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Determining optimum harvest stage of khejri pods through phytochemicals, minerals and sensory quality analysis

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ABSTRACT

Khejri pods were harvested 10, 15, 20, 25 and 30 days after pod setting (DAPS) to determine the ideal harvesting stage for fresh consumption and drying purpose. The proximate, phytochemicals, minerals, drying characteristics and sensory analysis were carried out at each stage. The results reveal maximum dry product yield (26.57±0.07% & 27.07±0.02%), rehydration capacity (305.67±1.15% & 269.30±1.52%) and high sensory score for rehydrated pods harvested at 10 and 15 days after setting. Phytochemicals, including total phenols, flavonoids, and total antioxidants were observed maximum in green tender pods; later on, a significant decline was observed in 30 days matured pods. The minerals such as potassium (1.32±0.02%), calcium (1300.76±7 ppm), zinc (31.86±1.2 ppm), and iron (35.60±2.5 ppm) content were observed at 10 days post setting, with a substantial decline as pod development progressed. The highest levels of proteins and fair quantity of crude fibres were also reported in pods harvested during the tender green stages. In conclusion, the study suggests that for getting optimum quality fresh and dried pods coupled with good sensory characters and to harness maximum nutraceutical benefits, khejri pods should be harvested at the tender green stage, specifically between 10-15 days after pod setting.

Key words: *Prosopis cineraria*, pod maturity, rehydration, sensory evaluation, antioxidants.

INTRODUCTION

Prosopis cineraria (L) Druce (Khejri), is a native leguminous tree species of hot arid and semi-arid regions of the Indian subcontinent. It is well-suited for traditional rainfed farming system in the Thar Desert due to its deep taproot system, minimal canopy shade, atmospheric nitrogen fixation, soil organic matter enrichment and non-competitive nature with ground crops for moisture and nutrients (Samadia *et al*., 16). Looking into the importance of khejri tree in supporting traditional farming system, ICAR-CIAH, Bikaner has developed dwarf and regular bearer improved varieties (Thar Shobha and Thar Amruta), vegetative propagation technique and optimized production technology to promote its commercial cultivation. Khejri trees produce tender pods and lush-green foliage during the harsh and scorching period of April and May months. Pods are extensively utilized for dried as well as fresh vegetable and leaf fodder provides protein rich feed for livestock. Dried young pods locally known as 'sangri' are extremely popular for vegetable and a key ingredient in 'panchkoota' a traditional Indian cuisine known for its numerous health benefits. Khejri pods exhibits plentiful functional phytochemicals, particularly polyphenols, tannins, flavonoids, antioxidants, saponins, and alkaloids due to simultaneous exposure to multiple stress conditions during pod development leads to metabolites biosynthesis (Liu *et al*., 11). The medicinal properties associated with khejri pods are antidiabetic, hypoglycaemic, anticancer, anti-inflammatory, and antiasthmatic effects (Kumar *et al*., 10). Recent, studies demonstrated a reduction in serum cholesterol levels in hypercholesterolemic rabbits fed with khejri pods and a significant decrease in low density cholesterol and triglycerides in cricket players after a 21-day intake of khejri pod-based supplements (Ram *et al*., 15; Pareek *et al*., 13). Given the medicinal and economic importance of khejri pods, it is essential to identify the ideal horticultural maturity stage at which the pods exhibit optimal sensory qualities for both fresh consumption and drying, and also accumulate the maximum amount of bioactive metabolites. Hence, the current study was undertaken to determine the optimum harvest stage for khejri pods on the basis of sensory characters, drying, rehydration quality, minerals and phytochemicals analysis.

