

# Development of low calorie RTS beverage rich in nutraceuticals and antioxidants from cactus fruit and kokum fruit by blending with coconut water

■ Vasundhra Devidas More, Sindhu, Shweta Saloni, Sujata and Vipul Jaglan

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See end of the Paper for authors' affiliation

Correspondence to :

**Vasundhra Devidas More**  
Department of Food  
Technology, Vignan's  
Foundation for Science,  
Technology and Research  
University, Vadlamudi, Guntur  
(A.P.) India

■ **Abstract** : Cactus fruit (Prickly pear) has received abundant attention with regard to its high nutraceutical value and also is also grown locally in various regions of Maharashtra. Therefore, the present study was carried out on preparation of RTS beverage blends from prickly pear, kokum and coconut water to improve its quality and flavor. These blends were packaged in 200 ml non-transparent stand pouch and tested for physico-chemical, sensory evaluation and microbial analysis. Chemical composition carried out of unblended pulp. The pH, total soluble solids (TSS), titratable acidity, color analysis, ascorbic acid were determined. The blend of cactus fruit and kokum at ratio (3:1) received the highest scores in overall acceptability. Therefore, it was evaluated for polyphenol content by using Folin–Ciocalteu reagent, antioxidant activity was measured using two in vitro assays 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) and sensory was carried out to check the acceptability of beverage after adding of low GI sugar and stevia. Pasteurization was carried out to increase shelf life.

■ **Key words** : Low calorie, RTS beverage, Antioxidants, Nutraceuticals, Cactus, Kokum

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**C**actus (plural: cacti, cactuses, or cactus) is a member of the succulent plant family Cactaceae. This means that they are native only to North America, South America, and the West Indies. This means that they are native only to North America, South America, and the West Indies. South America, and the West Indies.

The high water use efficiency of cactus is attributed to Crassulacean Acid Metabolism (CAM) which is present in rapidly growing cactus species such as *Opuntia ficus indica*, *O. megacantha* and *O. amychlea*

(Cactaceae) that produce forage for animals, vegetables, and fruits with 14% glucose (FAO, 1996).

In India an important part of the population is settled in rain fed dry areas which need perennial vegetation to protect them from erosion using drought hardy and economically viable plants.

*Opuntia* genus is widely known for its mucilage production. Mucilage, a complex carbohydrate with a great capacity to absorb water, should be considered a potential source of industrial hydrocolloid. Mucilage contains varying proportions of L-arabinose, D-galactose,

L-rhamnose, and D-xylose, as well as galacturonic acid (Saenz *et al.*, 2004). Nutritional content and bioactive chemical constituents of *Opuntia ficus-indica*.

The main constituent of *Opuntia ficus-indica* cladodes is water (80.95%), followed by small amounts of carbohydrates (3-7%), fibre (1-2%), and protein (0.5-1%).

The sugar moiety includes mucilaginous components containing polymers, such as chains of (1-4)-linked  $\beta$ -D-galacturonic acid and R(1-2)-linked L-rhamnose residues.

## ■ METHODOLOGY

### **Pulp extraction :**

The method was used in the process of separating the pulp from the seeds (Quadri *et al.*, 2012). The applied method was mechanical and was done by magnetically shaking a predetermined pulp sample at a 1,200-rpm speed for 40 min. The action decreased the fruit's viscosity and facilitated the removal of its seeds. The pulp from it was then centrifuged at a speed of 3,600 rpm for 20 min and then filtered through a 100-mesh cloth filter.

### **Standardization of blended RTS beverage preparation:**

Blended RTS beverages were prepared using 15% of blended pulp extracts of cactus fruit and kokum fruit, 15% of total soluble solid (TSS) and 0.4% of acidity at the time of preparation in the formulated blend. The blended pulp of ratio of cactus fruit and kokum fruit 60:40 with 15% of low GI sugar, 0.3% of acidity as % of malic acid and 40 ppm of potassium sorbet of 82% of coconut water. The controlled sample of RTS beverage having 100 per cent cactus pulp. Pasteurization was carried out at 98°C for 15 sec. The undamaged, disease free, mature and ripe fruits were obtained from Sound ripe cactus fruits were purchased from Jubeli market, Rajkot Hingol Gadh, Jasdhan.

### **Analysis of beverage :**

#### *Physical method :*

#### **Total soluble solids :**

The content of total soluble solids (TSS) in cactus fruit pulp and RTS beverage was determined with the help of refractometer. Care was taken that the refractometer was washed with distilled water and wiped

dry before every reading.

