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Bio active compounds and antioxidant activity of dehydrated karonda during storage

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Abstract
The present investigation was conducted at Department of Fruit science laboratory, College of Horticulture, Rajendranagar, during 2017-2018. The experiment was laid out in completely randomised block design. Dehydrated karonda prepared with 4% salt recorded the highest sensory score for overall acceptability (8.33). Ascorbic acid, total carotenoid content, total phenols, total flavonoids, antioxidant activity by FRAP and DPPH radical scavenging activity recorded significant decrease during six months storage period. There was no bacterial and mould growth during storage for six months. Organoleptic scores of 8.27, 8.47, 8.33, 8.27 and 8.33 were recorded for colour, flavour, texture, taste and overall acceptability, respectively on Nine point Hedonic scale. The product showed good overall acceptability at the end of six months storage period (7.07) and was in the "Like moderately" range on Nine point Hedonic scale.

Keywords: Karonda, dehydrated karonda, antioxidant activity, karonda processed products, storage studies

Introduction
Karonda, a hardy, evergreen bush belongs to the family Apocyanaceae. Karonda grows well even on marginal soils and less fertile soils, where most other fruits either fail to grow or give poor performance. It is found throughout India, mainly in the semi arid regions. Karonda can be grown as a hedge plant in the home gardens, fields & orchards. It also serves as Bio fence. (Tripathi *et al.*, 2014) [15]. Karonda is popular in indigenous systems of medicine like Ayurveda, Unani and Homeopathy as a source of several phytochemical constituents. It has good nutritional value and is useful in treatment of several illnesses such as intestinal worms, diarrhea, skin ailments etc. (Kumar *et al.*, 2013) [7]. Though karonda shows medicinal, therapeutic and health benefits, it is not a popular table fruit. Incorporation of karonda into various products may add dietary diversity, income to rural community, insurance towards food security. The research was conducted to study the feasibility of incorporation of Karonda (*Carissa carandas* L.) to process into dehydrated karonda.

Material and Methods
The experiment was conducted at Department of Fruit science laboratory, College of Horticulture, Rajendranagar, Hyderabad and Central Instrumentation Cell, PJTSAU. Mature (Half red half white) karonda fruits were obtained from Fruit Research Station, Sangareddy, SKLTSHU, for research work and all other ingredients from local market. Mature karonda fruits without bruises were sorted for further processing. The selected fruits were washed with tap water and subjected to blanching for three minutes. Fruits were cut into halves and seeds were removed using stainless steel knives.

Ascorbic acid (mg/100g)
Ascorbic acid was estimated by 2, 6 dichlorophenol - indophenol visual titration method (AOAC, 2000) [2]. The method is based on reduction of 2, 6 dichlorophenol – indophenol dye. The dye, which is blue in alkaline solution and red in acidic solution, is reduced by ascorbic acid to a colourless form. The reduction is quantitative and specific for ascorbic acid in solutions in the pH range of 1.0 - 3.5. 5 ml of 3% metaphosphoric acid extract of the sample was taken in a conical flask and titrated with standard dye. The end point was pink, which existed for at least 15 seconds.

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre value x Dye factor x Volume made x 100}}{\text{Aliquot taken x Volume of sample taken}}$$

Total carotenoids

1g (Z) of homogenised sample was saponified with working alcoholic KOH solution and incubated at 37°C for 20 minutes, shaking intermittently. Saponified mixture was then transferred to separating funnel and extracted with petroleum ether. Upper solvent was transferred into separate conical flask and the process of extraction was repeated till a clear white solvent layer was obtained indicating complete extraction. Amount of solvent was measured and noted as "A" ml. OD of solvent was measured as "B" at 450 nm (AOAC, 2000) [2].

$$\text{Total carotenoids (microgram/g sample)} = \frac{4 \text{ x A x B x } 1000}{Z \text{ mg}}$$