MATERIALS AND METHODS

Pods were collected from uniform trees of Khejri variety 'Thar Shobha' grown organically in the ICAR-CIAH, Bikaner experimental field. Inflorescences were tagged at bloom onset, and pods were harvested at 10, 15-, 20-, 25-, and 30-days post-setting. At each stage, pods were blanched in

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boiling water for 5 minutes with continuous stirring, then immediately dipped in cold water (10 °C) for 5 minutes to prevent overcooking. The pods were then shade-dried on perforated stainless-steel trays. Dry product recovery (%) was calculated using the following formula: (Fresh pods weight before drying/ dried pods weight) ×100. Dried pods were rehydrated in hot water for 4-hour period and rehydration per cent was calculated by following formula-

$$\text{Rehydration (\%)} = \frac{\text{Rehydrated pods weight}}{\text{Dried pods weight}} \times 100$$

A 10-member expert panel evaluated the color, texture, and taste of rehydrated samples using a 9-point hedonic scale. The panellists assigned scores for rehydrated pods color, taste, and texture, each ranging from 0 to 9. According to the scale, scores below 6.0 were deemed unacceptable, scores between 6 and 7.5 were considered acceptable, and scores from 7.6 to 9 were rated as excellent for vegetable and drying purpose.

The proximate analysis was carried out as procedure outlined by the Association of Official Analytical Chemists (AOAC, 1). The total phenols content was determined with the Folin- Ciocalteu reagent and expressed as mg gallic acids equivalents (GAE/g DW). Total flavonoids content (TFC) was estimated by the aluminium chloride colorimetric assay (Medini *et al*., 11). Total antioxidant activity (TAA) was determined on different scales by *in vitro* systems including cupric ion reducing capacity (CUPRAC); ferric reducing antioxidant power (FRAP), DPPH, and ABTS. CUPRAC and ABTS assay was carried out using ascorbic acid as reference compound (Apak *et al*., 2). FRAP assay was determined following the method described by Benzie and Strain (4). The DPPH scavenging assay was conducted according to the method of Berwal *et al*., (5) with some modifications. The per cent inhibition was calculated by using the following equation:

$$\text{\% Inhibition} = \left(\frac{\text{OD of Control - OD of Sample}}{\text{OD of Control}}\right) \times 100$$

Mineral analysis was conducted following the method outlined by Raghuramulu *et al*., (14) with minor adjustments. The mineral content was estimated using an Atomic Absorption Spectrophotometer (Shimadzu AA-7000), with AAS standard solution of 5% HNO_3 was used as a reference.

The statistical analysis was conducted using Web Based Agricultural Statistics Software Package 2.0 (Jangam and Thali, 9). The results were presented as mean values with their corresponding standard deviations and were subsequently subjected to a one-way analysis of variance (ANOVA), followed by a Duncan test ($p < 0.05$).

RESULTS AND DISCUSSION

The dry product recovery (%) was significantly affected by the pod harvesting stage (Table 1). Pods harvested 10 days after setting yielded 26.57±0.07%, increasing slightly to 27.07±0.02% at 15 days. However, the yield dropped to 23.89±0.21% at 20 days. It then increased significantly, peaking at 28.01±0.13% for pods harvested at 25 and 30 days. The lower yield in early harvests is due to high moisture content, while the peak yield at later stages is attributed to increased dry matter content from fibre, cellulose, and lignin deposition. The decrease at 20 days may result from seed development, which adds fresh weight without a corresponding increase in dry weight. Similar patterns were observed in the dehydration of vegetable-type cluster bean (Gurjar *et al*., 7) and *Phaseolus vulgaris* pods (Ismail *et al*., 8). Rehydration capacity, an indicator of dry product quality, was highest (305.67±1.15%) in pods harvested at 10 days and declined to 211.86±1.07% at 30 days (Table 1). The high rehydration in early stages is due to the porous cell walls, which contain less fibre, cellulose, and lignin, allowing better water absorption. In contrast, pods harvested at 20, 25, and 30 days had reduced porosity and elasticity due to higher fibre and lignin content. Similar findings were reported by Tufekci and Ozkal (17) in their study on dried okra flakes.

