Original Research Article

Development of karonda juice supplemented whey beverage with enhanced antioxidant activity

Abstract

This study assessed the antioxidant activity, sensory characteristics, and storage stability of whey-based beverages supplemented with karonda juice. Beverages prepared with varying concentrations of Karonda juice (6–14%) showed a significant increase in antioxidant activity with increasing juice concentration, while variations in sugar content showed no significant effect. The beverage containing 10% sugar and14% karonda juice showed highest acceptability by sensory evaluation. The antioxidant capacity of the supplemented beverages was significantly higher than that of paneer whey, with ABTS and DPPH assay values increasing to 5.65±0.03 and 3.99±0.04, respectively. The product remained acceptable for up to three weeks based on seonsory assessment. These findings suggest that karonda juice has the potential to enhance both the antioxidant capacity and sensory appeal of whey-based beverages, while maintaining an acceptable shelf life of three weeks under refrigerated conditions.

Key words: **Antioxidant activity; Karonda Juice; Whey, Beverage.**

1. Introduction

Karonda fruit (Carissa carandas), which resembles small-sized berries, is considered an underutilized fruit. It is widely cultivated in Gujarat, Uttar Pradesh, and Rajasthan (Tripathi et al., 2014). Traditionally, karonda fruit is used to prepare a number of traditional products, such as pickles, chutneys, sauces, and squash (Dhatwalia et al., 2021). It has also been historically used to treat ailments like acidity, stomachaches, burning sensations, and sore throat (Wani et al., 2013, Verma et al., 2015). Like other berries, Karonda contains anthocyanins, flavonoids, tannins and vitamin C (Singh et al., 2020). These compounds have antioxidants, anti-inflammatory, antidiabetic and antimicrobial properties whiche enable them to be used as functional ingredient (Arora et al., 2023). Reactive oxygen species (ROS) produced during oxidative metabolism are considered as a causative factor in several lifestyle-mediated diseases

(Kurek et al., 2022). The body has its defence system to neutralize the free radical oxygen species, oxidative stress occurs when reactive oxygen species exceed the body's antioxidant resistance mechanism (Lobo et al., 2010). There are few studies demonstrated utilization of pigments extracted from Karonda fruit in the development of various food ingredients (Itankar et al., 2011).

Whey is an important by-product in dairy processing, is classified into acid whey and sweet whey types based on the method used for milk protein precipitation. Acid whey is produced during paneer manufacture and sweet whey is produced during casein or cheese production. Whey is a good source of various micronutrients and protiens, particularly whey proteins play vital role in managing body weight. It also contain essential micerals such as calcium, phosphorous, potassium, magnesium, chloride, zinc, citrate and amino acids such as lysine, cysteine and methionine. Despite of nutritional and functional properties paneer whey is often regarded as a by-product, posing concerns regarding nutrient loss and environmental impact. Whey can be used for the development of beverages, particularly can be used in formulation of functional food. Fruit juices contain different consituents with functional properties and incorporating into whey to formulate whey-based beverages is an interesting approach to enhance whey utilization (Mamoun et al., 2011). Previously, orange and mango juice supplementation of whey has been studied to prepare protein and antioxidant-rich whey-based beverages (Whitaker, 2015; Ahmed et al., 2023). Hence, the present work was carried out to utilize karonda fruit juice in whey and to prepare and evaluate the antioxidant potential of enriched karonda whey beverages.

2. MATERIALS AND METHODS

The ripened Karonda fruits were harvested from Horticulture Farm, S. D. Agricultural University, Sardarkrushinagar. The fruits were sorted and thoroughly cleaned with potable water to remove any adhering dirt. The seeds were then removed manually and the juice was transferred to plastic container and stored at -20°C till further use.

Pasteurised paneer whey (containing 0.35 % fat, 0.52 % protein, 4.74 % lactose, 0.70 % ash and 6.31% total solids) was obtained from Banas dairy, Palanpur, Gujarat, Whey protein concentrate (WPC) was procured from Charotar Casein Company Pvt. Ltd., Nadiyad, Gujarat. Citric acid

Comment [MF1]: You should mentioned the protein percentage in WPC

Food grade citric acid (AR) procured from by Jay Chemicals, Palanpur. 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), 2,2'-azino-bis, 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Merck, India.

