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Changes in physico-chemical characteristics of kokum (*Garcinia indica*) based RTS beverage during storage

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Abstract

Kokum (*Garcinia indica*) is one of the important indigenous tree spice crops originated and grown in Western Ghats of India. The shelf life of the fruit is only about one week and the fruit is mainly used for culinary purposes. The rich bioactive profile of kokum makes it a highly nutritious and desirable fruit crop. Three kokum based Ready to Serve (RTS) juices were standardized and evaluated for quality changes during storage under ambient and refrigerated conditions. During storage, there was an increase in total soluble solids, titratable acidity, reducing sugars and total sugars under both the storage conditions. However, a decrease in pH was observed during storage for all the treatments. No indications of microbial growth were observed till 60 days of storage, however, after 60 days of storage few colonies of microbial growth could be detected under both the storage conditions. The study concluded that towards the end of storage for 75 days kokum RTS blended with roasted powdered cumin seeds stored in PET bottles under refrigerated condition was most preferred RTS with overall acceptability score of 8.16 which was followed by the same RTS stored under ambient condition with overall acceptability score of 7.10.

Keywords: *Garcinia indica*, kokum, storage, ready to serve beverage, quality

Introduction

Kokum (*Garcinia indica* Choisy) is one of the important indigenous tree spice crops originated and grown in Western Ghats of India, Coorg, Wayanad, and Goa. The ripe kokum fruit is either dark purple or red tinged with yellow in colour. Shape of the fruit varies from round to oval and it weighs around 21 to 85 g (Devi *et al.*, 2013) [8]. Kokum is an important minor fruit of culinary, pharmaceutical, nutraceuticals and industrial uses. Kokum is traditionally used to treat sores, dermatitis, diarrhoea, dysentery, ear infection, and to facilitate digestion (Sanjay, 2019) [18]. Kokum seeds are used for oil extraction. That oil is called kokum butter and used in curries, cosmetics, medicines and costly confectionery preparations in foreign countries.

Ready to serve (RTS) is a type of fruit beverage which contains 10% of fruit juice and 10% total soluble solids besides about 0.3% acid. It should contain good body, uniform colour and possess pleasing flavour and aroma characteristic of the fruit. It is not diluted before serving and hence it is known as ready to serve juice (RTS) (Srivastava and Kumar, 2002) [23]. The blending of fruit juices for the preparation of ready to serve (RTS) beverages with addition of spice extracts as health drinks are thought to be convenient alternative for its utilization in order to have some value added drinks which are of high quality in respect of both sensory and nutritional aspects (Bhardwaj and Pandey, 2011) [5]. Kokum juice is used as a weight loss supplement, since it is anorectic. It is used as a hepatotonic, cardi tonic, anti-tumour and as a cure for bleeding piles. This plant is also pharmacologically studied for chelating, free radical scavenging, anti-bacterial, anti-fungal, anti-cancer, anti-inflammatory, anti-obesity and anti-ulcer activities (Antala *et al.*, 2012) [2]. However, the shelf life of the fruit is only for about one week, but its rich bioactive profile makes kokum a highly nutritious and desirable fruit crop. Hence, the present study of evaluating the kokum Ready to Serve (RTS) beverage was undertaken with the objectives of evaluating the shelf life of different blended kokum RTS beverage and to study the physico-chemical qualities and sensory characteristics of kokum based RTS blended beverages.

Materials and Methods

Freshly harvested matured kokum fruits (Fig. 1) obtained from ICAR-Indian Institute of Spices Research (ICAR-IISR), Chelavoor farm, Kozhikode, Kerala was selected for the study. The fruits were thoroughly washed with water to remove surface impurities.

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Other raw materials like cumin and cinnamon were obtained from the local market at Kozhikode. After washing the fruits were cut into two halves and the seeds were removed. The pulp along with the skin was ground in a mixer grinder and then strained through stainless steel sieve to get smooth pulp. The pulp obtained was mixed with sugar and water in the ratio 1:1:2 to obtain a fruit syrup of 40°Brix. This was then boiled and concentrated to 45°Brix to obtain the concentrated kokum syrup.