Table 1. Changes in physical parameters of fresh pods, drying and rehydration characteristics, and sensory ratings of rehydrated khejri pods

Pod maturity stage (DAPS)	Dehydration and rehydration characteristics		Sensory rating		
	Dry pod recovery (%)	Rehydration (%)	Colour	Taste	Texture
10 days	26.57±0.07^{c}	305.67±1.15^{a}	8.91±0.053^{a}	8.67±0.227^{a}	8.84±0.134^{a}
15 days	27.07±0.02^{b}	269.30±1.52^{b}	8.47±0.049^{a}	8.35±0.147^{a}	8.41±0.183^{a}
20 days	23.89±0.21^{e}	247.48±0.57^{c}	6.60±0.205^{b}	6.42±0.172^{b}	6.24±0.255^{b}
25 days	24.40±0.22^{d}	229.00±1.41^{d}	5.49±0.279^{c}	5.24±0.254^{c}	4.28±0.307^{c}
30 days	28.01±0.13^{a}	211.86±1.07^{e}	3.76±0.251^{d}	3.54±0.354^{d}	2.90±0.189^{d}

*DAPS: Days after pod setting, There is no statistical difference between the means shown with the same letter in the same column ($p > 0.05$)

Pods harvested at 10- and 15-days post-setting were rated highly suitable for use as vegetables and drying, exhibiting excellent quality with high scores 8.91, 8.67 and 8.84 for color, taste, and texture, respectively with no significant differences between these two stages (Table 1). In contrast, rehydrated pods at 20 days maturity displayed a notable reduction in sensory scores (6.24-6.60), making them acceptable for vegetable use, though of inferior quality. The lowest sensory scores (2.90-5.49) were recorded for rehydrated pods at 25- and 30-days maturity, rendering them unacceptable for vegetable purposes. Similarly, Varshitha *et al*. (18) observed high sensory ratings (8.89) in terms of overall acceptability for okra pods harvested after 4 days after anthesis.

The proximate composition in *P. cineraria* pods exhibited significant variation across all maturity stages. The protein content ranges between 15.22±0.10 and 18.79±0.17% and reported maximum (18.79±0.17%) at 20 days followed by 10 days (17.02±0.19%) and minimum at 30 days (15.22±0.10%) maturity (Table 2). Similarly significant dynamic changes were noticed in fat content; found maximum (6.62±0.05%) at 15 days followed by at 10 days (4.35±0.75%) thereafter it was drastically reduced and observed minimum (1.84±0.55%) at 30 days maturity. Crude fibre content was considerably lower in initial pod maturity stages and varied from 13.55±0.48 to 11.77±0.04% between 10- and 25-days maturity. Maximum crude fibre (15.99±0.43%) was observed at 30 days when pods were matured and simple carbohydrates converted to complex polysaccharides. Carbohydrate concentration displayed minimal variation ranging from 51.47±0.15% to 56.52±0.15% from 10 to 30 days maturity. Similarly, ash content showed relatively consistent levels across various developmental stages, with the minimum (4.46±0.03%) observed in 20 days and the maximum (5.01±0.04%) at 30 days maturity. The observed variations in proximate composition across different pod development stages can be attributed to dynamic changes in processes such as photosynthate partitioning, cell division, enlargement, phytochemical conversions, and the trees' responses to abiotic stresses.

During the initial growth phases, total phenols were notably higher compared to the later stages (Fig. 1A). The maximum total phenols (175.83 mg GAE/g DW) were observed at 10 and 15 days (167.37 mg GAE/g DW) maturity with no significant difference between them. However, at 20- and

