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Evaluation of nutritional quality and microbial safety of karonda squash during storage

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Abstract
The research experiment was carried out to evaluate changes in nutritional quality, microbial safety and organoleptic scores of karonda squash during storage for six months. No significant change is recoded in pH whereas significant increase in TSS was recorded from third month of storage to six months. Titrable acidity, total and reducing sugars showed increasing trend during storage. There was no bacterial and mould growth during storage for six months. The organoleptic scores remained in acceptable range "Like moderately" at the end of storage period. The nutritional value, microbial safety and overall acceptability revealed the possibility of incorporating karonda fruits for preparation of squash.

Keywords: Karonda, squash, processing, value added products, nutritional quality, storage studies

Introduction
Potential underutilized tropical fruits of India like aonla, karonda, jamun, bael, carambola and phalsa are the only source of protective foods to meet the vitamins and minerals requirements of people living in villages. These fruits have been used in Indian system of medicine such as Ayurvedic and Unani since time immemorial due to their curative properties. Fruits have attractive colour, karonda is drought tolerant and India has a rich heritage of indigenous fruit types (Mitra *et al*., 2010) [15]. Karonda (*Carissa carandas*) belongs to the family Apocyanaceae with attractive Pink-white fruits at maturity. Ripe fruits have attractive colour, excellent flavour, source of bioactive compounds and antioxidants but cannot be relished in large quantities in fresh state. Fruits are seasonal, available mostly during July to September, perishable and can be stored in fresh condition only for three to five days. Value addition technology plays an important role in exploiting nutritional and therapeutic value of karonda. Karonda has been identified as a new addition to the available tropical fruit range with multiple uses (Arif *et al*., 2016) [3]. Under-exploited fruits play a very crucial role in supplementing the diet of the native people of India and value addition in karonda is one of the predominant opportunities for rural communities for sustainable livelihoods (Srivastava *et al*., 2017) [19]. Karonda is a useful food and medicinal plant of India, found to be widely distributed throughout subtropical and topical regions. The plant has been used as a traditional medicinal plant in Ayurvedic, Unani, and Homoeopathic system of medicine. The major bioactive constituents, which impart medicinal value to the herb, are alkaloids, flavonoids, saponins and large amounts of cardiac glycosides, triterpenoids, phenolic compounds and tannins (Virmani *et al*., 2017) [21]. The root, leaves, flowers and fruits of karonda showed many traditional values, pharmacological uses and phytochemical constituents, traditionally used to treat intestinal worms, scabies, biliousness, pruritus, snake bite poisoning, anemia, stomach ache, mouth ulcers, astringent, diarrhoea, ear ache, rheumatism, anthelmintic, anorexia, female libido and sore throat (Tesfaye and Ravichandran, 2018) [20].

Material and Methods
The experiment was conducted at Department of Fruit science laboratory, College of Horticulture, Rajendranagar, Hyderabad and Central Instrumentation Cell, PJTSAU. Ripe karonda fruits were obtained from Fruit Research Station, Sangareddy, SKLTSHU for research work and all other ingredients from local market.

pH: pH was determined using pH meter. pH meter was calibrated with the help of standard buffer solutions (pH 4.0 and 7.0).10 g sample was macerated with 100 ml distilled water.

The mixture was agitated and allowed to stand for 30 minutes. The supernatant was used to determine pH.

TSS (^{0}Brix): Total soluble solids were recorded with digital Refractometer (Model: HI 96801 Refractometer 0-85 ^{0}Brix Hanna Instrument).

Total and Reducing sugars (%): The titrimetric method as described by Ranganna (1986) [17] was adopted for the estimation of total and reducing sugars using Fehling A and B.

$$\text{Reducing sugar (\%)} = \frac{\text{Mg of invert sugar} \times \text{dilution} \times 100}{\text{Titre} \times \text{weight of the sample} \times 100}$$

$$\text{Total sugars as invert sugars (\%)} = \frac{\text{Factor x Volume made up x Dilution x 1000}}{\text{Titre value x Weight of sample taken}}$$

Titrable Acidity (%): Titrable acidity of the sample was measured by titrating a given sample against a standard alkali solution of known concentration using phenolphthalein as an indicator to a light pink colour. 10 g of homogenized sample was taken and made up to 100 ml in a volumetric flask. The contents were filtered through Whatman No.1 filter paper. 10 ml of aliquot was taken into a conical flask and titrated against standard solution of sodium hydroxide (0.1 N NaOH), using phenolphthalein as an indicator. The acidity was expressed in terms of percent citric acid equivalent adopting the following formula (AOAC, 2000) [2].