2.1 Chemical Characteristics of Karonda Juice

The juice was analysed for Total Solids (%),Total Soluble Solids (°Brix), pH and Titratable Acidity (% Citric acid) using the method of Ranganna (2012)

Comment [MF2]: Total solids

Comment [MF3]: total soluble solids (°Brix), pH and titratable acidity (% citric

2.2 Preparation of the karonda pulp extracts

To obtain the bioactive compounds in the karonda fruit pulp both the hydrophilic and lipophilic extracts were prepared by following the method described by Nilsson et al. (2005). To prepare the hydrophilic extract, 10 grams of pulp was mixed with acetate buffer (0.1M, pH 5.0) in a 1:4 (w/v) ratio. The mixture was homogenized using a laboratory blender (Indosati Scientific Equipment, India) for 3 minutes. The homogenized mixture was then centrifuged at 26,000 x g for 30 minutes at 4°C (REMI-502: REMI Elektro Technik Ltd). The supernatant and pellet were separated and stored at -20°C for further analysis.

The pulp pellets obtained from the hydrophilic extraction were used for lipophilic extraction. One gram of water-insoluble fraction of pulp pellets was mixed with acetone at 1:8 (w/v) ratio. The mixture was vortex mixed for 30 minutes and then centrifuged at 1000 x g for 10 min (REMI-502: REMI Electcktro Technik Ltd). The supernatant was collected and stored at -20 ^{o}C for further antoxidant analysis.

2.3 Antioxidant activity assay

The antioxidant activity of Karonda fruit extract was assessed using the ABTS (2,2'-azino-bis, 3-ethylbenzothiazoline-6-sulfonic acid) and the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.

2.3.1 ***ABTS (2,2'-azino-bis, 3-ethylbenzothiazoline-6-sulfonic acid) assay***

The antioxidant activity of hydrophilic and lipophilic extracts karonda fruit juice was determined using ABTS method as described by (Re et al., 1999) with some modifications. The stock solution of 7.0 mM ABTS solution and 2.45 Mm potassium persulfate was prepared. The solutions were mixed and kept at room temperature for 12-16 h in the dark. The ABTS stock solution was diluted with acetate buffer (approx 1: 90) till the absorbance showed 0.70 at 734 nm using the spectrophotometer (Model-SL210, Elico equipment, India). Karonda fruit extracts of (200 µl) appropriately diluted with each extract (hydrophilic and lipophilic) were added to the 2 ml ABTS solution. Then the absorbance was taken at 734 nm using UV-VIS spectrophotometer. The standard curve constructed by plotting absorbance against Trolox concentrations ranging from 25 - 200 µM. The results are expressed in mM Trolox equivalents (TE)/g of the fruit juice.

2.3.2. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant activity of hydrophilic and lipophilic extracts of karonda fruit juice was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) followed by the method described by (Brand-Williams et al., 1995). A DPPH stock solution (100 mM) was prepared in methanol. The hydrophilic and lipophilic extracts of karonda fruit were suitably diluted with their corresponding organic solvents. Suseqently, a 100 µL of the diluted extracts were mixed with 3.9 mL of DPPH solution (1:25 diluted in methanol) and incubated at 37 ^{o}C for 120 min. The absorbance of reaction mixture was recorded at 515 nm using a UV-VIS spectrophotometer (Model-SL210, Elico equipment, India). For the blank determination, 100 µL methanol was used in place of the extracts and absorbance was measured immediately against methanol. and a standard curve was obtained by plotting absorbance against Trolox concentrations ranging from 100-1000µM. The results are expressed in mM Trolox equivalents (TE)/g of the fruit juice.

2.3.3 Total phenolic content

The total phenolic content of karonda fruit pulp was determined by using Folin Ciocalteu's method (Kähkönen et al., 1999; Suntornsuk et al., 2002). A 400 µl aliquot of the approximately diluted sample extract was mixed with 2 ml of Folin-Ciocalteu's reagent (0.2 N). The mixture was left undisturbed for 3 min, 1.6 ml of 7.5% sodium carbonate solution added. The resulting mixture was incubated in the dark at room temperature for 30 min. For blank determination, 400 µL of distilled water was taken in place of the samples. The absorbance was recorded against a

blank at 765 nm using a UV-VIS spectrophotometer (Model-210, Elico equipment, India). The standard curve showed linearity in the range of 10-100 µg/ml gallic acid. Results were expressed as µmol of gallic acid equivalent (GAE) per gram fruit pulp.