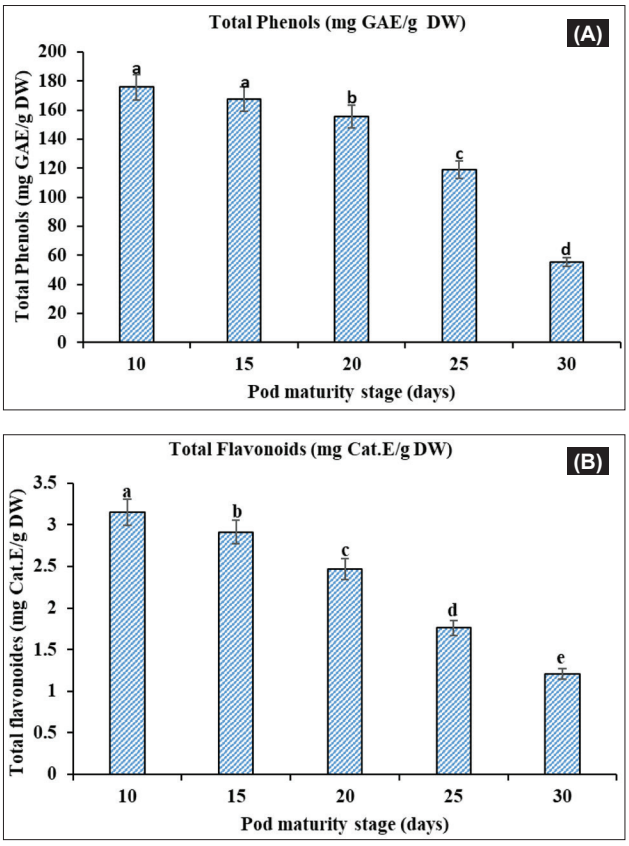

Fig. 1. Changes in total phenols (A) and flavonoids (B) in *P. cineraria* pods development stages

DW: Dry weight; GAE: Gallic acid equivalent; Ct.E: Catechole Equivalent; There is no statistical difference between the means shown with the same letter in the bar diagram (*p >0.05)*.

Table 2. Proximate analysis of khejri pods at different maturity stages

Pod maturity stage (DAPS)	Protein content (%)	Fat content (%)	Crude fiber (%)	Carbohydrate (%)	Ash content (%)
10 days	17.02±0.19^{b}	4.35±0.75^{b}	13.55±0.48^{c}	54.42±0.86^{d}	4.78±0.03^{bc}
15 days	15.79±0.02^{c}	6.62±0.05^{a}	12.44±0.28^{e}	54.42±0.32^{d}	4.64±0.03^{b}
20 days	16.97±0.11^{a}	3.06±0.11^{c}	11.77±0.04^{f}	55.89±0.02^{c}	4.46±0.03^{d}
25 days	16.72±0.01^{b}	2.76±0.57^{d}	12.97±0.37^{d}	58.02±0.53^{a}	4.72±0.05^{c}
30 days	15.22±0.10^{d}	1.84±0.22^{e}	15.99±0.43^{a}	51.47±0.15^{e}	5.01±0.04^{a}

There is no statistical difference between the means shown with the same letter in the same column (*p >0.05)*

25-days maturity, phenols showed a significant reduction to 155.85 and 118.91 mg GAE/g DW, respectively. Subsequently, an extreme decline was reported in total phenols at 30 days and measured 50.42 mg GAE/g DW, marking 3.48 times decrease compared to the initial concentration. Similarly, the total flavonoids exhibited a continuous decrease as the pod's maturity progressed and reported maximum (3.15 mg Ct. E/g DW) at 10 days, followed by a significant decline at 15-, 20-, 25-, and 30-days maturity, with values of 2.91, 2.46, 1.74, and 1.21 mg Ct. E/g DW, respectively (Fig. 1B). The results suggests that both total phenols and flavonoids concentrations are highest during the initial growth phases of pod development, gradually decreasing as the pods mature. Our results are in agreement with the findings of Asati *et al*. (3) who reported high phenols and flavonoids in young pods as compared to matures pods in *P. cineraia* samples collected from variable geography of arid and semi-arid region. The observed decline in both phenols and flavonoids may be associated with the plant's physiological changes during pod development, where the synthesis or accumulation of these compounds decreases. The reduction in total phenolic content as pods mature was attributed to the oxidation of polyphenols catalysed by polyphenol oxidase (Fawole *et al*., 6). The high flavonoid accumulation during initial pod development stages and subsequent decline during maturity stage may be due to the degradation of flavonoids and their possible utilisation in the biosynthesis of other compounds.

The data from antioxidant activity assays, as presented in Fig. 2A, 2B, 2C, and 2D, revealed significant variations among pod maturity stages. The highest antioxidant activity was observed at 10- and 15-days maturity, with no significant difference between the two stages in all the *in vitro* anti-oxidant assays performed. At 10 days maturity, antioxidant activity was measured at 174.68±3.8 mg AAE/g DW, 48.55±2.6 mg AAE/g DW, 97.47±3.1%, and 59.22±2.5% in CUPRAC, ABTS, DPPH and FRAP assays, respectively. Antioxidant activity exhibited a significant decline at 20- and 25-days maturity in CUPRAC and FRAP assays, while DPPH and ABTS assays showed a steady reduction at the same stages. The minimum antioxidant potential was observed in mature pods, *i.e*., at 30 days, in all the assays performed. Our findings align with those of previous researchers who documented high antioxidant activity in both the pods and leaves of *P. cineraria*. Asati *et al*. (3) observed a noteworthy 86.36% scavenging potential for FRAP in the ethanolic extract of immature green pods. These findings suggest that the optimal harvesting time for *P. cineraria* pods, in terms of