Total phenols

Estimation of total phenolic contents was carried out using Folin-Ciocalteu reagent and absorbance was measured at 750 nm in spectrophotometer the results are expressed as Gallic Acid equivalent mg/100gms (GAE mg /100gm). A 0.2ml methanol extract of sample was taken as an aliquot. To this 0.5ml of Folin-Ciocalteu reagent (Diluted 1:1) was added. 10ml sodium carbonate was added to the sample and the content was made upto 12ml with distilled water and incubated for 60 minutes at 37°C. The resulting blue colour complex was measured at 750 nm on a UV spectrophotometer. Total flavonoid content was expressed as gallic acid equivalents. (Kamalaja and Prashanthi, 2016) [6].

$$\textbf{Total Phenols (GAE mg/100g)} = \frac{\text{Standard concentration x Sample OD x Volume made up x 100}}{\text{Standard OD x Aliquot taken x sample weight (g) x 1000}}$$

Total flavonoids

Known volume of methanol extract of sample was taken and volume was made up to 5 ml with distilled water. 0.3 ml of sodium nitrite was added to methanol extract. After 5 minutes, 0.6 ml of 10% aluminium chloride was added and mixed. Then 2ml of 1N sodium hydroxide was added to the mixture. This was followed by the addition of 2.1ml of distilled water to it. Then the solution was mixed well. The absorbance of resultant pink colour was measured at 510nm in a UV visible spectrophotometer against blank. Total flavonoid content was expressed as rutin equivalents. (Kamalaja and Prashanthi, 2016) [6].

$$\text{Total Flavonoids (RE mg/100g)} = \frac{\text{Standard concentration x Sample OD x Total Volume made up x 100}}{\text{Standard OD x Aliquot taken x sample weight (g) x 1000}}$$

DPPH radical scavenging activity

The antioxidant activity was determined by the ability of extract to scavenge DPPH (2, 2- diphenyl-1- picryl-hydrazyl) radical. The reduction of the DPPH radical was determined by measuring the absorption of the resulting oxidised solution at 517 nm against methanol blank. To 1ml of methanol, 3ml of DPPH was added and used as control. Methanol was used as blank. Total Antioxidant capacity of sample by DPPH assay was expressed as Trolox Equivalents mg/100g sample (Kamalaja and Prashanthi, 2016) [6].

$$\text{Percent inhibition} = \frac{AC - AE}{AE} \times 100$$

Where, AC - Absorption of control
AE - Absorption of extract or standard

$$\text{TAC by DPPH assay TE mg/100g} = \frac{\text{Std. Conc. x Sample \% Inhibition x Volume Made Up x 100}}{\text{Sample \% Inhibition x Aliquot Taken x Sample Weight (g) x 1000}}$$

Ferric reducing antioxidant power (FRAP) assay

The procedure described by Benzie and Strain (1996) [3] and modified by (Tadhani *et al*., 2007) [13] was used to evaluate Ferric reducing antioxidant power of karonda and products. The principle of this method is based on the reduction of a ferric-2,4,6-tris (2-pyridyl-s-triazine) complex (Fe^{3+} -TPTZ) to its ferrous coloured form (Fe^{2+} - TPTZ) at acidic pH, in the presence of antioxidants. The results are expressed as FRAP Trolox Equivalent mg/100g (TE mg /100g). Known volume of methanol extract of sample was taken and volume was made up to 0.3ml with distilled water. 1.8ml of FRAP reagent was added and allowed to incubate at 37 °C for 10 minutes. The blue coloured complex was measured at 593nm using double beam U.V. spectrophotometer. Total antioxidant activity by FRAP was expressed as Trolox equivalents (Kamalaja and Prashanthi, 2016) [6].

Microbial count

The media used were Nutrient agar (NA) for Total Bacterial Count and Rose Bengal Agar (RBA) for Total Mould count. All media used were prepared according to the manufacturer's instructions.