2.3.4 Ascorbic acid content

The ascorbic content (vitamin C) of karonda fruit juice was estimated using N-bromosuccinamide method described by (Suntornsuk et al., 2002). A 5 ml of aliquot diluted sample was added to 1 ml acetic acid (1 %) and 5 ml potassium iodide solution (4 %), then mixed thoroughly. Following this, 3 ml of diethyl ether was added. The mixture was then titrated against standard N-bromosuccinamide solution (0.5 mg/ml). After allowing the layers to separate, the endpoint of the titration was identified by the appearance of a brown color in the ether layer due to liberated iodine, which was compared against a non-titrated control mixture. For the blank determination, 5 ml of Milli-Q water was utilized in place of the sample, indicating the volume of bromosuccinamide reagent required to produce a distinct brown color in the ether layer. The ascorbic acid content was subsequently calculated using a specific formula.

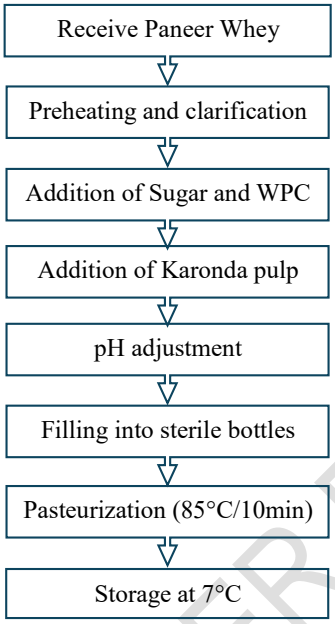
$$Ascorbic\ acid\ \left(\frac{mg}{100g}\right) = \frac{\text{Reagent factor} \times (\text{Sample reading} - \text{blank reading}) \times \text{Final volume}}{(\text{Aliquot of sample taken} \times \text{Wt. of sample})} \times 100$$

Where Reagent factor = $1/(\text{Standard reading} - \text{blank reading})$

2.3.5 Preparation of karonda whey beverages

Karonda whey beverage was prepared using paneer whey added with varying concentrations of karonda pulp, following the method described by De et al. (2008) with minor modifications (Figure 1). The clarified whey obtained from paneer production was preheated to 45 ^{o}C. To this, 10 % sugar and 0.5% WPC 80 (whey proten concentrate) were added followed by addition Karonda Juice (6, 8,10,12,14 %). The mixture was adjusted to pH 4.0 using 2.5M citric acid, filled into sterilized glass bottles. The pH and WPC levels were decided through initial trials, where the pH was varied from 2.5 to 6.5, and WPC concentrations between 1% and 3%, to assess the thermal stability of whey protein stability. The bottles were sealed with caps and pasteurised at 85 ^{o}C for 10 min and immediately cooled. The developed beverage was stored at 7 °C for subsequent analysis and storage studies.

Figure 1. Flow chart for preparation of karonda juice supplemented whey beverage



2.3.5 Proximate analysis of developed beverage

The developed beverage was analysed for Total Solids (%),Total Soluble Solids (°Brix), pH, Titratable Acidity (% Citric acid) using the method of Ranganna (2012). The Ascorbic acid content was measured by N-bromosuccinamide method. The antioxidant activity was measured by ABTS and DPPH radical Scavenging assay and Total Phenolic content by Folin Ciocalteu's method.

Comment [MF4]: total solids (%),total soluble solids (°Brix), pH, titratable acidity (% citric

2.3.5 Sensory evaluation

The sensory analyses of karonda whey beverage samples were done by a panel of seven judges using a 9-point Hedonic Scale. The panelists assessed and recorded scores for sensory attributes including taste, aroma, mouth feel, appearance and overall acceptability.

2.3.5 Storage studies of beverage

The antioxidant and microbial analysis of karonda whey beverages was conducted periodically at every seven day interval over a storage period of 4 weeks. The microbial analysis included the determination of total bacterial count (TBC), yeast and mold and coliform Chauhan et al., (2002).

2.3.6 Statistical analysis

The experiments were conducted in triplicate, and the resulting data were subjected to statistical analysis. Analysis of Variance (ANOVA) was used to test for significance of differences and mean done using the critical difference (CD) value at a 5% level of significance.