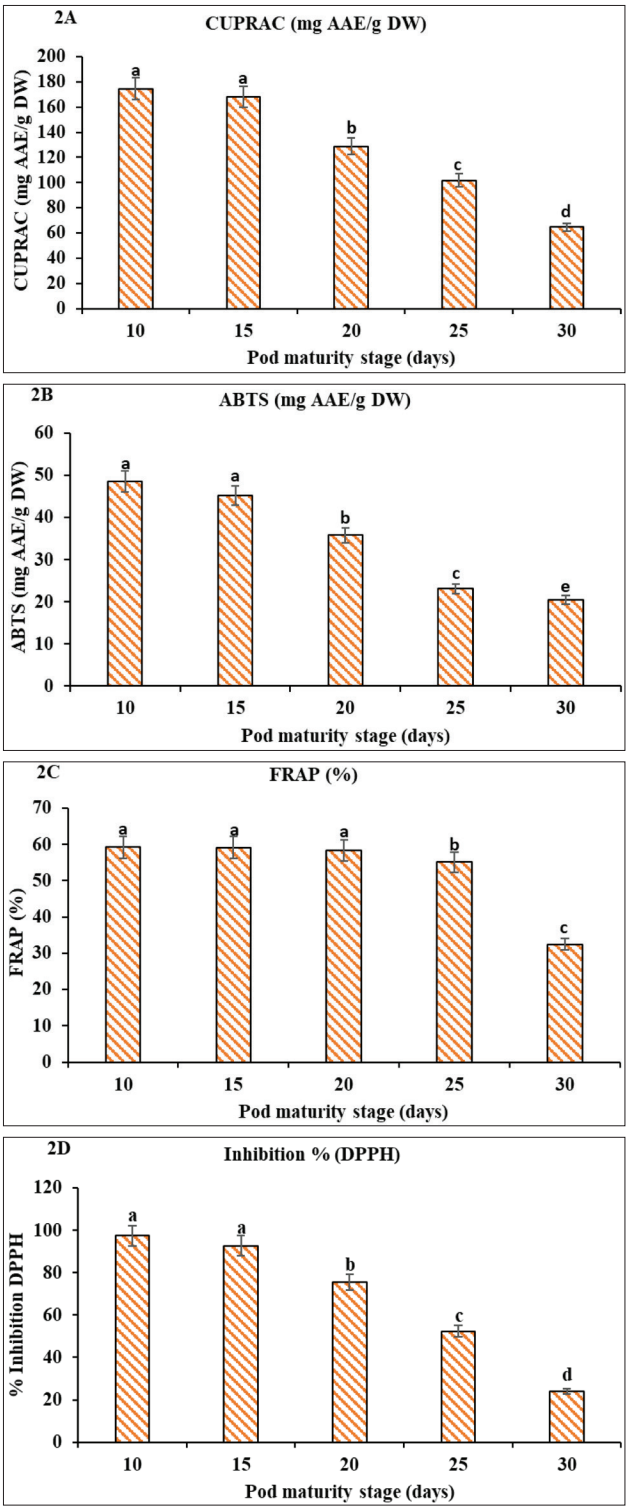

Fig. 2. Changes in total antioxidants activity in different pod maturity stages in khejri pods, TAA: Total antioxidants activity; DW: Dry weight; AAE: Ascorbic acid equivalent; There is no statistical difference between the means shown with the same letter in the bar diagram *(p >0.05)*

Table 3. Variation in minerals content in khejri pods at different maturity stages