Ascorbic acid (mg/100 g): 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.

$$\text{Ascorbic acid (mg/100 g)} = \frac{\text{Titre value x Dye factor x Volume made x100}}{\text{Aliquot taken x Volume of sample taken}}$$

Microbial load: 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 9 ml of sterilized distilled water. Thereafter, 1 ml 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 ^{o}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 9 ml of sterilized distilled water. Thereafter, 1 ml 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 24 ^{o}C for 3 - 5 days and the numbers 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, taste, texture, and overall acceptability (Jones, 1955 [8] and Marek *et al*., 2007) [13] developed for the purpose. Score card was prepared keeping in view of the quality characteristics of the products. Descriptive terms were given to various quality attributes like Texture, colour, taste, flavour and overall acceptability.

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

Results and Discussion

The best acceptable karonda squash based on organoleptic evaluation was studied for changes in chemical composition, moisture content, microbial count and organoleptic evaluation during storage for six months.

1. pH

The changes occurred in pH during the storage of karonda squash during six months storage are presented in Table 1. The pH of karonda squash was 3.03 immediately after preparation. A gradual change in pH was recorded during storage. Though pH of karonda squash decreased from 3.03 and reached 2.96 at the end of six months, significant difference was not observed throughout storage. Similar findings were reported by Karpagavalli and Amutha (2015) [10] in pomegranate squash for 180 days and Akubor *et al*. (2017) [1] in pineapple squash stored for 6 months.

2. Total Soluble Solids (^{0}Brix)

The changes recorded in TSS during the storage of karonda squash during six months storage are presented in Table 1. TSS 52.17 ^{0}Brix was recorded at the beginning of storage and showed increase in TSS throughout the storage period. Significant increase was recorded from third month of storage to six months (53.83 ^{0}Brix) at ambient temperature. The increase in TSS content of squash during storage might be due to the degradation of polysaccharides like starch, cellulose, pectins into simple sugars in the presence of organic acids (Kumar and Deen, 2018) [12]. Similar findings were reported

by Karpagavalli and Amutha (2015) [10] in pomegranate squash stored for 180 days, Mohire *et al*. (2016) [16] in karonda syrup during 3 months storage period, Chaudhary *et al*. (2017) [6] in storage of mango squash for five months, Kavitha and Goud (2018) [11] in ber squash during 90 days storage and Kumar and Deen (2018) [12] in wood apple squash during storage period for five months while Akubor *et al*. (2017) [1] observed decrease in TSS in pineapple squash stored for 6 months.

3. Titrable acidity (%)

The titrable acidity during the storage of karonda squash for six months was recorded and presented in Table 1. Titrable acidity of karonda squash showed increasing trend during storage period for six months. Titrable acidity of freshly prepared squash was 1.42% and later increased to 1.50% during storage. However, no significant change in titrable acidity was observed during initial two months of storage, but significant increase was observed during the latter part of storage, which could be due to degradation of pectic substances which in turn was responsible for increase in acidity in fruit products (Bajpai and Vasure, 2017) [4]. Similar results were reported by Bajpai and Vasure (2017) [4] in karonda squash, Karpagavalli and Amrutha (2015) [10] in pomegranate squash stored for 180 days, Harshita *et al*. (2016) [7] in mango ready-to-serve drink and squash for three months storage period, Mohire *et al*. (2016) [16] in karonda syrup during 3 months of storage period, Kumar and Deen (2018) [12] in wood apple squash and Akubor *et al*. (2017) [1] in pineapple squash stored for 6 months. However, Kavitha and Goud (2018) [11] reported significant decrease of acidity in ber squash during storage for 90 days.

4. Total Sugars (%)

The total sugar content in karonda squash during the storage of six months was recorded and presented in Table 1. Total sugar content of freshly prepared squash was 48.06%, whereas it increased to 50.71% at the end of storage period. It was observed that there was an increase in total sugar content throughout the storage period. The total sugars increased significantly with increase in storage duration from fifth month onwards, which might be due to hydrolysis of polysaccharides like pectin, starch etc. into simple sugars and inversion of non-reducing sugar to reducing sugars. Similar results were reported by Harshita *et al*. (2016) [7] in mango ready-to-serve drink and squash stored for three months, Mohire *et al*. (2016) [16] in karonda syrup during 3 months of storage period, Chaudhary *et al*. (2017) [6] in storage of mango blended squash during five months storage period, Kavitha and Goud (2018) [11] in ber squash and Kumar and Deen (2018) [12] in wood apple squash and during storage.