3. RESULTS AND DISCUSSION

The karonda fruit juice was acidic had titratable acidity of 0.48±0.02 and pH of 4.25±0.02 (Table 1). The antioxidant activity of the karonda fruit juice as determined by DPPH and ABTS assays, is shown in Table 2. The antioxidant capacity (DPPH assay) of hydrophilic exract was highest with 3.44±0.06 µmol TE/g as compared with lipophilic extract with 1.22±0.0.2 µmol TE/g ($P \leq 0.05$). The results of antioxidant capacity obtained from ABTS assay also confirmed the trend obtained with DPPH method. In contrast to DPPH assay results, the ABTS assay showed a significantly higher antioxidant capacity values for both hypdrohillic and lipophilic extracts *(*$P \leq 0.05$). The karonda juice extracts with highest antioxidant capacity as determined in ABTS and DPPH assay contained highest content of total phenolds as measured by the Folin-Ciocalteu method (**Table 2**). The hypdrohillic exract of karonda juice contained significantly higher levels of total phenolic content (5.56±0.14 µmol GAE/g). Azeez et al. (2016) also reported high antioxidant properties (DPPH, total phenolics and total flavonoids) of Karonda fruit. The high contents of flavonoids in different varieties of Karonda was demonstrated by Bons and Paul (2020). ABTS and DPPH are based on reduction of ABTS and DPPH free radical of samples but value from DPPH assay might be lower than those from ABTS assay (Brand-Williams et al., 1995; Re et al., 1999). Wang et al. (1996) revealed that some compounds which have ABTS scavenging activity may not show DPPH scavenging activity (Arts et al., 2004) and found that some products of ABTS scavenging reaction may have a higher antioxidant capacity and can further react with remaining ABTS radicals.

Table 1. Chemical characteristics of karonda fruit pulp

Parameter	Values
Total Solids (%)	16.12± 0.42
Total Soluble Solids (°Brix)	15.2± 0.20
pH	4.28± 0.02
Titratable Acidity (% Citric acid)	0.48± 0.02

Data are presented as means ± SD (n=3)

Table 2: Antioxidant and total phenolic content of karonda pulp

Karonda fruit pulp		Hydrophilic Extract	Lipophilic Extract	Total antioxidant activity
Antioxidant capacity (µmol *TE/g)	ABTS	4.78 ± 0.04[a]	1.58 ± 0.03[a]	6.36 ± 0.04[a]
	DPPH	3.44 ± 0.06[a]	1.22 ±0.02[a]	4.66± 0.04[a]
Total phenolic content (µmol GAE**/g)		5.56 ± 0.14[a]	2.32 ± 0.10[a]	7.88± 0.10[a]

*Means with different superscripts in each column were significantly different (LSD test, p<0.05) from each other. Data are presented as means ± SD (n=3) *-TE- trolox equivalent **-GAE- gallic acid equivalent*

3.1 Antioxidant activities and sensory properties of whey based beverage supplemented with Karonda juice

Table 4 presents the antioxidant activity of beverage samples prepared with varying levels of sugar and karonda juice, evaluated using DPPH and ABTS assays. The ABTS radical scavenging activity of the beverages increased progressively with higher fruit pulp levels, yielding values of 1.80, 2.48, 3.27, 3.89, and 5.66. Similarly, the DPPH radical scavenging activity also showed an upward trend, with values of 1.10, 1.63, 2.23, 2.68, and 3.84. As shown in Table 4, increasing the fruit pulp concentration from 6% to 14% resulted in a significant ($p<0.05$) enhancement of antioxidant activity in both assays. However, variations in sugar levels did not lead to any significant ($p<0.05$) differences in antioxidant activity (Table 3) These findings are similar with (Chavda et al., 2016), who reported antioxidant activity in whey protein-enriched cranberry,

blackberry, and strawberry beverages containing 10% sugar was not significantly affected when assayed by ABTS and DPPH method. The antioxidative properties of karonda fruits are attributed to their high vitamin C content and phenolic compounds (Azeez et al., 2016; Sakhale et al., 2012). These bioactive compounds may act independently or synergistically with whey proteins to enhance antioxidant activity (Kaushik Khamrui et al., 2001).

Table 3: Sensory attributes of protein enriched whey beverages.