Total Bacterial Count

One gram of each sample was homogenized with 9ml of sterilized distilled water. Thereafter, 1ml homogenized sample was serially diluted for 10 times (10^{-1} - 10^{-10}). From each dilution test tube, one ml liquid was spread on to the Nutrient Agar plate. The inoculated plates were inverted and incubated at 37°C for 48 hr. The bacterial colonies were counted with the help of colony counter. Individual colonies were counted and multiplied with the dilution factor to get the microbial population in gram of sample. The plates giving a range between 30 and 300 colonies were considered to be taken into account. The total colony count, referred as colony forming units (cfu) was calculated as below:

$$\text{CFU} = \frac{y}{dx}$$

Where, y = Number of colonies formed
d = Dilution
x = Volume of sample taken

Total mould count

One gram of food sample was homogenized with 9ml of sterilized distilled water. Thereafter, 1ml homogenized

sample was serially diluted for 10 times (10^{-1} - 10^{-10}). From each dilution test tube, one ml sample was spread on to the Rose Bengal agar plate. The inoculated plates were inverted and incubated at 24ºC for 3 - 5 days and the number of colonies were counted.

Organoleptic evaluation

Organoleptic scoring was done by a panel of 15 members using a scorecard for sensory acceptability of 9 points hedonic scale with corresponding descriptive terms ranging from 9 'like extremely' to 1 'dislike extremely', for colour, flavour, texture, taste and overall acceptability (Jones, 1955; Marek *et al.*, 2007) [5, 8] developed for the purpose.

Statistical analysis

The analysis of variance of the data obtained was done by using Completely Randomized Design (CRD) and interpreted.

Results and Discussion

Trials were conducted to develop dehydrated karonda with minimal processing using salt and mature karonda fruits. The process for preparation of dehydrated karonda was standardised and presented in Plate No. 1. The karonda fruit pieces were dehydrated at 55 ºC ± 1 ºC with varying salt concentration. The best acceptable products were subjected to organoleptic evaluation by a panel consisting of 15 members on 9-point hedonic scale and the results are presented in Table No. 1 and Fig. 1. The product was found to be appealing and acceptable, which can be used for masticatory purpose, cooking dal or chutney during off-season. The variations tried are presented in Table No. 1. The best variation was selected for further study, based on sensory evaluation data (Fig. 1).