Ready to Serve (RTS) kokum beverage was prepared from concentrated kokum syrup by adding boiled and cooled water in the ratio 1:6 (Fig. 2). The total soluble solids at this stage were reduced to 12.3°brix. In order to raise the concentration of kokum juice to 15°Brix, sugar was added and the juice was boiled, stirred and cooled. After cooling, potassium meta bi sulphite (KMS) was added at the rate 0.1% as preservative and stirred thoroughly. 100 ml of kokum juice was then filled in to the polyethylene terephthalate bottles (PET) and stored under ambient and refrigerated storage conditions. Triplicate samples were drawn at 7 days interval for the first 28 days and then the samples were drawn at 15 days interval for a period of 75 days. The detailed description of the process is described in flow chart. The other two treatment combinations followed were kokum juice mixed with roasted and powdered cumin seeds (2.1 g in 3000 ml kokum juice) and kokum juice mixed with cinnamon powder (0.3 g in 3000 ml of kokum juice). The spices were added just before the addition of

preservatives when the juice was in warm condition.

A three factor completely randomized block design was followed to determine the effect of the three treatment combinations of kokum based RTS beverages on various quality parameters during storage. The beverages were kept under ambient and refrigerated storage conditions for a period of 75 days. Freshly prepared kokum based RTS beverage was initially analyzed for its quality and thereafter at regular intervals during the storage period. Three replicates of each treatment were taken for quality analysis at regular intervals of 0, 7, 14, 21, 28, 45, 60 and 75 days.



Fig 1: Kokum fruits

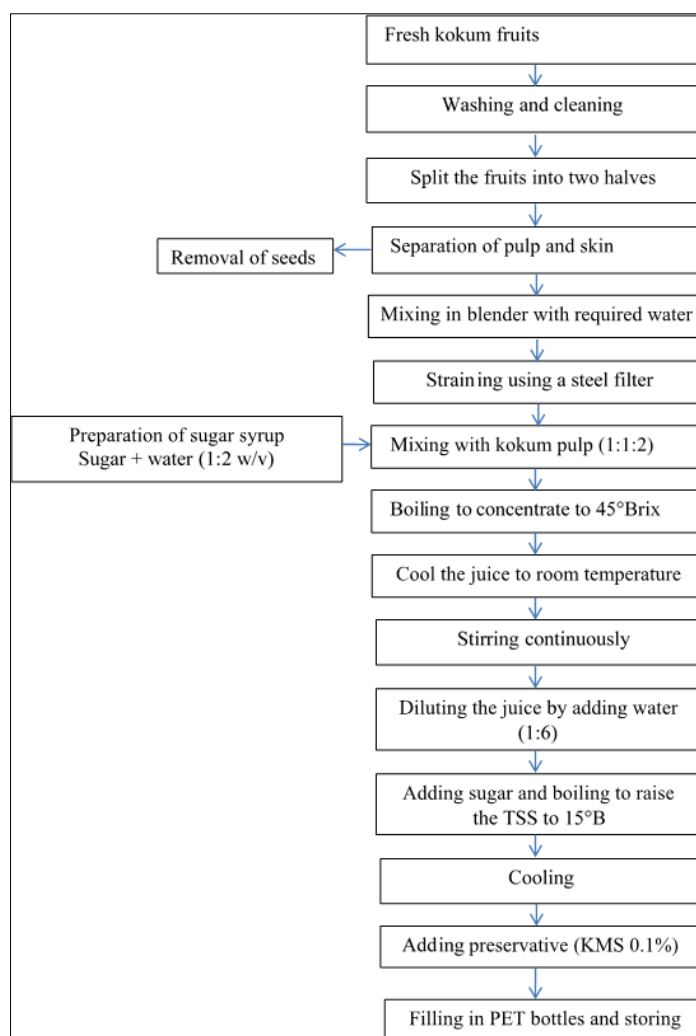


Fig 2: Flow chart for the preparation of Kokum RTS beverage

Quality analysis

Total soluble solids was measured by using hand refractometer (ERMA, 0-32 and 28-62) by placing a drop of RTS on prism of refractometer and observing the coincidence of shadow of the sample with the reading on the scale and is expressed as °Brix. The pH value of the sample was determined by using a digital pH meter (Cole-Parmer, USA) and the pH meter was standardized with distilled water of pH 7.0.