Sugar level (%)	Sensory	Fruit juice (%)				
		6	8	10	12	14
6	Taste	6.7±0.22^{a}	8.1±0.13^{b}	7.5±0.11^{c}	6.5±0.16^{a}	6.3±0.28^{a}
	Aroma	6.5±0.22^{a}	8.2±0.26^{b}	7.1±0.14^{c}	7.0±0.20^{c}	6.4±0.10^{a}
	Mouthfeel	7.0±0.20^{a}	8.5±0.31^{b}	7.5±0.14^{c}	6.5±0.16^{d}	6.0±0.17^{e}
	Colour & appearance	7.0±0.23^{a}	8.2±0.18^{b}	8.3±0.12^{b}	8.5±0.23^{b}	8.5±0.18^{b}
	Overall acceptability	6.9±0.24^{a}	8.3±0.21^{b}	7.5±0.10^{c}	6.7±0.23^{a}	6.5±0.27^{a}
8	Taste	7.0±0.21^{a}	7.8±0.17^{b}	8.2±0.36^{c}	7.0±0.33^{a}	6.4±0.24^{d}
	Aroma	7.0±0.11^{a}	7.5±0.23^{b}	8.5±0.31^{c}	7.0±0.26^{a}	6.3±0.27^{d}
	Mouthfeel	6.5±0.22^{a}	8.0±0.11^{b}	8.5±0.23^{c}	6.8±0.32^{d}	6.0±0.20^{e}
	Colour & appearance	6.6±0.12^{a}	8.0±0.20^{b}	8.2±0.10^{b}	8.3±0.20^{b}	8.5±0.10^{b}
	Overall acceptability	6.8±0.29^{a}	7.9±0.34^{b}	8.4±0.25^{c}	7.0±0.21^{d}	6.6±0.33^{a}
10	Taste	6.3±0.31^{a}	6.9±0.26^{b}	7.5±0.23^{c}	8.5±0.17^{d}	8.6±0.12^{d}
	Aroma	6.5±0.29^{a}	7.0±0.27^{b}	7.6±0.14^{c}	8.5±0.19^{d}	8.5±0.25^{d}
	Mouthfeel	6.5±0.3^{a}	7.2±0.24^{b}	7.0±0.22^{b}	8.45±0.2^{c}	8.5±0.16^{c}
	Colour & appearance	6.8±0.27^{a}	8.3±0.31^{b}	8.2±0.13^{b}	8.6±0.24^{b}	8.7±0.16^{b}
	Overall acceptability	6.5±0.39^{a}	7.0±0.27^{b}	7.7±0.26^{c}	8.5±0.22^{d}	8.6±0.26^{d}

Means with different superscripts in each column were significantly different (LSD test, $p<0.05$) from each other.
Data are presented as means ± SD (n=3)

Table 4: Antioxidant attributes of protein enriched whey beverages.

Fruit pulp (%)	Antioxidant activity (µmol *TE/ml)					
	ABTS	DPPH	ABTS	DPPH	ABTS	DPPH
	Sugar 6%		Sugar 8%		Sugar 10%	
6	1.80±0.04^{a}	1.10±0.03^{a}	1.84±0.03^{a}	1.14±0.02^{a}	1.87±0.05^{a}	1.21±0.04^{s}
8	2.48±0.03^{b}	1.63±0.04^{b}	2.53±0.02^{b}	1.65±0.03^{b}	2.52±0.03^{b}	1.68±0.03^{b}
10	3.27±0.03^{c}	2.23±0.02^{c}	3.36±0.02^{c}	2.28±0.02^{c}	3.39±0.03^{c}	2.30±0.02^{c}
12	3.89±0.02^{d}	2.68±0.03^{d}	3.93±0.01^{d}	2.76±0.03^{d}	3.99±0.02^{d}	2.75±0.02^{d}
14	5.66±0.02^{e}	3.84±0.02^{e}	5.7±0.03^{e}	3.96±0.01^{e}	5.65±0.04^{e}	3.99±0.03^{e}

Means with different superscripts in each column were significantly different (LSD test, $p<0.05$) from each other. Data are presented as means ± SD (n=3)

The sensory evaluation of whey based beverage supplemented with Karonda juice based on taste, aroma, mouthfeel, colour and appearance and over all acceptability characteristics is presented in Table 5. The addition of varying levels of juice from 8 to 14% did not affect the color of the beverage ($p>0.05$). This could be due to high anthocyanin content of karonda (22.57 mg cyaniding equivalent of fruit weight) which impart bright red colour to the beverage at low pH values (Azeez et al., 2016; Wahyuningsih et al., 2017). The overall acceptability scores for beverages fortified with 6.0, 8.0, 10.0, 12.0 and 14.0 per cent karonda fruit pulp with 10.0 per cent sugar were 6.5, 7.0, 7.7, 8.5 and 8.6. According to hedonic scale employed the results indicate that the beverage with 10% sugar supplemented with 14% sugar was liked very much. The addition of upto 15% pineapple and blackberry juice because of low amount of soluble solids and 10% sugar to paneer whey to develop beverage has been reported (Purkiewicz and Pietrzak-Fiećko, 2021). Similarly, (Shukla et al., 2000) reported ready-to-serve whey beverage by incorporating 10% sugar and 30% litchi juice was acceptable by senory panel.

