

**DEVELOPMENT OF CARBONATED AND NON
CARBONATED SUGARCANE JUICE
BEVERAGES**

Dissertation

**Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of**

**DOCTOR OF PHILOSOPHY
in
FOOD TECHNOLOGY
(Minor Subject: Processing and Food Engineering)**

By

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CERTIFICATE I

This is to certify that the dissertation entitled, “**Development of Carbonated and Non Carbonated Sugarcane Juice Beverages**” submitted for the degree of **Ph.D.** in the subject of **Food Technology** (Minor Subject: Processing and Food Engineering) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Mr. Bharat Sidram Agarkar (Admn. No. L-2013-A-18-D)** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that the dissertation entitled, “**Development of Carbonated and Non Carbonated Sugarcane Juice Beverages**” submitted by **Mr. Bharat Sidram Agarkar (Admn. No. L-2013-A-18-D)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Ph.D.** in the subject of **Food Technology** (Minor Subject: Processing and Food Engineering) has been approved by the Student’s Advisory Committee after an oral examination on the same in collaboration with an external examiner.

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ABSTRACT

The non carbonated sugarcane juice beverages blended with juices of kinnow (10-15 %), aonla (5%), lemon (2-2.5%) and ginger (2.5-3%) and sugarcane juice beverage with lemon and ginger juice (3% each) were found superior. The pasteurization of sugarcane juice beverages at 82^oC temperature for 5 min along with 120ppm sodium benzoate and processing of bottled juice in boiling water for 30 min was found optimum. The beverages stored at refrigerated temperature (4^oC) scored slightly high for all the sensory and chemical parameters than stored at room temperature (30±4^oC). There was slight decrease in values of total soluble solids (1.6%), pH (3.63%), ascorbic acid (1.35%), total sugars (2.38%) and viscosity (1.71%) for all the beverages after 3 months during storage of 6 months while the values for titratable acidity and reducing sugars were increased 4.91% and 7.9%, respectively. The total polyphenol and total flavonoids were decreased by 20 per cent while the antioxidant activity was decreased by 25 per cent during the storage of six months. The carbonated sugarcane juice beverages containing juices of kinnow and aonla each 2-3%; lemon and ginger each 1.5% and sugarcane juice 41-43% were found superior while beverage contained only lemon and ginger juice 1.25 % each with sugarcane juice 47.5% was found acceptable. The pasteurization temperature of 82^oC for 5 min was found optimum for blended juices which was used as base for the carbonated sugarcane juice beverages. The beverages carbonated at 80psi carbonation level containing 60ppm sodium benzoate were found superior. The beverages stored at refrigerated temperature (4^oC) scored slightly high score for all the sensory and chemical parameters than the stored at room temperature (30±4^oC). The significant reduction in CO₂ content of beverages was observed during storage for 6 months and beverages were found acceptable. The more CO₂ retention was observed in refrigerated samples than stored at room temperature while statistically no significant difference. The both beverages showed stability till 3 months while further storage slightly decreased the values. The products were found acceptable after six months storage.

Key words: Aonla juice, Carbonated beverage, Ginger juice, Kinnow juice, Lemon juice, Non carbonated beverage, Sugarcane juice.

Signature of Major Advisor

Signature of the Student

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CONTENTS

CHAPTER	TOPIC	PAGE NO.
I	INTRODUCTION	1-4
II	REVIEW OF LITERATURE	5-17
III	MATERIAL AND METHODS	18-30
IV	RESULTS AND DISCUSSION	31-77
V	SUMMARY	78-83
	REFERENCES	84-91
	SUBMITTED/ ACCEPTED/ PUBLISHED RESEARCH ARTICLES	
	VITA	