Moisture content of the fresh kokum was determined by toluene distillation method (ASTA, 3). About 10 g of sample was taken in the distilling flask of Dean and Stark apparatus and about 150 ml of toluene was added and boiled slowly. The volume of water collected in the receiver trap to the mass of kokum taken gave the moisture content of the fruit. The procedures described by Sadasivam and Manickam, (2008) [16] was used to determine Titratable acidity (titrating with sodium hydroxide), reducing sugars (Nelson-Somogyi method), total sugars (phenol sulphuric acid method), Fat (Soxhlet extraction method with petroleum ether by gentle heating for 6 hours), crude fibre content (acid and alkali treatment followed by incineration of the residue), protein (Lowry's method and the intensity was checked in Shimadzu UV-1800 spectrophotometer at 660 nm) and total carbohydrate content (Anthrone method).

Microbial analysis was done by total plate count method (Frazier, 2006) [9]. The sample was prepared by dilution plating technique where 1 ml of squash was taken and 9 ml of distilled water was added in test tube. Sensory evaluation of the product involved the use of sight (to evaluate the appearance, colour and the global aspect of eyes), tactile (to test the taste and texture) and olfaction (to smell the flavour). Semi-trained panel of 15 members were used to evaluate all the sensory attributes of the kokum based RTS beverage. Each attribute was translated in a numeric scale using a 9 point hedonic score system (Ranganna, 1991) [14]. The data obtained during storage of different kokum based RTS beverage were statistically analysed using AGRES statistical software (version 7.01, Pascal Intl software solutions).

Results and Discussion

Variation in total soluble solids

The total soluble solids of kokum RTS, kokum RTS blended with roasted cumin powder and kokum RTS blended with cinnamon powder beverages on the initial day of storage was 15°Brix. There was a decrease in total soluble solids (TSS) in

all the treatments on the 7th days and then a gradual increase was observed. After 75 days of storage, the values increased again to 15, 15 and 15°Brix for all the three treatments of kokum based RTS beverages when stored under ambient conditions and the corresponding values under refrigerated condition were 15.33, 15.33 and 15.33°Brix (Fig. 3). The analysis of variance showed that the treatments of RTS and storage conditions had no significant effect on total soluble solids of kokum beverage. However, the storage period and interaction between conditions and storage period had significant effect on the total soluble solids of kokum based RTS beverages.

Increase in the TSS of the product during storage could be due to the hydrolysis of the polysaccharides (such as starch and pectin) into simple sugars such as monosaccharides (such as glucose and fructose) and oligosaccharides (sucrose) and other constituents (Hemalatha *et al.*, 2018) [10]. Similar changes in TSS during storage were reported in functional ready to serve (RTS) drinks made from a blend of aloe vera, sweet lime, amla, and ginger (Lokesh and Mishra, 2017) [12]. Waskar and Gaikwad, (2004) [25], studied the storage of pomegranate, kokum and mango based blended juice packed in three packaging containers such as glass, polyvinyl chloride (PVC) and high density polyethylene (HDPE) at three different storage conditions and found that the total soluble solids of the blend increased from an initial value of 18.4 °Brix to 21.20, 20.40, and 20.20 °Brix when stored in HDPE containers at room temperature (12.64 to 31.13 °C), in cool chamber (18.91 to 21.24 °C) and in cool storage (5 °C) after 150 days, respectively. Sindumathi and Premalatha, (2013) [22] reported that papaya blended with pineapple ready to serve (RTS) beverage in the ratio of 50:50, filled in glass bottles and kept under ambient storage conditions showed a gradual increase in total soluble solids from 15 to 18°Brix during 120 days of storage. Rathod *et al.* (2014) [15] observed that storage of bael fruit RTS beverage blended with aonla in the ratio of 40:60, filled in polyethylene terephthalate bottles (PET) bottles and kept under refrigerated conditions for 45 days, total soluble solids increased from 10 to 13°Brix. Thamilselvi *et al.* (2015) [24] reported that in lime based herbal RTS beverage of three treatments in which lime was control, lime RTS with tulsi extract (15%) and lime RTS with bermuda grass extract (15%), stored in glass bottles under ambient condition for 150 days, the total soluble solids varied from 15° to 15.90, 15.70 and 15.90°Brix for the three 3 treatments, respectively.