3.2 Characterization of whey based beverage supplemented with Karonda juice

The proximate chemical composition, chemical characteristics and antioxidant properites of whey based beverage supplemented with Karonda juice (14%) is presented in Table 5. The pH and titratable acidity of were 4 and 0.35% (citric acid), respectively. The beverage contained low fat content and high amount of carbohydrates and protein, the protein content was 1.28%, which is slightly higher than the values (0.62-0.82%) reported by previous studies in different berries. The chemical characteististics were very similar to those values reported by Chavda et al. (2016) who reported values of TS 18.44- 18.63%, ash 0.73-75%, carbohydrate 11.69-17.17%, protein 0.6-0.8%. and TSS 17.40-17.80% in different varieties of cranberry, blackberry and strawberry beverage. On the other hand the antioxidant capacity of the beverage, as determined by the ABTS assay, was 5.65 ± 0.04 µmol TE/mL, which is approximately 16.6-fold higher than that of plain paneer whey (0.34 µmol TE/mL). Similarly, the antioxidant capacity measured by the DPPH assay was 3.99 ± 0.03 µmol TE/mL, resulted a 33.3-fold increase compared to plain paneer whey (0.12 µmol TE/mL). These finding are similar with the observations of Arpit (2008) who reported the endogenous antioxidant capacity of paneer whey as 0.30 µmol TE/L using the

ABTS method. Further, the antioxidant capacity increased by 1.67-fold in mango whey beverages, 2.94-fold in pineapple, 8.6-fold in strawberry, and 6.83-fold in blackberry whey beverages. The substantial increase in antioxidant activity observed in these beverages can be attributed to high ascorbic acid (1.15±0.02 mg/ml) and total phenolic content (21.89±0.01 mg GAE/100 mL) (Vinson et al., 2001). This suggests that bioactive compounds in karonda juice play pivotal role in antioxidant capacity of beverage.

Table 5: Physicochemical, proximal, and antioxidant characteristics of whey based beverage supplemented with Karonda juice

Parameters	Karonda (14 %)
pH	4.0±0.01
Total Solids (%)	18.42±0.03
Protein (%)	1.28±0.01
Fat (%)	0.72±0.02
Ash (%)	0.82±0.04
Carbohydrate* (%)	15.6
Energy Value kcal/100g	74
Titratable acidity ((%) Citric acid)	0.38±0.02
Vitamin C (mg/ml)	1.15±0.02
Total phenolic content (mg GAE**/100ml)	21.89±0.01
Antioxidant Capacity (µmol TE/ml)	
ABTS	5.65±0.04
DPPH	3.99±0.03

Data are presented as means ± SD (n=3) *By difference

3.3 Evaluation of whey based beverage supplemented with Karonda juice during the storage

The antioxidant capacity of the selected beverage (formulation 5) decreased during storage at 7°C, from 5.65 ± 0.03 to 4.61 ± 0.03 µmol TE/mL (ABTS assay) and from 3.99 ± 0.04 to 2.58 ± 0.04 µmol TE/mL (DPPH assay) (Table 6). This reduction may be due to loss of Vitamin C and

