et al*., 2014). In contrast, TPC of jujube (Zozio *et al*., 2014) and mango (Palafox-Carlos *et al*., 2012) fruits decreased with ripening while TPC of papaya fruit increased with ripening (Zuhair *et al*., 2013). The considerable differences of TPC in these fruits during ripening indicated genetic and physiological state could affect the degree of phenolic compounds biosynthetic pathway. The increased level of antioxidant activity during ripening might be a self-defensive response against the effects of oxidative stress (Smirnoff, 1995). Since, antioxidants can scavenge reactive oxygen species, the tissue exhibiting high antioxidant activities would better resist oxidative stress than tissue with lower antioxidant potential (Lester, 2008).

Antioxidant capacity somewhere correlates with some other compounds present in the sample such as total phenols and ascorbic acid. FRAP values in present studies, during ripening correlate with increasing values of ascorbic acid. There are diverse chemical compounds that are present in any fruit which contribute towards its antioxidant activity but the levels of one or two antioxidants in foodstuffs do not necessarily reflect their total antioxidant potential.

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