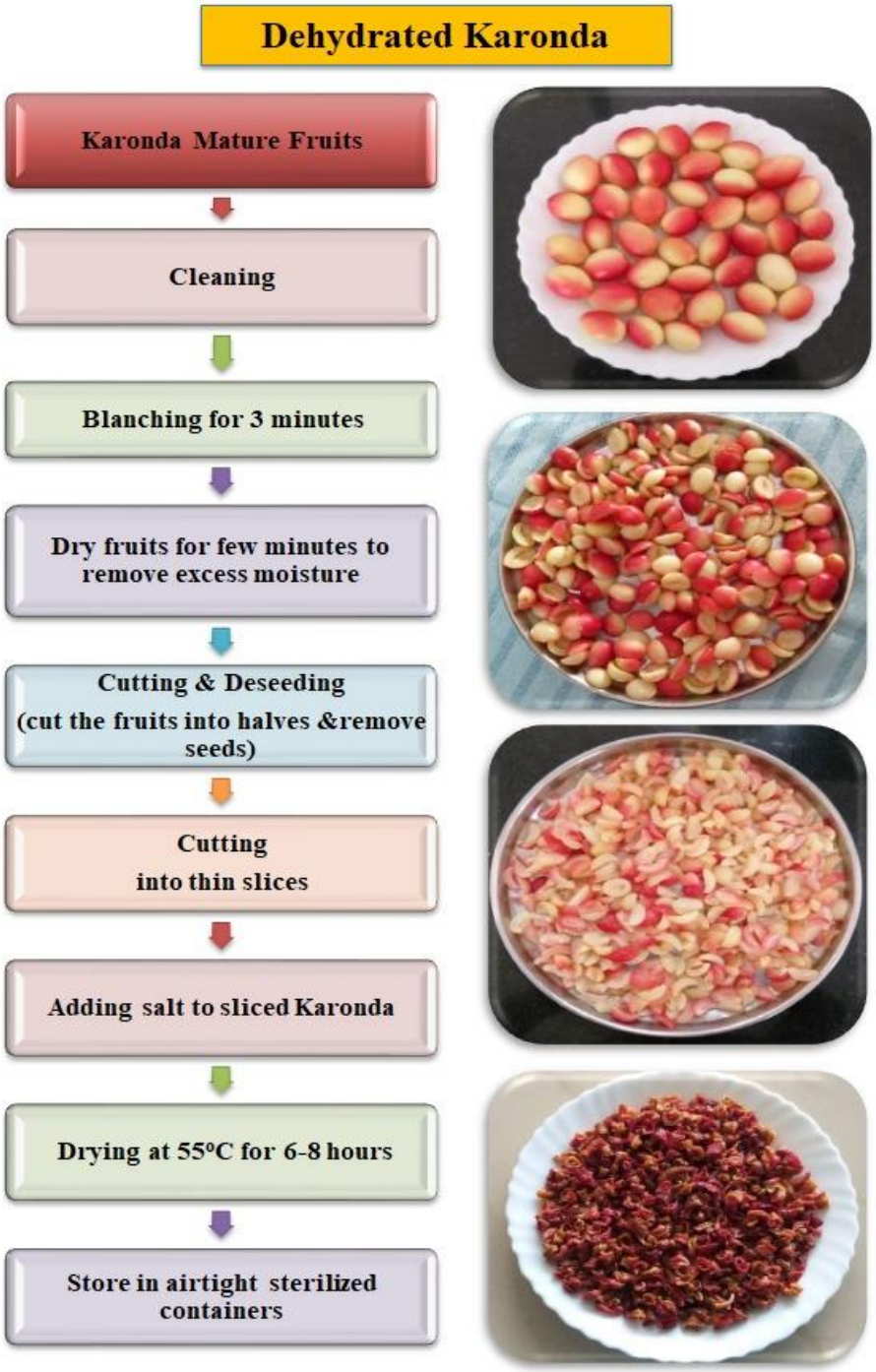

Plate 1: Preparation of Dehydrated Karonda

Dehydrated karonda prepared with 4% salt recorded the highest sensory score for colour (8.27) and was on par with Trial-I but differed significantly from Trial-III for colour. Highest sensory score for flavour was reported in Dehydrated karonda prepared with 4% salt (8.47) which differed significantly from other two variations. Organoleptic evaluation for taste showed the highest score in 4% salt variations (8.27) and differed significantly from other two variations. Dehydrated karonda with 4% salt showed highest overall acceptability (8.33) and is in "Like very much range" on 9 point Hedonic scale. This might be due to the best acceptable colour, flavour, texture and taste. Thus, it was evident that, with minimal processing, postharvest fruit losses can be reduced and there is a possibility to extend availability in off season.

Table 1: Standardization trials of Dehydrated Karonda

Ingredients	Trial 1	Trial 2	Trial 3
Karonda fruit	1000g	1000g	1000g
Salt	20g (2%)	40g (4%)	60g (6%)
Remarks		Accepted for further study based on organoleptic scores	