Pod maturity stage (DAPS)	K (%)	P (ppm)	Mg (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)	Fe (ppm)	Ca (ppm)	Na (ppm)
10 days	1.27±0.02^{a}	24.10±0.42^{ab}	29.19±0.42^{a}	31.86±1.2^{a}	23.05±0.5^{a}	9.65±0.6^{a}	35.60±2.5^{a}	1300.76±7^{c}	113.05±2.8^{d}
15 days	1.11±0.03^{b}	22.35±0.21^{c}	29.03±0.70^{b}	29.28±0.9^{b}	15.67±0.2^{b}	7.78±0.1^{c}	32.54±1.2^{b}	1105.34±10^{d}	100.51±1.6^{f}
20 days	1.02±0.00^{c}	24.70±0.59^{a}	29.05±0.50^{b}	27.51±1.5^{c}	15.52±0.7^{c}	7.92±0.5^{b}	20.53±1.5^{e}	1378.63±18^{b}	194.79±3.7^{a}
25 days	1.02±0.01^{c}	18.84±0.25^{d}	28.86±0.60^{d}	24.64±0.7^{d}	15.72±0.5^{b}	7.45±0.3^{d}	10.18±0.6^{f}	982.26±10^{e}	152.25±2.4^{b}
30 days	0.83±0.02^{d}	21.72±0.60^{c}	28.95±0.25^{c}	25.10±1.8^{e}	12.77±0.3^{d}	6.90±0.5^{e}	23.33±1.9^{d}	969.04±5^{f}	145.14±1.6^{c}

There is no statistical difference between the means shown with the same letter in the same column (*p >0.05)*

antioxidant content, is during the early developmental stages between 10-15 days after pod setting.

Potassium content was highest at 10 and 15 days of maturity (1.32±0.02% and 1.11±0.03%, respectively), and dropped to 0.83±0.02% at 30 days. Phosphorus peaked at 10 days (24.10±0.42 ppm), decreased at 15 days, increased slightly at 20 days, and reached a low of 18.84±0.25 ppm at 25 days. Magnesium remained stable throughout pod development. Zinc, manganese, copper, and iron concentrations in pods varied with maturity (Table 3). The highest levels of zinc (31.86±1.2 ppm), manganese (23.05±0.5 ppm), copper, and iron (35.60±2.5 ppm) were all observed at 10 days. These levels steadily declined as the pods matured, reaching their lowest at 25 days, with zinc at 24.64±0.70 ppm and iron at 10.18±0.6 ppm. However, iron levels surged again at 30 days to 23.33±1.9 ppm.

The highest calcium content was observed at 20 days (1378.63±18 ppm), followed by 10 days (1300.76±7 ppm) in tender pods. The lowest calcium content (969.04±5 ppm) was estimated in stage 30 days maturity pods. Khejri pods were found to be a rich source of sodium, with significantly high levels (194.79±3.7, 152.25±2.4, 145.14±1.6 ppm) observed at 20-, 25-, and 30-days maturity, respectively, while the minimum sodium content (100.51±1.6 ppm) was found at 15 days.

The elevated mineral content observed in the young pod could be associated with a more efficient nutrient accumulation during the initial stages of pod development, potentially influencing a reduced rate of vegetative-to-generative growth. Calcium, potassium and zinc are pivotal in numerous plant signal transduction pathways as well as in processes such as cell division and the formation of cell walls which justify comparatively higher concentration during early pod growth stages.

The findings reveal that *P. cineraria* pods exhibit substantial proteins, crude fibres and medicinally important phytochemicals during the early stages of development *i.e*. pods harvested between 10-15 days after setting. In addition to the phytochemicals, essential minerals vital for human health, such as potassium, calcium, zinc, and iron, were found to be adequately high during the tender green stage, with the exception of sodium, which was more abundant in mature pods. Likewise, optimum sensory, dehydration and rehydration characters were also observed during initial pod maturity stages. To get optimal quality dry pods and to maximize the medicinal benefits derived from *P. cineraria*, it is recommended to harvest pods between 10 to 15 days after setting.

AUTHORS' CONTRIBUTION

P.S. Gurjar: conceptualization, methodology, writing original draft, D.K. Samadia: conceptualization, editing, resources, M.K. Berwal: investigation, data curation, V.V. Apparao: mineral analysis, A.K. Verma: data curation, H. Ram: formal analysis, resources

DECLARATION

The authors confirm no conflicts of interest with respect to the concerned manuscript.

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