LIST OF TABLES

Table No.	Title	Page No.
3.1	Combination of treatments used for recipe standardization of non carbonated sugarcane juice beverages	21
3.2	Combination of treatments used for recipe standardization of carbonated sugarcane juice beverages	22
4.3	Optimization of pasteurization temperature and time of sugarcane juice beverages	33
4.4	Optimization of level of preservative for non carbonated sugarcane juice beverages pasteurized at 82 ⁰ C for 5 min.	34
4.5	Optimization of level of preservative for carbonated sugarcane juice beverages (juice base pasteurized at 82 ⁰ C for 5 min and cooled to 15 ⁰ C)	35
4.6	Physicochemical and phytochemical constituents of juices of sugarcane, kinnow, aonla, lemon and ginger	36
4.7	Mineral content of the of juices of sugarcane, kinnow, aonla, lemon and ginger (mg/100ml)	37
4.8	Sensory evaluation of non carbonated sugarcane juice beverages	40
4.9	Physicochemical and phytochemical constituents of non carbonated sugarcane juice beverages	42
4.10	Mineral content of the of non carbonated sugarcane juice beverages (mg/100ml)	43
4.11	Effect of storage period on physicochemical and phytochemical constituents of non carbonated sugarcane juice beverages	49-50
4.12	Effect of storage temperature on physicochemical and phytochemical constituents of non carbonated sugarcane juice beverages (6 months storage)	51-52
4.13	Effect of storage period and storage temperature on microbiological quality of non carbonated sugarcane juice beverages	55
4.14	Sensory evaluation (overall acceptability) for optimization of carbonation level and standardization of recipe for the carbonated sugarcane juice beverages	56
4.15	Sensory evaluation of carbonated sugarcane juice beverages at 80 psi carbonation level	57
4.16	Physicochemical and phytochemical constituents of carbonated sugarcane juice beverages (80psi carbonation)	61
4.17	Mineral content of the of carbonated sugarcane juice beverages (mg/100ml).	62
4.18	Effect of storage period on physicochemical and phytochemical constituents of carbonated (80 psi) sugarcane juice beverages	70-71

Table No.	Title	Page No.
4.19	Effect of storage temperature on physicochemical and phytochemical constituents of carbonated sugarcane juice beverages (6 months storage)	72
4.20	Effect of storage period and storage temperature on CO ₂ content (mg/100ml) of carbonated sugarcane juice beverages	76
4.21	Effect of storage period and storage temperature on microbiological quality of carbonated sugarcane juice beverages	77

LIST OF FIGURES

Table No.	Title	Page No.
1	Effect of blending on the phytochemicals of non carbonated sugarcane juice beverages	43
2	Effect of storage period on sensory qualities of non carbonated sugarcane juice beverages	44-46
3	Effect of storage temperature on sensory qualities of non carbonated sugarcane juice beverages (6 months storage)	48
4	Effect of storage period and storage temperature on colours (Tintometer colour units) of non carbonated sugarcane juice beverages	53-54
5	Effect of carbonation levels on sensory qualities of carbonated sugarcane juice beverages	59-60
6	Effect of blending on phytochemicals of carbonated sugarcane juice beverages	63
7	Effect of carbonation levels on phytochemicals of carbonated sugarcane juice beverages	63-64
8	Effect of storage period on sensory qualities of carbonated sugarcane juice beverages	65-67
9	Effect of storage temperature on sensory qualities of carbonated (80psi) sugarcane juice beverages (6 months storage)	68-69
10	Effect of storage period and storage temperatures on colours (Tintometer colour units) of carbonated sugarcane juice beverages	74-75
11	Effect of storage period and storage temperature on CO ₂ Gas: Volume (CO ₂ Gas dissolved by I volume of water) of carbonated sugarcane juice beverages	76-77

LIST OF PLATES

Plate No.	Title
1	Non-carbonated sugarcane juice beverages
2	Carbonated sugarcane juice beverages

CHAPTER I

INTRODUCTION

Sugarcane (*Saccharum officinarum*) is widely cultivated as a major crop in tropical and subtropical regions of the world. India ranks second largest producer of sugarcane having 5.06 million ha area under cultivation and annual crop production of 366.8 million tonnes. The Uttar Pradesh and Maharashtra are the major states who grows sugarcane with the annual crop production 126.9 million tonnes and 98.9 million tonnes, respectively. The sugarcane is also grown in Punjab with annual production of 6.6 million tonnes (Anonymous 2016). The 80 per cent of world demand of sugar is met by sugarcane and the rest is from sugar beet.