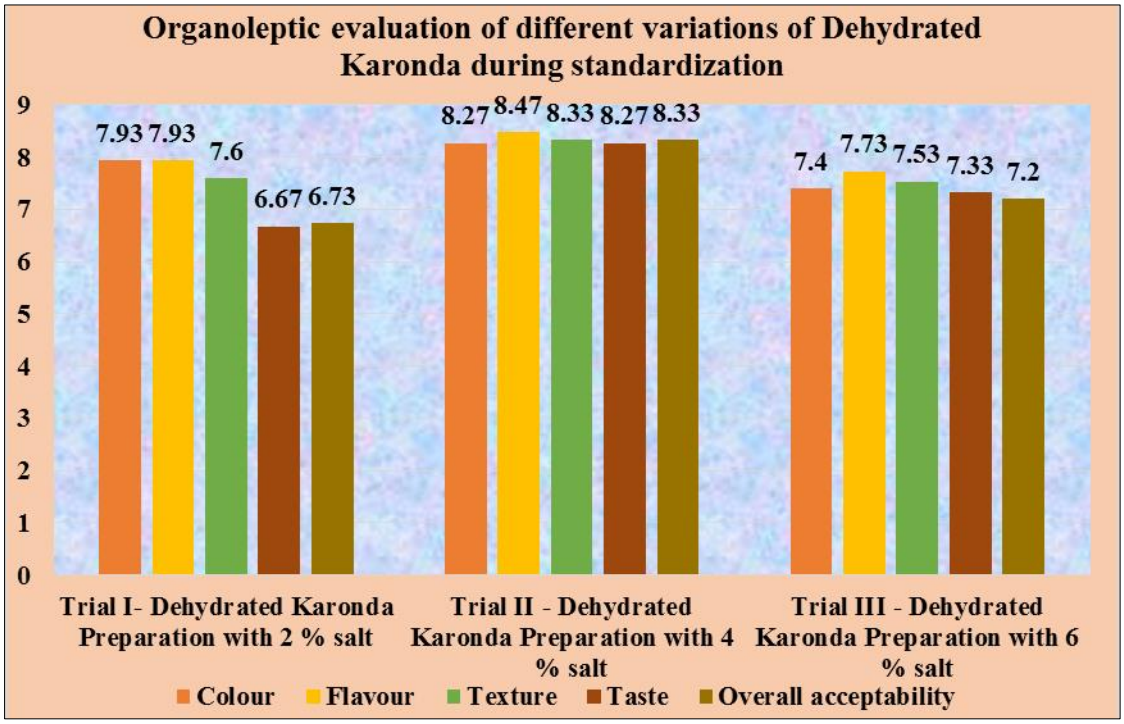

Fig 1: Organoleptic evaluation of different variations of dehydrated karonda during standardization

Storage studies

The best acceptable dehydrated karonda was studied for changes in bioactive compounds and antioxidant activity during storage for six months.

Dehydrated karonda

The changes that occurred in ascorbic acid content during the storage of Dehydrated Karonda are presented in Table No. 2. Ascorbic acid content was highest in dehydrated karonda immediately after preparation (30.80mg/100g), whereas lowest was recorded after six months storage period (17.28 mg/100g). Almost 43% of ascorbic acid was lost during storage. This might be due to degradation of ascorbic acid into dehydroascorbic acid as well as to furfural during storage. Similar results were reported by Pareek and Kaushik (2012) [9], Suhasini *et al.* (2015) [12], Bishnoi *et al.* (2016) [4]. Saxena *et al.* (2016) [11] reported decrease in ascorbic acid content throughout storage of karonda powder for four months and the loss was in the range of 56 to 69 percent.

Total carotenoids (mg/100g)

The changes recorded in total carotenoids during six months storage of dehydrated karonda are presented in Table No. 2. Highest content was recorded in freshly processed dehydrated karonda (0.394 mg/100g). It was observed that total carotenoid content decreased significantly during the third month and continued upto six months. Lowest content was recorded at the end of six months (0.351 mg/100g). This decrease might be due to auto oxidation of carotenoids, leading to loss of total carotenoids and due to its highly unsaturated chemical structure, which made it very susceptible to thermal degradation and oxidation. Similar results were reported by Pop *et al.* (2016) [10] who demonstrated that significant losses in total carotenoid content occurred during the storage of dried apricots.

Total phenols (GAE mg/100g)

The changes recorded in total phenols during the storage of dehydrated karonda for six months are presented in Table No.2. Highest content was recorded in freshly processed dehydrated karonda (260.74mg/100g). Total phenol content decreased significantly from fourth month storage and continued upto six months (188.20 mg/100g). Oxidation and polymerization reactions might have resulted in reduction of phenolic content.

Similar results of decreasing trend of total phenols content

were reported by Bishnoi *et al.* (2016) [4] in (frozen and dehydrated aonla pulp) during six months storage period. Thakur *et al.* (2018) [14] also reported a similar significant decrease in total phenols during storage of wild aonla preserve for six months.