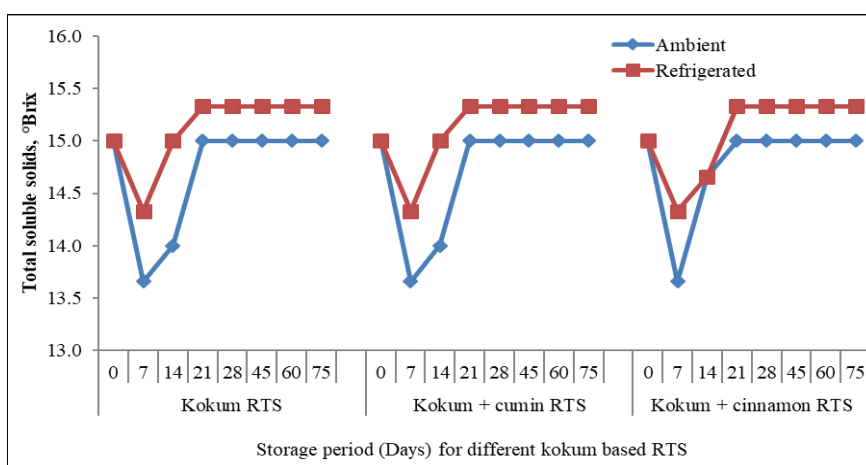


Fig 3: Variation in total soluble solids during storage of different kokum based RTS beverages

Variation in pH

The changes in pH of different treatments of kokum based RTS beverages under different storage conditions for 75 days of storage is presented in (Fig. 4). A decrease in pH was observed during storage of kokum RTS, kokum RTS blended with cumin and kokum RTS blended with cinnamon beverages. On the initial day of storage, the values were 3.17, 3.10, and 3.10, respectively and after 75 days of storage the values decreased to 2.41, 2.45 and 2.51, respectively when stored under ambient conditions and under refrigerated conditions, the corresponding values were 2.60, 2.58 and 2.69, respectively. The analysis of variance showed that the storage conditions, treatments of RTS, period of storage and their interactions had significant effect ($p \leq 0.01$) on the pH of kokum RTS.

Jan and Masih (2012) [11] reported a significant decrease in pH during storage of pineapple juice blended with carrot and orange juice and stored in PET bottles for 21 days under refrigerated conditions. This decrease in pH could be due to increase in titratable acidity, as acidity and pH are inversely proportional to each other. It was observed that the maximum pH (4.18) was recorded in the pineapple juice blended with

carrot and orange juice in the ratio (50:20:30). Malav *et al.* (2014) [13] reported that orange based RTS beverage blended with pomegranate, aonla and ginger juice mixed in the ratio (90:10:10) when stored in sterilized plastic bottles under ambient condition for 90 days, the pH decreased from 3.27 to 2.93. Sasikumar *et al.*, (2013) [20] observed that therapeutic RTS beverage from aloe vera gel, aonla fruit juice and ginger juice extracts in the ratio of 50:25:25 stored in glass bottles at refrigerated conditions, the pH value decreased from 4.90 to 4.50 during storage for six months. A decrease in pH from 4.39 to 3.98 was reported by Dambalkar *et al.*, (2015) [7] during storage of RTS beverage prepared from beet root, orange, ginger juice and carrot juice prepared in the ratio of 50:30:10:10 and stored in glass bottles under refrigerated conditions for 3 months. Sanjay, (2019) [18] studied the preservation of kokam fruit juice with and without preservatives and observed that pH of kokum juice reduced from 6.08 to 3.17 for juice stored under ambient conditions without addition of preservatives towards the end of storage period for 28 days, while it reduced to 3.38 and 3.62 for juices preserved with 4% citric acid and 3% benzoic acid as preservative, respectively.