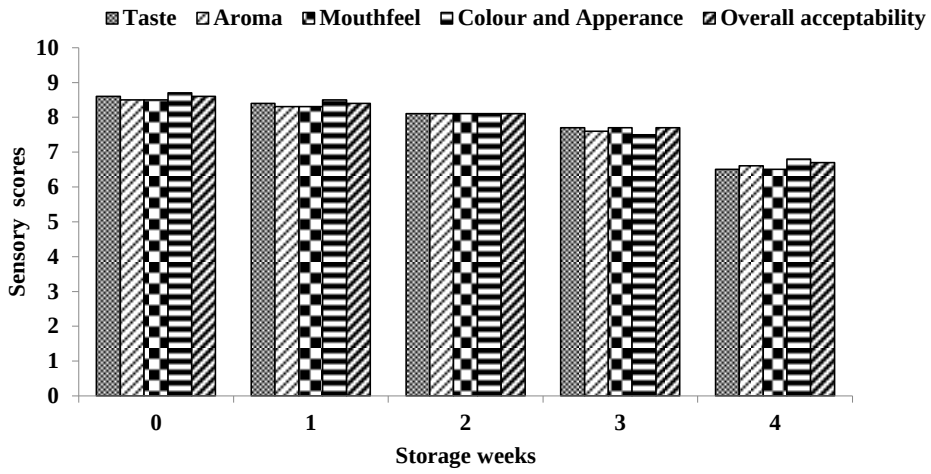
total phenolic content which may affect the antioxidant capacity (P≤0.05). Although the contribution of ascorbic acid to the antioxidant capacity of fruit beverages is less than that of the total phenols. A similar result was obtained by (Sakhale et al., 2012) when evaluating the antioxidant capacity of whey with mango juice during storage. They reported a ascorbic acid reduction from 9.8 to mg/100g after 30 days of storage at refrigeration. Conversely, decrease in pH 4.0 at beginning of the storage 3.83 at the end of storage might be due to the slight growth of microorganism in the beverage. The total bacterial count of freshly prepared was at 0.59 log CFU/ml. Yeast and mold counts, although absent in the fresh beverage, but increased significantly during storage due to the aciduric nature of the product (Table 6). In contrast, coliform counts remained undetectable throughout the storage duration. The observed increase in total bacterial counts can be attributed to the proliferation of acidophilic bacteria in the beverages. These findings align with the results of (Chavan et al., 2015) who reported an increase in the standard plate count of chhana whey beverage from 2.0 to 7.9 CFU/ml after 30 days during refrigeration. Similarly, (Kaushik Khamrui et al., 2001) concluded that acidification of beverages helps inhibit microbial growth, thereby extending shelf life to approximately three weeks at refrigeration temperature. On the other hand sensory quality of beverage remained acceptable for up to 3 weeks of storage with an overall acceptability score of 7.7 on the 9-point hedonic scale (Figure 2). Beyond this period, the sensory score was 6.7 indicating that storage time significantly affected sensory acceptance (P>0.05).

Table 6: Chemical and microbiological changes of Karonda whey beverage during storage at 7 ± 2°C.

Storage Period (week)	Chemical Parameters				Antioxidant Activity		Microbiilogical Parameters		
	pH	Total solids	Vitamin C (mg/100 ml)	TPC (µmol GAE /ml)	ABTS (µmol TE/ml)	DPPH (µmol TE/ml)	TBC (CFU/ml)	Yeast and Mold (CFU/ml)	Coliform (CFU/ml)
0	4.00	17.20^{a}	1.15±0.01^{a}	21.89±0.1^{a}	5.65±0.03^{a}	3.99±0.04^{a}	0.59	0	ND
1	3.89	17.25^{a}	1.04±0.03^{b}	21.78±0.1^{a}	5.49±0.02^{a}	3.81±0.02^{a}	0.88	0	ND
2	3.86	17.10^{a}	0.98±0.01^{c}	21.39±0.09^{a}	4.78±0.01^{b}	3.59±0.03^{a}	1.22	0.28	ND
3	3.84	17.15^{a}	0.74±0.02^{d}	20.47±0.13^{c}	4.67±0.02^{b}	3.53±0.02^{a}	1.56	0.54	ND
4	3.83	17.20^{a}	0.57±0.01^{e}	20.6±0.13^{c}	4.61±0.03^{b}	2.58±0.04^{c}	2.67	0.98	ND

ND=Not Detected; Means with different superscripts in each column (a, b, c, d, e) were significantly different ($p<0.05$), means ± SD (n=3)

Figure 2: Sensory attributes of protein enriched karonda beverage at 7 ± 2°C



4. CONCLUSIONS

The analysis of antioxidant properties of karonda fruit juices led to several interesting findings. The results of this study indicated that antioxidant activity of paneer whey can be enhanced with supplementation of karonda juice. This approach can be used to develop whey based beverage with enhanced antioxidant capacity. During the storage of the developed beverage was acceptable upto the 3 weeks of storage with adequate acceptance based on sensory evaluation. Moreover the developed beverage is a potential functional product with antioxidant activity was mainatained during storage at refrigeration temeperature.

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