The most of the Asian countries, Latin America and also other countries grows sugarcane commercially and sugarcane juice is a common drink. In India, sugarcane juice is commonly extracted by crushing the sugarcane by mechanically operated small machines or sometimes by manually or bullock operated wooden charkas both in rural and urban areas. The urban and rural population is habitual for thirst quenching and delicious drink made up from sugarcane juice with lemon, mint and ice. It contains 75-85 per cent water, 0.3-3.0 per cent reducing sugar, 10-21 per cent non-reducing sugar, also contains minerals, enzyme and organic acids (Swaminathan 1995). The sugarcane juice possesses many medicinal properties as helps to strengthen the body organs (stomach, kidneys, heart, eyes, brain and sex organs). It helps kidney to keeps the urinary flow clear and to perform their functions properly. It is also important to cure health problems like high acidity, gonorrhoea, enlarged prostate and cystitis (Karthikeyan and Samipillai 2010). Sugarcane juice is commonly used as a remedy for jaundice and used as a major source for preventive and healing for sore throat, cold and flu. The diabetic can enjoy this sweet drink without fear due to its low glycemic index and keep the body healthy. It helps to hydrates the body quickly when exposed for long time exposure to heat and physical activity (Subbannayya *et al* 2007).

Sugarcane juice has simple sugars so spoils quickly after extraction. The sugarcane juice become a popular delicious drink only by prolonging its shelf life by preventing the spoilage of juice using appropriate method of preservation. After harvesting of the sugarcane, endogenous enzyme (invertase) is activated and become a cause of deterioration of sugarcane (Thulasimani and Chidambaram 2006a). It becomes great substitutes for soft drinks like cola, pepsu, limca, sprite etc. The hygienic conditions are mainly not maintained during the process of transport of sugarcane from field to the point of extraction and processing of juice. Moreover, the sugarcane juice is commonly served as fresh, unpasteurized and is the major cause for the health hazards.

Kinnow Mandarin (*Citrus reticulata*) is a hybrid of two citrus cultivars King and Willow Leaf which is classified as kinnow mandarin (Saeed *et al* 2006). As the Punjab has

the highest area under the cultivation of kinnow (46,000 hectare) and approximately 2200 hectares gets added every year. The Punjab state had registered a production of 988,000 tonnes of kinnow in 2013-14. It is also grown in other states like Rajasthan, Haryana, Himachal Pradesh, Jammu & Kashmir and Utter Pradesh (Komal 2014). Kinnow mandarin is quite important as it has attractive colour, distinctive flavour and being rich source of vitamin C, vitamin B, β -carotene, calcium and phosphorous. It has many kinds of uses mainly in beverages, industrial and medicinal products (Sogi and Singh 2001). The kinnow juice turns bitter soon after the extraction due to conversion of limonate-a-ring-lactone into limonin. The limonin is bitter compound formed during storage of juice (Premi *et al* 1994). This bittering limits the processing of fruit. The kinnow juice was blended with aonla juice and spice extracts like ginger for improvement of the taste, aroma, palatability nutritive value and also to reduce bitterness. There is a huge potential to utilize this fruit in value added food products such as juice, nectar, squash and RTS drinks. These citrus drinks are the most popular and widely accepted fruit drinks in a global market (Benzaman *et al* 2001 and Gorinstein *et al* 2004).