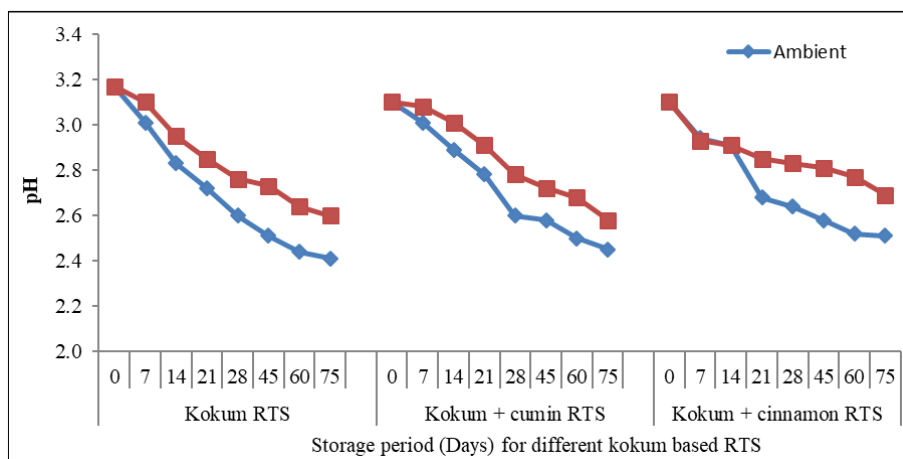


Fig 4: Variation in pH during storage of different kokum based RTS beverages

Variation in titratable acidity

The titratable acidity of kokum based RTS beverages, increased from an initial value of 0.49, 0.35 and 0.36% for kokum RTS, kokum RTS blended with cumin and kokum RTS blended with cinnamon, respectively (Fig. 5). After 75 days of storage, the values increased to 0.75, 0.76 and 0.51%, respectively for the three treatments when stored under ambient conditions and the corresponding values when stored under refrigerated conditions were 0.65, 0.62 and 0.50%. Analysis of variance showed that the treatments of RTS, storage conditions, storage period and their interactions had significant effect ($p \leq 0.01$) on titratable acidity of kokum based RTS.

Waskar and Gaikwad, (2004) [25] reported that the titratable acidity of blended pomegranate, kokum and mango juice stored packed in HDPE bottles decreased from an initial value of 1.62% at the beginning of storage to 1.35, 1.53 and to 1.5% when the blends were stored at room temperature (12.64 to

31.13 °C), in cool chamber (18.91 to 21.24 °C) and in cool storage (5 °C) for 150 days, respectively. A significant increase in titratable acidity during storage of pineapple juice blended with carrot and orange juice was reported by Jan and Masih, (2012) [11]. It was observed that maximum increase in acidity (from 0.54 to 1.36%) was recorded in the pineapple juice blended with carrot juice and orange juice in the ratio 50:20:30. The increase could be due to the addition of citric acid and higher level of orange juice of the treatment combinations studied. Similarly, Sindumathi and Premalatha, (2013) [22] observed that the acidity of papaya-pineapple RTS blended in the ratio of 50:50 increased from 0.31 to 0.54% during storage for 120 days in glass bottles under ambient storage conditions. Further, Sasikumar (2015) [19] reported that the acidity of functional beverages prepared from aloe vera and bael fruit using different proportions increased from 0.86 to 0.89% after 2 months of storage under refrigerated condition when stored in glass bottles.

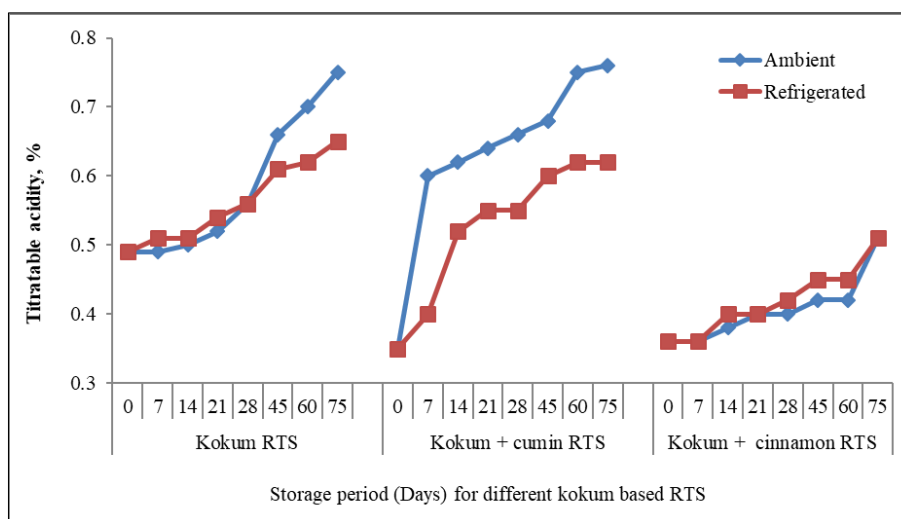


Fig 5: Variation in titratable acidity during storage of different kokum based RTS beverages