Total flavonoids (RE mg/100g)

The total flavonoids during the storage of dehydrated karonda for six months was estimated and presented in Table No. 2. Highest total flavonoids content (525.83mg/100g) was recorded in freshly prepared dehydrated karonda and decreased significantly from second month of storage till six months(327.93mg/100g). The decrease in flavonoids content may be due to storage at ambient temperature.

Ferric reducing antioxidant power assay (FRAP Assay) (TE mg/100g)

The antioxidant activity was measured using FRAP assay during the storage of dehydrated karonda for six months and presented in Table No. 2. Highest FRAP value (36.39 TE mg/100g) was recorded at 0 month and decreased significantly from the fourth month of storage till six months (28.56 TE mg/100g). This significant decrease might be due to decrease in ascorbic acid, total phenols, total carotenoids and total flavonoids.

DPPH (TE mg/100g)

The antioxidant activity was measured using DPPH free radical scavenging activity and expressed as Trolox equivalents. Results of storage of dehydrated karonda for six months was recorded and presented in Table No. 2. Highest antioxidant activity (350.80 TE mg/100g) was recorded at 0 month and decreased significantly from the fourth month of storage till six months (255.14 TE mg/100g). However, maximum decrease in antioxidant activity was observed in the sixth month of storage. This significant decrease might be due to decrease in ascorbic acid, total phenols, total carotenoids and total flavonoids.

Table 2: Changes in Bioactive compounds and antioxidant activity of Dehydrated Karonda during storage

Storage Period	Bioactive compounds and antioxidant activity of Dehydrated Karonda					
	Ascorbic acid (mg/100g)	Total Carotenoids (mg/100g)	Total Phenols (GAE mg/100g)	Total Flavonoids (RE mg/100g)	FRAP (TE mg/100g)	DPPH (TE mg/100g)
0 Months	30.80	0.394	260.74	525.83	36.39	350.80
1 Month	29.64	0.392	260.15	514.20	36.29	345.54
2 Months	29.42	0.391	253.80	498.52	36.18	340.95
3 Months	29.02	0.384	246.57	465.21	34.84	336.40
4 Months	26.41	0.374	228.51	427.91	32.94	309.30
5 Months	22.60	0.365	210.86	383.30	30.65	281.83
6 Months	17.28	0.351	188.20	327.93	28.56	255.14
S.Em+	0.69	0.001	5.40	4.82	0.55	5.15
C.D. at (5%)	2.10	0.003	16.55	14.77	1.69	15.76

Moisture content (%)

Moisture content of dehydrated karonda was studied at monthly intervals and presented in Table No. 3. Moisture content of freshly prepared dehydrated karonda was found to be 6.56%. Though moisture content of the product increased throughout the storage period, significant difference was observed only after two months of storage period.

Similar results of significant increase was reported by Suhasini *et al.* (2015) [12] in osmotically dehydrated karonda during storage period for 4 months. Similar results were reported by Saxena *et al.* (2016) [11] in karonda powder and revealed that moisture content increased during storage for 4 months.

Total bacterial count (CFU/ml): Dehydrated karonda was tested for bacterial growth at monthly intervals and presented in Table No. 3. The results revealed that there was no bacterial growth throughout the study period. This may be due to low moisture content of product and hygienic processing and storage conditions.

Total Mould Count (CFU/ml)

Dehydrated karonda was tested for mould growth at monthly intervals and presented in Table No. 3. The results revealed that there was no mould growth throughout the study period. This may be due to low moisture content of product and hygienic processing and storage conditions. Similar results were reported by Saxena *et al.* (2016) [11] in karonda powder and reported no yeast and mould growth during storage of sun-dried and cabinet-dried samples for 4 months.