#### **pH :**

The pH of cactus fruit pulp and RTS beverage was measured by pH meter after standardization.

#### *Chemical methods :*

The moisture, ash, crude fibre and fat, contents were determined using AOAC (1998) while protein content was determined by Micro Kjeldahl method AOAC (1998). The carbohydrate content was by difference (Ranganna, 1995).

#### **Moisture content :**

Moisture is the major component of food. The moisture content of any food is determined not only to analyze the chemical composition of food material on moisture free basis but also to assess the shelf life of the product. Moisture content was determined according to AOAC (1998) method. About 5 g of sample was weighed accurately on the balance, and then it was spread uniformly into a Petri dish and put in a hot oven at  $105 \pm 1^\circ\text{C}$  for 6 hours. After drying; the covered dish was transferred to the desiccators and weighed soon after it reached the room temperature. The procedure was repeated till constant weight of dried matter was obtained. The loss in moisture was recorded as the moisture content. It is shown in following formula.

#### **Total ash :**

Ash content was determined in percentage as described in described in AOAC (1998). The ash of the food stuff is the inorganic residue which remains after burning of organic matter. Muffle Furnace was used to determine total ash. 3 g of sample was placed into a pre-weighed, dry porcelain crucible and was placed in muffle furnace for 6 hrs at about  $600^\circ\text{C}$ . It was then cooled in desiccators and weighed without delay. The ash content was determined as :

#### **Crude protein :**

The crude protein content was estimated according to the Kjeldahl's method as described in AOAC (1998).

#### **Crude fat :**

The crude fat content in each composite flour sample was determined by taking 6g ground sample and running

through Soxhlet apparatus for 2-3 hours using petroleum ether as a solvent by following the procedure described in AOAC (1998).

#### **Crude fibre :**

The crude fibre was estimated according to the procedure as outlined in AACC (1998).

#### **Titration acidity :**

Acidity of cactus pulp and RTS beverage of cactus fruit was determined by A.O.A.C. (1990) method.

25ml of fruit juice was taken in 250ml conical flask and to this 2 drop of phenolphthalein indicator was added and titrated against 0.1 N sodium hydroxide till a permanent pink color was obtained.

#### **Total sugar :**

Total sugar in cactus fruit pulp and RTS beverage of cactus was determined by the method described by Rangana(1997).

#### **Determination of reducing sugar :**

5ml Fehling's A and B solution was taken in 300ml conical flask and diluted with 40ml water. The pulp in burette was added slowly in hot boiling Fehling solution till the appearance of slight red color. Now three drops of methylene blue indicator was added and titration was continued till brick red precipitate occurred by destroying the blue coloration.

#### **Determination of total sugar :**

For the estimation of total sugar, 20ml solution was taken in a beaker and 5ml of concentrated HCl was added that solution was boiled on water bath for 5 min for the hydrolysis to convert the non-reducing sugar into reducing sugar. After cooling, excess of acid was neutralized by sodium carbonate solution. The solution was transferred in 100ml volumetric flask and volume was taken in burette and titrated with the Fehling solution A and B similar as was done in reducing sugar.

#### **Ascorbic acid :**

Ascorbic acid in cactus fruits and wine was determined by A.O.A.C. (1990). 5ml L-Ascorbic acid solution with same amount of  $\text{HPO}_3$  solution was titrated against 2, 6-Dichlorophenol-indophenol. The end point was judged by light pink color.

#### **Total polyphenol detection method :**

Total phenol content (TPC) in tea extracts was determined Spectrophotometrically according to a modified method of Lachman *et al.* (1998).

#### **DPPH radical scavenging activity assay (Yeddes *et al.*, 2013 a and b) :**

The antioxidant capacity of the methanol extracts was tested by DPPH (1,1-diphenyl-2-picrylhydrazyl) according to method adopted by Yen and Duth. The DPPH method is the most used for the evaluation of the antioxidant properties and antiradical activity of natural products.

#### **Sensory evaluation :**

Sensory evaluation plays an important role while deciding the quality of products. Sensory evaluation was carried out using 9 point Hedonic scale.

#### **Micro biological load determination :**

##### *TPC method :*

Micro biological load determination was done by total plate count (TPC) method. In this method, two solutions were made. One was phosphate buffer saline (PBS) solution and the other one was plate count agar (PCB) solution (media).