The Aonla (*Emblica officinalis* Geartn.) is the richest source of vitamin C, contains 160 times more vitamin C than that of apple (Barthakur and Arnold 1991). The fruit contains a leucanthocyanin as it retards the oxidation of ascorbic acid. The gallic acid present in aonla fruit has antioxidant effect. The fresh fruits of aonla contains 454.40 mg/100ml of ascorbic acid, 7.53 per cent total sugar and also contains calcium, iron and phosphorus (14.91, 0.62 and 11.81 mg/100ml, respectively). It has high potential for processing (Dachiya and Dhawan 2001). Aonla fruit is used in the treatment of haemorrhage, diarrhoea, gastric disorders, dysentery, headache, constipation, jaundice, diabetes, cough and liver enlargement. It is used as a ingredient in triphala to treat chronic dysentery, biliousness and other disorders. It helps in maintaining digestive system healthy (Anand 1970, Parrotta 2001, Goyal *et al* 2007).

The many researchers in their study report proved that aonla has dominant medicinal properties like antibiotic, adaptogenic, antitumor, diuretic, antiscorbutic, antiviral, hepatoprotective, laxative, cardiogenic, antiulcerogenic and hypoglycemic properties (Pragati *et al* 2003, Mishra *et al* 2009). The Mathur *et al* (1996) reported the hypolipidaemic effect of fruit juice of aonla, while Perianayagam *et al* (2004) reported anti-pyretic and analgesic activity in ethanolic and aqueous extract.

The aonla has excellent nutritional profile and physicochemical properties so it is processed into different types of products. This fruit has sour and astringent taste. It is utilized in different forms as raw, processed, cooked and in the form of a pickle. In the market different aonla products (preserves, juice, jam, cheese, candy, powder, beverage and chutney) are available and those are liked by the consumers being the richest source of vitamin C and antioxidants (Goyal *et al* 2007 and Mishra *et al* 2009).

Lemon (*Citrus limon*) is an important therapeutic plant. Lemon juice is rich in vitamin C and useful for a series of health benefits. The colour of lemon is green to yellow and used in preparation of various foods as it possesses distinctive flavor and has ability to enhance spicy flavor in certain foods. The lemon juice is a commercial product of lemon fruit. The lemon has strong antibacterial, antiviral and immune-boosting powers. It is commonly used as a weight loss aid, digestive aid and liver cleanser. The lemon juice contains immunity promoting nutrients like citric acid, calcium, magnesium, vitamin C, bioflavonoids, pectin and limonene those helps against infection (Al-Juhaimi 2013)

Ginger (*Zingiber officinale*) is the herb which promotes the release of bile from the gallbladder so important for those people suffering from gallstones. It may also decreases joint pain from arthritis and may have blood thinning and cholesterol lowering properties which is useful in treating heart disease (Fahlberg 1969).

The tender ginger rhizomes are more juicy and fleshy with very mild taste. It can be stewed in boiling water for making ginger tea along with honey. The mature ginger rhizomes are fibrous and nearly dry. The juice extracted from old ginger roots is highly potent and oftenly used as a spice to flavor different dishes. It also acts as a useful food preservative and it used to kill the harmful bacteria like *Salmonella* (Afshari *et al* 2007).

Ginger has great potential as an antibiological as it content high amount of phytochemicals such as gingerol and gingerol related compounds, paradol, terpenoids, zerumbone, zingerone, shogol and flavonoids. Due to these compounds it exhibit various pharmacological and biological activities (antioxidant, anti-tumor, anti-inflammatory, antianalgesic, anti-microbial, hepaticprotective, anti cancerous, anti-proliferation and antiinvasion). It was also reported that more work needs to be done for the benefit of improving human health (Marwat and Khan 2015).

Mint (*Mentha spicata*) is the herb and considered as a stimulant, antispasmodic, carminative, stomachic and diuretic. It is mainly used for curative treatment in gas pain, rheumatism, toothache and muscle pain. Mint has antioxidant properties due to presence of constituents like menthone, rosemarimic acid, menthol and carvone.