Variation in reducing sugars

On the first day of storage, the reducing sugars of beverages from kokum RTS, kokum RTS blended with cumin and kokum RTS blended with cinnamon were 14.56, 15.12 and 12.19%, respectively and after the storage period of 75 days the values increased to 45.56, 45.51 and 42.23%, respectively when stored under ambient conditions and the values changed to 42.22, 42.19, 40.38%, respectively when stored under refrigerated conditions (Fig. 6). The analysis of variance indicated that the storage conditions, treatments of RTS, period of storage and their interactions had significant effect ($p \leq 0.01$) on the reducing sugars of kokum RTS.

Byanna *et al.* (2013) [6] during standardization of sweet orange and kokum blended RTS beverage using sugar

substitute with 50% + 50% fructose observed an increase in reducing sugars from 12.17 to 12.86% when the beverage was stored in pre-sterilized glass bottles under ambient conditions for 180 days. The studies conducted by Bafna, (2014) [4] on the use of kokum fruit as RTS beverage stored in polyethylene terephthalate (PET) bottles under refrigerated condition (4 °C) for 75 days reported that reducing sugars increased from 12.18 to 12.50 g/100ml during the storage period. Sasikumar *et al.* (2013) [20] reported that the reducing sugars increased from 11.21 to 15.80% in RTS beverages prepared from aloe vera gel and aonla fruit juice blended in the ratio 50:25 when packed in glass bottles and stored at refrigerated conditions for 6 months.

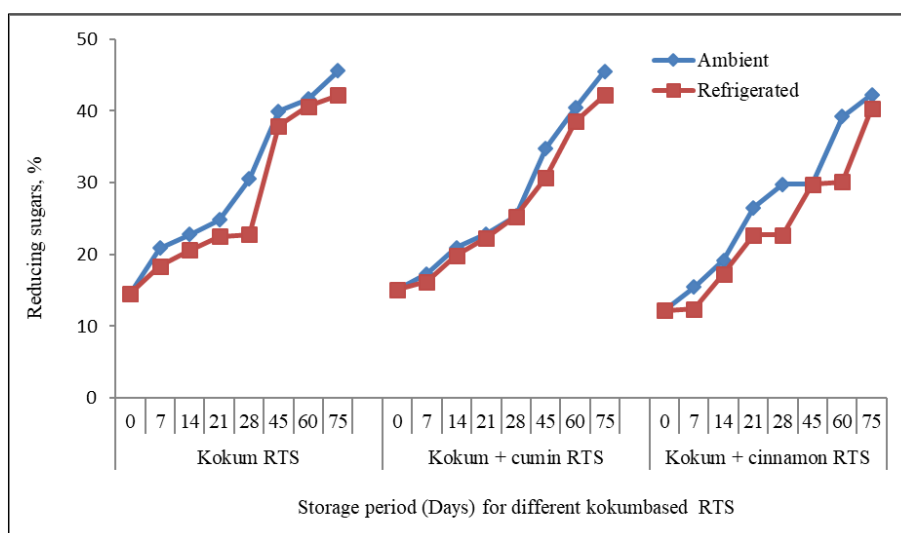


Fig 6: Variation in reducing sugars during storage of different kokum based RTS beverages