Table 3: Moisture content, Total Bacterial and Mould count of Dehydrated Karonda during storage

Storage Period	Moisture content, Total Bacterial and Mould count of Dehydrated Karonda		
	Moisture Content (%)	Total Bacterial Count (Log CFU/g)	Total Mould Count (Log CFU/g)
0 Months	6.56	0.00	0.00
1 Month	6.63	0.00	0.00
2 Months	6.73	0.00	0.00
3 Months	6.83	0.00	0.00
4 Months	6.95	0.00	0.00
5 Months	7.03	0.00	0.00
6 Months	7.17	0.00	0.00
S.Em+	0.06	0.00	0.00
C.D. at (5%)	0.18	0.00	0.00

Organoleptic evaluation of dehydrated karonda

After confirming microbiological safety of dehydrated karonda through TBC and TMC at monthly intervals, the dehydrated karonda was subjected to organoleptic evaluation by a panel of 15 members, on a 9-point hedonic scale at monthly intervals and presented in Fig. 2. The results revealed that sensory scores for colour, flavour, texture, taste and overall acceptability decreased with advancement of storage period. The colour of the product was maintained upto three months, but showed significant decrease during later period of storage. The product showed little or no difference in taste and overall acceptability upto three months but decreased significantly in later period of storage. The decreasing trend of overall acceptability of the product might be due to decrease in colour scores and flavour of the product during storage. Even though organoleptic scores decreased when compared to fresh product, dehydrated karonda showed good overall acceptability at the end of six months storage period (7.07) and was in the "Like moderately" range.

Similar results were reported by Suhasini *et al.* (2015) [12] for overall acceptability of osmotically dehydrated slices of karonda during four months of storage. Ankush *et al.* (2019) [1] also reported that the appearance and overall acceptability scores decreased significantly in solar dried ber, aonla flakes and bael slices after 3 months of storage at ambient conditions.