#### **Statistical analysis :**

Statistical analysis for all the data were performed by using one way analysis of variance (ANOVA) and the statistical significance was given in terms of p-values, with differences of 95% confidence level ( $p < 0.05$ ) being considered statistically significance for the result of all analysis and various parameters. The final results obtained were expressed as the mean values  $\pm$  standard deviation.

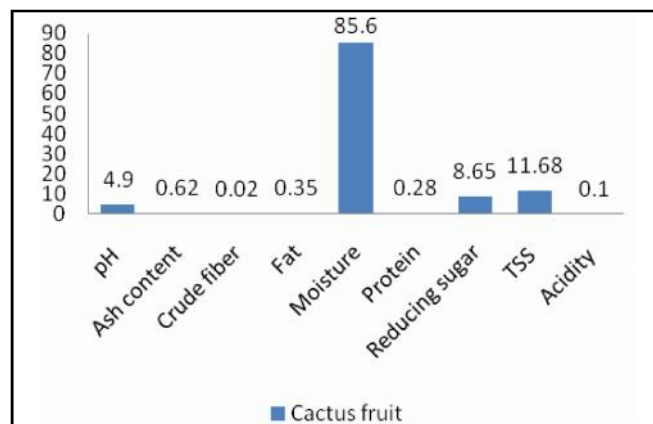
## **■ RESULTS AND DISCUSSION**

Chemical composition of cactus fruit pulp for various important chemical constituents and properties including TSS, pH, acidity, ascorbic acid, crude fibre, non-reducing sugar and Moisture content are in agreement with the same reported in the past (Paredes and Rojo, 1973; Askar and El Samahy, 1981; Sawaya *et al.*, 1983; Pimienta, 1990; Sepulveda and Saenz, 1990; Rodriguez *et al.*, 1996). The earlier reported components present in cactus pear pulp, protein (0.21–1.6%), fat (0.09–0.7%), fibre (0.02–

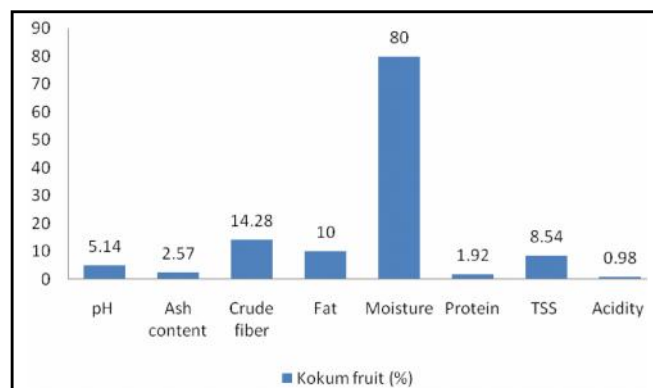
3-15%) and ash (0.4–1%) are the ones comparable to the present findings within natural variations expected under ecological and biological conditions (Fig. 1 to 4).

Parameters	Cactus fruit pulp (%)	Kokum fruit (%)	Coconut water (%)	Stevia powder (%)
pH	4.90±0.03	5.14	4.81	5.33
Ash content	0.62±0.65	2.57	0.39	12.06
Crude fiber	0.02±0.23	14.28	1.10	5.03
Fat	0.35±0.46	10.00	0.20	6.13
Moisture	85.6±0.63	80.00	94.99	10.73
Protein	0.28±0.76	1.92	0.72	13.68
Non reducing / Total sugar	8.65±0.42	-	1.67	4.50
TSS	11.68±0.65	8.54	5.60	5.05
Acidity	0.10±0.50	0.98	0.06	-s

Values are mean ± SD of duplicate samples



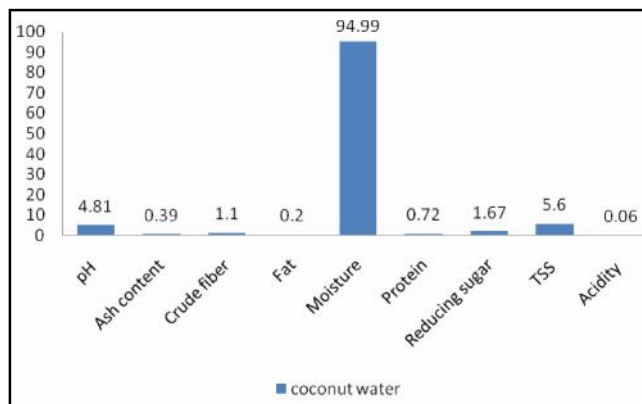
**Fig. 1 : Chemical analysis of cactus fruit pulp**