5. Reducing sugars (%)

The reducing sugars during the storage of karonda squash for six months was recorded and presented in Table 1. Reducing sugar content of 12.48% was recorded in freshly prepared karonda squash and increased significantly from fifth month of storage till six months (16.40%). The reducing sugars increased significantly with increase in storage duration, which might be due to hydrolysis of polysaccharides and inversion of non-reducing sugar to reducing sugars. Similar results were reported by Karpagavalli and Amutha (2015) [10] in pomegranate squash stored for 180 days, Harshita *et al*. (2016) [7] in mango ready-to-serve drink and squash stored for three months, Mohire *et al*. (2016) [16] in karonda syrup stored for 3 months, Chaudhary *et al*. (2017) [6] in mango blended squash, which increased constantly from 48.15 to 50.70% during five months storage period.

6. Ascorbic acid (mg/100 g)

The changes that occurred in ascorbic acid content during the storage of karonda squash during storage are presented in Table 1. Ascorbic acid content was highest in karonda squash immediately after preparation (9.19 mg/100g), whereas lowest was recorded after a six-month storage period (4.98 mg/100g). Almost 45.8% of ascorbic acid was lost during storage. Oxidation by trapped oxygen in glass bottles may be the reason for reduction in ascorbic acid content of the drink (Kumar and Deen, 2018) [12]. Similar results were reported by Karpagavalli and Amutha (2015) [10] in pomegranate squash stored for 180 days, Harshita *et al*. (2016) [7] in mango ready-to-serve drink and squash stored for three months, Mohire *et al*. (2016) [16] in karonda syrup stored for 3 months, Akubor *et al*. (2017) [1] in Pineapple squash during 6 months storage, Chaudhary *et al*. (2017) [6] in mango blended squash during five months storage period, Kavitha and Goud (2018) [11] in ber squash during 90 days storage and Kumar and Deen (2018) [12] in wood apple squash.

Table 1: Changes in chemical properties of Karonda Squash during storage

Storage Period	Chemical properties of Karonda Squash					
	pH	TSS (º Brix)	Titrable acidity (%)	Total Sugars (%)	Reducing Sugars (%)	Ascorbic acid (mg/100 g)
0 Months	3.03	52.17	1.42	48.06	12.48	9.19
1 Month	3.03	52.27	1.42	48.08	12.53	9.13
2 Months	3.02	52.40	1.43	48.13	12.77	8.64
3 Months	3.01	52.60	1.44	48.19	13.78	8.04
4 Months	2.99	52.93	1.48	48.87	14.86	7.18
5 Months	2.98	53.37	1.48	49.98	15.77	6.17
6 Months	2.96	53.83	1.50	50.71	16.40	4.98
SEm±	0.02	0.09	0.01	0.35	0.13	0.20
C.D. at (5%)	NS	0.27	0.02	1.06	0.40	0.62
C.V.	0.94	0.29	0.54	1.23	1.60	4.62

7. Moisture content (%)

Moisture content of karonda squash was studied at monthly intervals and presented in Table 2. Moisture content of freshly prepared karonda squash was found to be 37.71%. Significant decrease in moisture content was observed after two months storage and moisture content at the end of storage period and was found to be 36.31%. Similar results were reported by Wani *et al*. (2013) [22] in karonda jam, Kanwal (2017) [9] in guava jam and Singh and Saxena (2019) [18] in karonda jelly for a period of nine months.

8. Total Bacterial Count (TBC)

Karonda squash was tested for bacterial growth at monthly intervals and presented in Table 2. The results revealed that there was no bacterial growth throughout the study period. High sugar content of squash and hygienic processing and storage conditions might have resulted in microbiologically safe squash. Akubor *et al*. (2017) [1] reported an increase in the total plate count from 10 cfu/g to 30 cfu/g, which is within permissible limit during six months storage period of pineapple squash. Kumar and Deen (2018) [12] reported that the microbial growth in wood apple squash during storage showed an increase up to two months of storage at ambient temperature and thereafter showed continuous decrease. The increase in the sugar content and acidity in squash might be responsible for decrease in microbial growth at later stages of storage.

9. Total Mould Count (TMC)

Karonda squash was tested for mould growth at monthly intervals and presented in Table 2. The results reveal that there was no mould growth throughout the study period. High sugar content of squash, acidity and hygienic processing and storage conditions might have resulted in microbiologically safe squash. Similar results were reported by Singh and Saxena (2019) [18] in karonda Jelly. Brandao *et al*. (2018) [5] showed yeast and filamentous fungi growth in functional mixed cerrado fruit jam, but the results, during storage and at both temperatures, were within the standards required by law.