Variation in total sugars

The initial content of total sugars of kokum RTS was 15.03, while for kokum RTS blended with cumin and kokum RTS blended with cinnamon it was 15.45, and 15.66%, respectively. After storage for 75 days under ambient conditions, the values increased to 51.00, 50.45 and 49.42 per cent, respectively and when stored under refrigerated conditions, the corresponding values were 49.26, 50.27, and 46.58%, respectively (Fig. 7). The analysis of variance

showed that the treatments of RTS, storage conditions, period of storage and their interactions had significant effect ($p \leq 0.05$) on the total sugars of kokum RTS. Waskar and Gaikwad, (2004) [25] reported that the total sugars of blended pomegranate, kokum and mango juice decreased from 17.48% during storage at room temperature for 150 days to 18.52, 21.02 and 19.82% in packaging containers like glass, polyvinyl chloride (PVC) and high density polyethylene (HDPE) bottles while the corresponding decrease in values

under cold storage conditions were 18.40, 20.18 and 19.44%, respectively. Bafna (2014) [4] studied the storage of kokum fruit as RTS beverage for 75 days and reported that the total sugars increased from 16.32 to 16.88 g/100ml under refrigerated condition in polyethylene terephthalate bottles. Amaravathi *et al.* (2014) [1] in her studies on pineapple ready to serve beverages prepared with spices extracts of ginger (3.0%), pepper (0.2%), green chillies (0.3%), cardamom

(0.2%) and nutmeg (0.4%) stored in glass bottles and kept under ambient conditions for a storage period of 180 days found that the total sugars increased from 13.58 to 13.61%. Malav *et al.* (2014) [13] studied the composition of orange beverages blended with pomegranate, aonla and ginger juice in ratio 90:10:10, filled in sterilized plastic bottles stored under ambient conditions for 90 days and reported that total sugars increased from 10.57 to 10.77%.

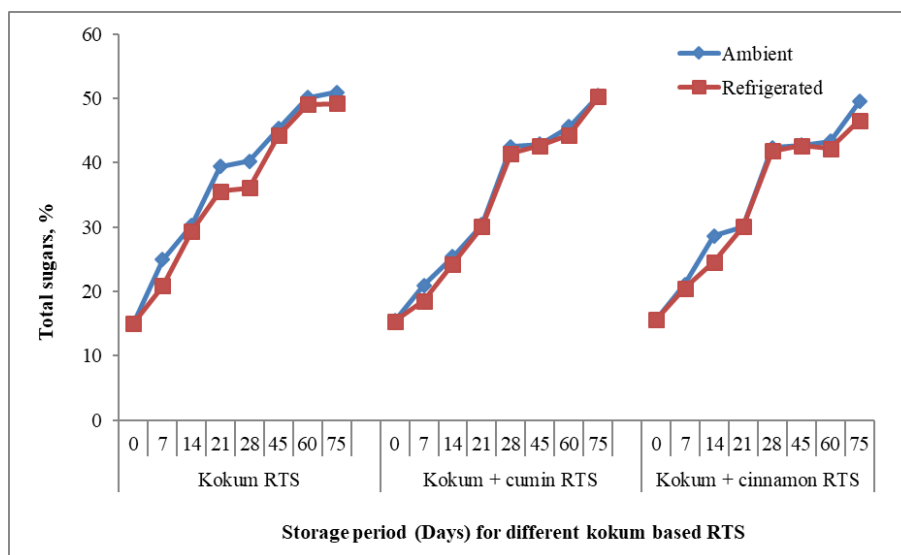


Fig 7: Variation in total sugars during storage of different kokum based RTS beverages

Changes in total plate count

The kokum based RTS beverage showed no indications of microbial growth up to 60 days of storage for all the three storage treatments. However, after 60 days of storage, few colonies of microbial growth could be detected under both storage conditions for all the three treatments studied (Table 1). Bafna (2014) [4] studied that during storage of kokum fruit

RTS beverage under refrigerated condition in polyethylene terephthalate bottles for 75 days, the total plate count varied from 12 cfu/ml on the initial day of storage to 17 cfu/ml on the 75th day of storage and further reported that according to PFA standards the microbial load should be less than 50 (cfu/ml) which meant that RTS beverage was safe and fit for consumption up to 75 days with respect to microbial count.