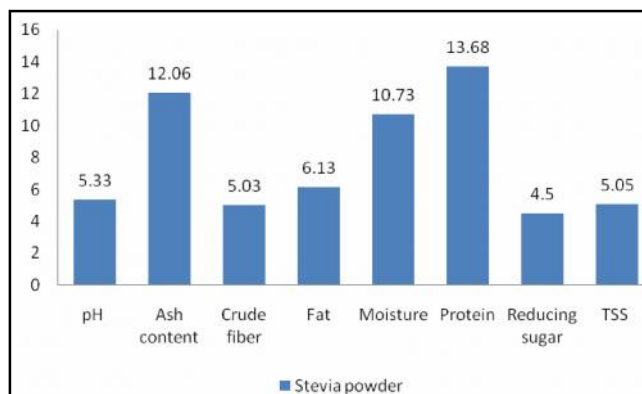
**Fig. 2 : Chemical analysis of kokum fruit**

**Physico-chemical properties of RTS beverage of cactus fruit :**

The data presented in Table 1 is on chemical



**Fig. 3 : Chemical analysis of coconut water**



**Fig. 4 : Chemical analysis of stevia powder**

composition of RTS beverage of cactus fruit (as finalized in sensory test). The values were - TSS (11.04%), pH (3.4), Reducing sugar (8.24%), Acidity (0.4%), Total phenol (1050 Mg GAE/L), protein (0.72%), Crude fibre (0.54%) present in cactus fruit RTS beverage were having the similar composition as reported in literature, although some minor variations RTS beverage cactus fruit in the values were observed in (Suliman *et al.*, 2014) that these are in agreement with reported observation by earlier worker.

These values were compared with control sample which was cactus fruit pulp and there was significant difference ( $p < 0.05$ ) in various parameters of physicochemical analysis as compared to blended RTS beverage; decrease in pH was observed which is due to malic acid added, also there was small increase in crude fibre, fat, protein due to addition of kokum fruit and coconut water.

**Table 2 : Physicochemical properties of RTS beverage of cactus fruit sensory evaluation**

Parameters	Control sample	RTS beverage
pH (%)	4.90± 0.03	3.4± 0.05
Ash (%)	0.62±0.65	0.70± 0.35
Crude fibre (%)	0.02±0.23	0.54± 0.62
Fat(%)	0.35±0.46	0.45±0.72
Water content (%)	85.6±0.63	88.79 ±0.49
Protein (%)	0.28±0.76	0.72±0.21
Reducing sugar (%)	8.65±0.42	8.24± 0.58
Non reducing sugar (%)	11.68±0.65	1.03±0.85
TSS (%)	0.10±0.50	11.04±0.16
Acidity (%)	4.90± 0.03	0.40±0.53
Total phenol (Mg GAE/L)	-	1050
DPPH (µmol TE/L)	-	1256.67

Values are mean ± SD of duplicate samples

### Sensory evaluation :

Organoleptic evaluation is generally the final acceptance of the food product as per consumer point of view (10 youth panelists including male and females were selected from batch of food technology, NAFARI Pune). The panelist were trained on the tasting terminology and requested to evaluate the various RTS beverage samples for color, taste, flavor, appearance and overall acceptability Thus the evaluation was conducted on the products from prickly pear, kokum and coconut water. Taste, odor, color, mouth feel, appearance and overall acceptability were evaluated (Table 2).

#### Trial 1 :

In this trial variations made with cactus fruit pulp, and ratio of malic acid to stevia powder. according to sensory parameter like taste, aroma, color, mouth feel sample which was having composition of pulp 15%, stevia 45mg, malic acid 0.1% was more acceptable as compared to other samples.

In this trial there were also variations of cactus fruit pulp; Cactus fruit had a taste similar to raspberries or watermelon, with a slight bite like kiwi fruit. The fruit was filled with small seeds which make it grainy to the tongue.

#### Trial 2 :

In this trial variations made with increase in amount for ratio of malic acid to stevia powder according to

panelist member sample with composition 15% pulp, 65mg/100ml of stevia powder, 0.4% malic acid had less deviation value and more average of acceptability so this sample was perfect combination of malic acid to stevia.