Table 2: Moisture content, total bacterial and mould count of karonda squash during storage

Storage Period	Moisture content, Total Bacterial and Mould count of Karonda Squash		
	Moisture Content (%)	Total Bacterial Count (Log CFU/g)	Total Mould Count (Log CFU/g)
0 Months	37.71	0.00	0.00
1 Month	37.68	0.00	0.00
2 Months	37.57	0.00	0.00
3 Months	37.39	0.00	0.00
4 Months	37.15	0.00	0.00
5 Months	36.74	0.00	0.00
6 Months	36.31	0.00	0.00
SEm±	0.09	0.00	0.00
C.D. at (5%)	0.29	0.00	0.00
C.V.	0.44	0.00	0.00

10. Sensory evaluation of Karonda Squash

Microbiological safety of karonda squash was established through TBC and TMC at monthly intervals. The microbiologically safe karonda squash was subjected to organoleptic evaluation by a panel of 15 members, on a 9-point hedonic scale at monthly intervals and presented in Fig.1. The results reveal that the prepared product, immediately after preparation, scored 8.67, 8.07, 8.0, 8.40 and 8.33 for colour, flavour, texture, taste and overall acceptability, respectively. The product showed "like very much" range on Nine-point Hedonic scale. The storage studies revealed that sensory scores for colour, flavour, texture decreased with advancement of storage period but showed significant difference only after four months storage. However, the product showed acceptable score for colour (8.00), flavour (7.33) and texture (7.20) at the end of 6 months storage period. The results revealed that sensory scores for taste and overall acceptability decreased with advancement of storage period but showed significant difference only after three months storage. However overall acceptability score of squash remained in "Like moderately to Like very much range" throughout the storage period. Similar results were reported by Chaudhary *et al*. (2017) [6] in mango and aloevera squash, Kavitha and Goud (2018) [11] in ber squash, Mishra *et al*. (2017) [14] in squash prepared from a blend of mango pulp and aloe vera gel and Kumar and Deen (2018) [12] in wood apple squash.

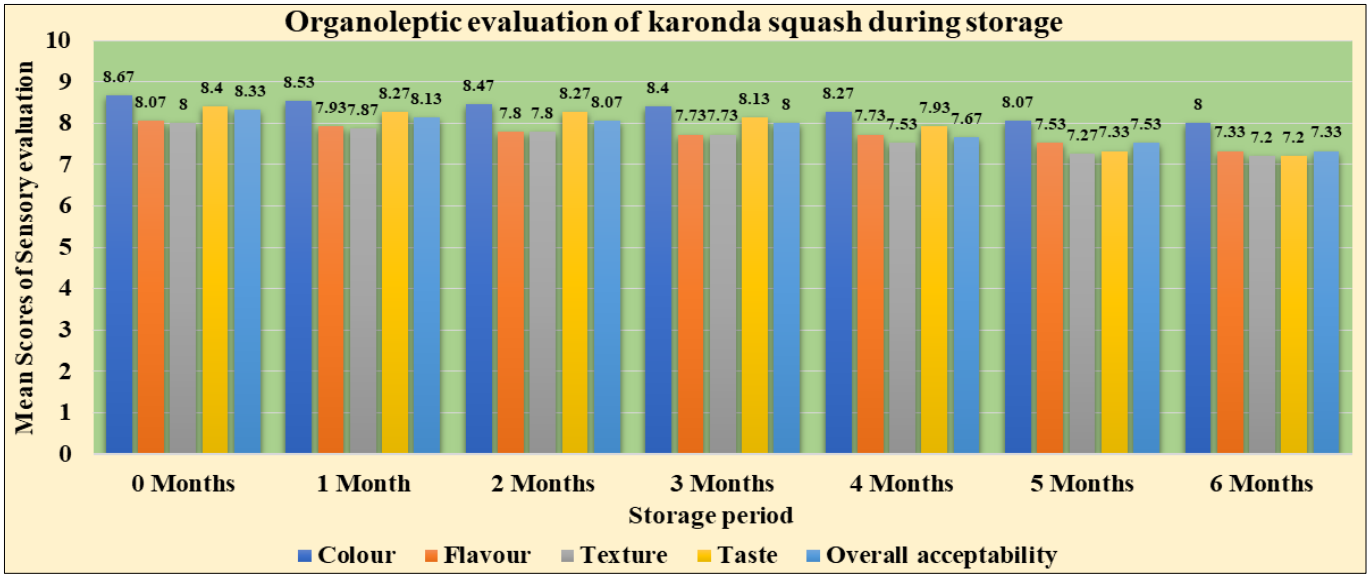

Fig 1: Organoleptic evaluation of karonda squash during storage

Conclusion

The results revealed that karonda fruit can be processed into squash which can be stored safely for about six months with acceptable sensory score. Nutritional quality, Organoleptic evaluation and microbiological safety results show a promising future of incorporating karonda in preparation of value-added products like squash in the processing industry.

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