Table 1: Variation in microbial count of kokum juices at different storage conditions and for different storage periods

Storage conditions	Storage Period, Days	Microbial count, cfu/ml		
		Treatments in kokum RTS beverage		
		kokum RTS	kokum + cumin RTS	kokum + cinnamon RTS
Ambient	0	ND	ND	ND
	15	ND	ND	ND
	30	ND	ND	ND
	45	ND	ND	ND
	60	2×10 ¹	2×10 ¹	3×10 ¹
	75	3×10 ¹	3×10 ¹	4×10 ¹
Refrigerated	0	ND	ND	ND
	15	ND	ND	ND
	30	ND	ND	ND
	45	ND	ND	ND
	60	1×10 ¹	1×10 ¹	2×10 ¹
	75	2×10 ¹	2×10 ¹	2×10 ¹

Note: ND - Not detectable

Variation in sensory analysis of different RTS beverage

The sensory analysis in terms of overall acceptability for different kokum RTS was done at 15 days storage interval for a period of 3 months showed that kokum RTS blended with cumin had the best flavour and taste with an overall acceptability score of 7.75 when stored under refrigerated condition followed by that stored under ambient condition with an acceptability score of 7.10 (Fig. 8). The analysis of

variance showed that the treatments of RTS, storage conditions, period of storage and their interactions had significant effect ($p \leq 0.01$) on the total organoleptic quality of kokum RTS.

Siddharth and Sharma, (2013) [21] studied the storage qualities of concord grape juice blended with kokum extract of different concentrations (from 0 to 0.6%) and reported that the highest overall acceptability in maintaining the sensory

characteristics was obtained when the concentration of kokum extract was 0.4% followed by 0.6%. However, minimum loss of anthocyanins during cold storage for a period of 4 months was recorded when 0.2% kokum extract was added. Organoleptic evaluation of kokum RTS under refrigerated condition in polyethylene terephthalate bottles for 75 days, showed a gradual reduction in the overall acceptability from

8.4 to 7.75, but however the change was nonsignificant (Bafna, 2014) [4]. Sahu and Kadeppagari, (2017) [17] studied sensory attributes of kokum drinks formulated with the addition of cumin and cardamom and reported that the sample containing the cumin and cardamom powders (0.2%) and roasted salt (1%) has better acceptability than the one which did not contain these additives.

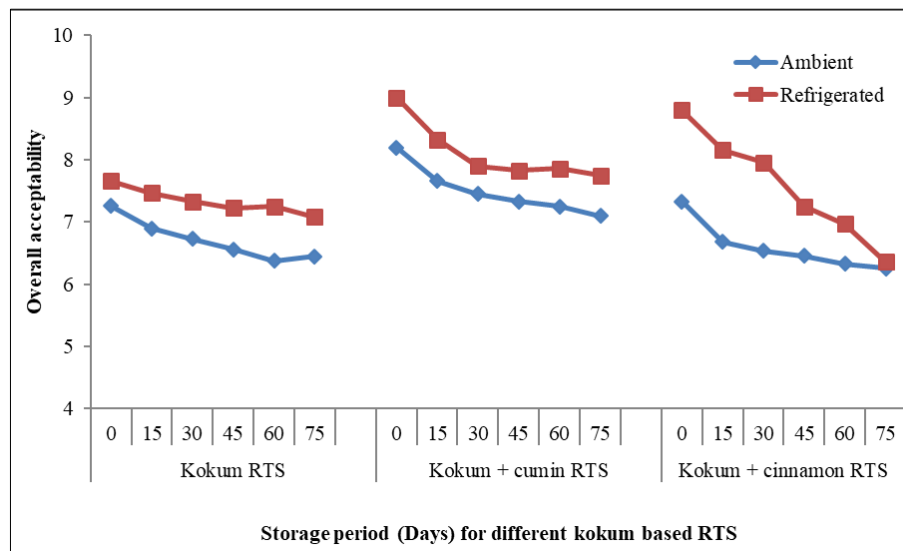


Fig 8: Variation in overall acceptability during storage of different kokum based RTS beverages

Conclusion

Three treatments of kokum based ready to serve (RTS) drinks - kokum RTS, kokum RTS blended with roasted cumin and kokum RTS blended with cinnamon were standardized and its quality during storage were studied for 75 days under ambient and refrigerated storage conditions. A decrease in pH during storage for all the treatments was observed. However, the total soluble solids, titratable acidity, reducing sugars and total sugars increased during storage. No indications of microbial growth were observed till 60 days of storage, however, after 60 days of storage, few colonies of microbial growth could be detected under both the storage conditions. From the study was concluded that kokum RTS juice with powdered cumin stored under refrigerated conditions followed by that stored in ambient conditions were most preferred RTS with overall acceptability score of 7.75 and 7.10, respectively.

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