In this trial there were also variations of cactus fruit pulp, Cactus fruit had a taste similar to raspberries or watermelon, with a slight bite like kiwi fruit. The fruit was filled with small seeds which make it grainy to the tongue.

#### Trial 3 :

In this trial blending with coconut water and variations made with increase in amount for ratio of malic acid to stevia powder.

According to sensory parameter like taste, aroma, color, mouth feel, all samples were acceptable by color and aroma but sample with 15% pulp, 85ml/100ml of coconut water, 60mg/100ml stevia powder, 0.4% malic acid was acceptable by taste.

In this trial there was also variations of cactus fruit pulp, cactus fruit had a taste similar to raspberries or watermelon, with a slight bite like kiwi fruit. The fruit was filled with small seeds which make it grainy to the tongue.

#### Trial 4 :

In this trial blending with coconut water and addition of kokum fruit pulp and variations made with increase in amount for ratio of malic acid to stevia powder.

According to sensory panelist member the sample with 15% pulp, 82 ml/100ml, 5 g/100 ml kokum fruit pulp, 60 mg/100ml stevia powder, 0.4% malic acid was having highest average and very less standard deviation so we could say this sample was acceptable combination of cactus fruit pulp, kokum, coconut water, stevia, malic acid.

The addition of kokum pulp which enhanced taste of beverage it also contained antioxidants which helps to increase nutraceutical value of final beverage.

#### Trial 5 :

In this trial there was replacement of stevia powder with low GI sugar.

According to panelist sample with 15% pulp, 82ml/100ml coconut water, g/100ml kokum fruit pulp, 6g/100ml low GI sugar, 0.4% malic acid was more acceptable also it had highest average value and very less standard

Table 3 : Microbial growth (Total plate count) in RTS beverage during storage				
Storage days	Control sample	Hot filled sample	Single pasteurized sample	Dual pasteurized sample
1	3.20	1.43	2.43	1.38
7	6.42	5.78	6.00	4.73
14	7.53	7.59	7.70	7.02
21	8.13	8.06	7.38	7.93
28	7.09	8.07	7.84	7.68

deviation. So it was selected as acceptable combination of cactus fruit pulp, kokum, low GI sugar, coconut water, malic acid.

Replacement of stevia powder with low GI sugar due to more desirable taste. In this trial there was also variations of cactus fruit pulp, Cactus fruit had a taste similar to raspberries or watermelon, with a slight bite like kiwi fruit. The fruit was filled with small seeds which make it grainy to the tongue.

#### Microbial analysis of RTS beverage of cactus fruit:

Total plate count (After incubation at 31°C)

The dual pasteurized and hot fill reduced total plate count compared to control sample and was slightly lower than single pasteurized beverage. The decreases may be occur by physical and chemical phenomena that occur during the pasteurization treatment, on day 7, experimental sample exhibited the lowest microbial count compared with control sample and single pasteurized beverage, after 2 weeks of storages all sample shows similar microbial count  $>7\log\text{CFU/mL}$ .

RTS beverage had a low pH (3.4-3.6) and high acidity (0.40– 0.45%), which creates antimicrobial environment. Complying with national and international health specifications for pasteurized juices and drinks, fresh pasteurized treated beverage did not exceed 2 logCFU/mL for total plate count and. About spore determination, microbial load was not detected in any of the samples and neither during storage (Table 3).

#### Conclusion :

On the basis of the results of this study it was concluded that formulation of mixed (blend) fruit juice from prickly pear with kokum fruit and coconut water is possible to satisfy consumer taste and preferences. Blending of prickly pear with kokum at ratio (3:1) gave better sensory score quality. To the best of our knowledge the present work is the first trial to perform these blends, so the study will be extended to clarify the effect of

different storage temperature and times on the Shelf life of selected blended beverage. So blending of prickly pear juice with kokum can be a boon to the growers in getting a good remuneration for their produce and to consumers in getting acceptable and antioxidant rich beverage at reasonable price.

#### Authors' affiliations:

**Sindhu**, Department of Food Technology, Bundelkhand University, Jhansi (U.P.) India

**Shweta Saloni**, Department of Food Processing and Technology, Bilaspur University, Bilaspur (C.G.) India

**Sujata**, Department of Home Science (Food and Nutrition), Mahant Darshan Das Mahila College, Muzaffarpur (Bihar) India

**Vipul Jaglan**, College of Dairy Science and Technology, Lala Lajpat Rai University of Veterinary and Animal Science, Haryana Agricultural University, Hisar (Haryana) India

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