The fruit beverages have great nutritional, medicinal and calorific values as compared to synthetic beverages. The utilization of fruits for the preparation of various processed products becomes limited due to high acidity, astringency, bitterness and such other factors in some of the fruits, inspite of having great nutritional qualities. The blending of juices helps to improve the aroma, taste and nutrients of the beverages. The different studies have been carried out previously so as to formulate various blended fruit beverages using kinnow, aonla, pomegranate and ginger juice (Bhardwaj and Mukherjee 2011). The blending of fruit juices also helps to reduces the cost of production (Kalra and Revathi 1981, Khan *et al* 1988). The blends of juices of kinnow, aonla and ginger are commercially used (Bhat *et al* 1982).

Sugarcane, kinnow, aonla and lemon are valued very much for their refreshing juice in spite of nutritional and medicinal properties, ginger juice for its pleasant flavor. Therefore, blending these juices for the preparation of sugarcane juice beverages is thought to be a convenient and economic alternative for utilization of these fruits. The sugarcane juice has a great demand in the market, but the problem of its safety processes and storage life.

Though sugarcane juice is a more popular drink in Indian community, still it is very rare in a commercially packaged form. It is need of time to develop an effective treatments and procedures to increase the nutritional quality and shelf life of the sugarcane juice for its safety and broad circulation in market. The considerable efforts have been aimed for stabilizing the sugarcane juice quality during processing, storage, handling and distribution.

Taking into considerations of past studies and research, a novel approach is being made to formulate the carbonated and non carbonated sugarcane juice beverages by blending with other fruits juices like kinnow, aonla, lemon and ginger/mint extract without addition of sugar and acids with optimizing the processing conditions and recipes for different blends. There is a need to conduct the systematic work on development and strengthening of shelf life of carbonated and non carbonated sugarcane juice beverages from sugarcane juice blended with different fruits juices for health benefits. Further assessment of these beverages for effect of storage on nutritional and sensory qualities is also important. This type of formulated health beverages will be boon to soft drinks category and will serve better choice to replace the existing non nutritious artificial soft drinks as recently Government has made compulsion to the MNCs through Food Laws of FSSAI to fortify the existing drinks by natural juices at least 5 percent (2.5 % for lemon) to continue them.

In India, available sugarcane juice sold by the local vendors is neither shelf stable nor health safe and only a few attempt are being made to develop technology for carbonated and carbonated sugarcane juice beverages from sugarcane juice blended with fruit juices and fresh ginger/mint to meet the daily need of vital nutrients and phytochemicals of the consumers. Therefore, the present study was undertaken to develop the carbonated and non carbonated sugarcane juice beverages using the blends with natural fruit juices with the following objectives:

1. To optimize the formulation and processing conditions for development of carbonated and non carbonated sugarcane juice beverages from sugarcane juice blended with fruit juices, fresh ginger and mint.
2. To assess the physicochemical and sensory qualities of developed beverages.
3. To study the effect of blends and carbonation levels on the bioactive compounds of developed carbonated and non carbonated beverages.
4. Evaluation of storage stability of prepared carbonated and non carbonated beverages.

CHAPTER II

REVIEW OF LITERATURE

This chapter deals with the review of research work done by the various research scientists relevant to the present study on “Development of carbonated and non carbonated sugarcane juice beverages”. The juices of kinnow, aonla, lemon and extract of fresh ginger/mint were used to blend the sugarcane juice for the development of taste, flavour and stability of developed beverages. The available literature has been reviewed under the following categories.