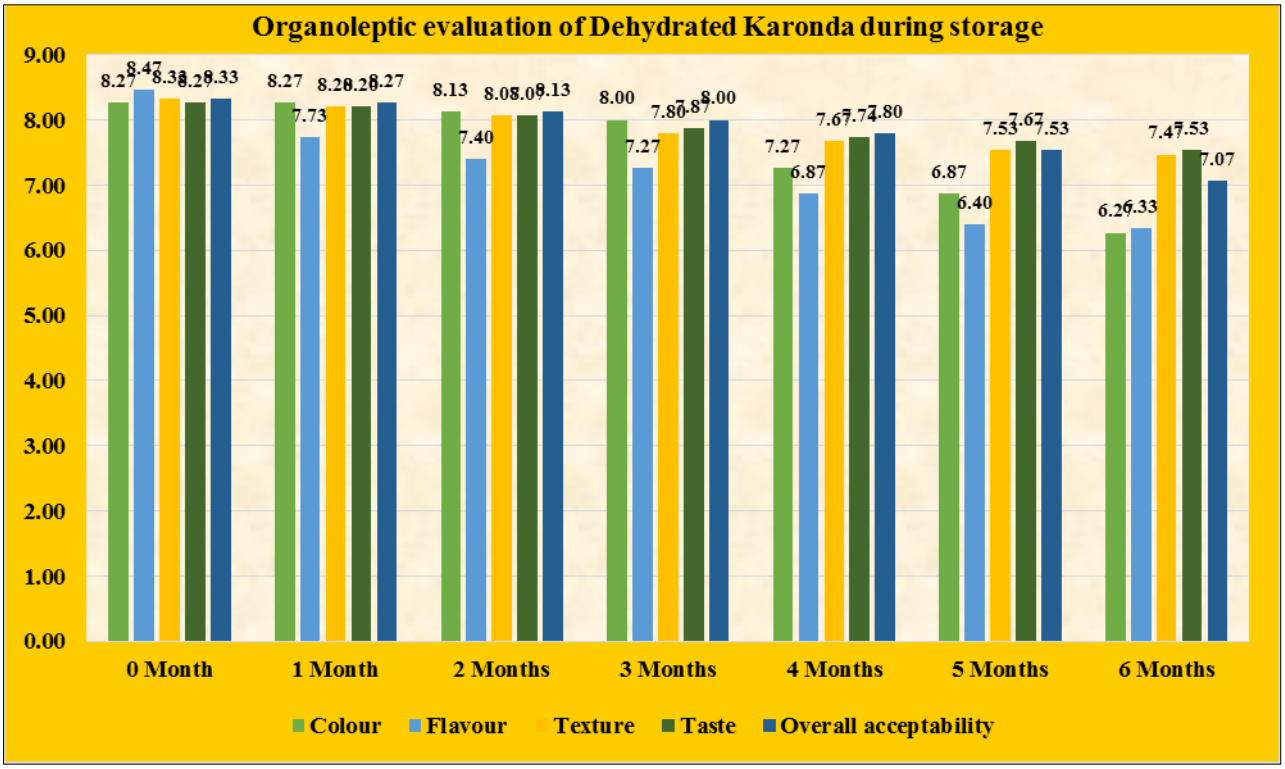

Fig 2: Organoleptic evaluation of Dehydrated Karonda during storage

Conclusion

The results revealed that karonda fruits can be processed into acceptable and safe product through simple technique. The product can be stored safely for six months with acceptable sensory scores. The nutritional quality, microbial safety, bio active compounds, antioxidant activity and organoleptic scores revealed the possibility of dehydration of Karonda with minimal processing technique.

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References

1. Ankush, Aggarwal RK, Sharma R, Bhardwaj SK. Changes in physico-chemical and sensory attributes of some wild fruits dried in indirect solar dryer. Int. J Chem. Stud 2019;7(2):1388-1392.
2. AOAC. Official methods of analysis. Association of Analytical chemists. Washington, D.C. 17th edition 2000.
3. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Analytical Biochemistry 1996;239:70-76.
4. Bishnoi JP, Gehlot R, Siddiqui S. Change in chemical constituents and overall acceptability of frozen and dehydrated Aonla fruit pulp during storage. Journal of Applied and Natural Science 2016;8(3):1615-1617.
5. Jones LV, Peryam DR, Thurstone LL. Development of a scale for measuring soldiers food preferences. Food Research 1955;20:512-520.
6. Kamalaja T, Prashanthi M. Manual on bioactive compounds in foods-Analysis, AICRP-Home science, Foods and Nutrition component, PJTSAU, Rajendranagar, Hyderabad 2016.
7. Kumar S, Gupta P, Gupta VKL. A critical review on Karamarda (*Carissa carandas* Linn) International Journal of Pharmaceutical and Biological Archives 2013;4(4):637-642.
8. Marek S, Stainslaw K, Piotr T, Marek S. Rheological and sensory properties of dessert sauces thickened by starch-xanthan gum. Journal of Food Engineering 2007;79:1144-1151.
9. Pareek S, Kaushik RA. Effect of drying methods on

quality of Indian gooseberry (*Emblica officinalis* G.) powder during storage. Journal of scientific and industrial research 2012;71:727-732.
10. Pop AE, Bunea A, Florina C, Carmen S, Adela P. Stability of carotenoids in dried Apricots (*Prunus armeniaca* L.) during storage. Bulletin UASVM Food Science and Technology 2016;73(1):93-98.
11. Saxena D, Misra KK, Rai R. Studies on suitability of cultivars, picking dates and drying methods for the preparation of karonda (*Carissa carandas* L.) fruit powder. Indian Journal of Horticulture 2016;73(2):267-273.
12. Suhasini L, Vanajalatha K, Padmavathamma AS, Rao PV. Physico-chemical properties of osmotically dehydrated Karonda (*Carissa carandas* L.). The Asian Journal of Horticulture 2015;10(1):53-59.
13. Tadhani MB, Patel VH, Subhash R. In vitro antioxidant activities of Stevia rebaudiana leaves and callus. Journal of Food Composition and Analysis 2007;20(3):323-329.
14. Thakur NS, Thakur N, Thakur HA, Kumar P. Standardization of method for the preparation of Preserve from Wild Aonla (*Phyllanthus emblica* L.) fruits and its quality evaluation during storage. Int. J Chem. Stud 2018;6(1):1841-1847.
15. Tripathi PC, Karunakaran G, Sankar V, Senthil Kumar R. Karonda - A Potential fresh fruit of future. Technical Bulletin 7/2014, ICAR-IIHR, Central Horticultural Experiment Station, Chettalli, Kodagu, Karnataka 2014,14p.