2.1 Sugarcane juice

2.2 Kinnow juice

2.3 Aonla juice

2.4 Lemon juice

2.5 Ginger extract

2.6 Mint extract

2.7 Blended juice beverages

2.8 Carbonated fruit juice beverages

2.1 Sugarcane juice

The process for bottled cane juice which consists of pasteurization at 80⁰ C for 10 min, with addition of 70 ppm of potassium metabisulphite as preservative, hot bottling, sterilization at 100⁰C for 30 min and cooling for storage was developed by Kaur *et al* (1995). They revealed that due to blending with 0.3% lemon juice and 0.1% ginger juice, the flavour of the sugarcane juice was improved. The pasteurization of juice and refrigerated storage at 8-10⁰C showed short shelf-life of only 2 days, where as the pasteurization, adding potassium metabisulphite, hot bottling and sterilization improved the shelf life of juice for more than 6 months. It was also observed that *Leuconostoc mesenteroides* activity was absent in both plain and treated juices.

The development of sugarcane juice beverage was studied by Chauhan *et al* (1997). It was observed that the beverage containing 55 per cent sugarcane juice, 2.58 per cent lemon juice, 2 per cent ginger juice, 0.4 per cent mint extract, 0.2 per cent colour and 40 per cent water scored the maximum sensory score. It was also reported that the resultant product could be safe for more than 6 months at room temperature (18 to 30⁰C) with slight changes in chemical composition and favour.

In the study conducted by Chauhan *et al* (2002) on development of sugarcane juice beverage, they reported that the pH, total soluble solids and total sugars decreased, whereas, titrable acidity and reducing sugars increased significantly (P<0.01) during storage of sugarcane juice beverage. The total plate counts and yeast and mold counts were increased

appreciably and coli were found absent in sugarcane juice beverage during storage. The significantly ($P < 0.01$) higher changes were reported in different attributes at room temperature than refrigeration temperature. The sugarcane juice beverage containing citric acid and potassium metabisulphite resulted in minimum changes of sensory qualities during storage at room and refrigeration temperature. The sugarcane juice had acceptable sensory qualities and storage stability during storage of 90 days at room as well as refrigeration temperature.

The process for the preparation of sugarcane juice concentrate using citric acid alone and in combination with sodium benzoate was studied. The treated samples were packed in glass bottles. This study was conducted by Singh *et al* (2002) and it was observed that concentrate when treated with citric acid (0.5 per cent) and sodium benzoate (500 ppm) in combination depicted the best result on sensory evaluation for appearance, clarity, colour, flavour and taste. The shelf life was increased up to 8 months.

The sugarcane juice was preserved by heating it to a different temperature (75, 85 and 95⁰C) alongwith addition of potassium metabisulphite as a preservative and citric acid. In the study Bosse *et al* (2006) reported that, pH was decreased at elevated temperature and time of pasteurization. The reducing sugars was increased as the temperature of pasteurization increased whereas sucrose levels decreased.

The quality of cane juice was also affected due to acid and enzymatic inversion reported by Singh *et al* (2006). The two kinds of invertase enzymes, namely neutral invertase (NI) and acid invertase (AI) are present in sugarcane. These enzymes are having direct correlation with sucrose and reducing sugar contents during plant growth. These enzymes are responsible for the inversion of sucrose (Siswoyoa *et al* 2007).

The study was conducted by Mao *et al* (2007) on maintaining the quality of sugarcane juice with blanching and ascorbic acid. The study showed the changes in physiochemical characteristics of fresh sugarcane juice stored at 10⁰C. It was observed that blanching before squeezing the juice from sugarcane stem and use of ascorbic acid at 0.1 per cent improved the quality of sugarcane juice by inhibiting degreening and browning. It also reduced the activity of polyphenol oxidase (PPO) and sugarcane neutral invertase (SNI) in fresh sugarcane juice. The addition of ascorbic acid was found more effective than blanching.

The processing of sugarcane juice was studied by De Oliveira and Garcia (2007). In the study the pure sugarcane juice was mixed with fresh lemon and pineapple juice and subjected to a heat treatment at 75⁰C temperature for 25 min and/or dose of 2.5 kGy gamma radiation. The products were stored in high density polyethylene bottles. They reported that processing of the sugarcane juice reduced the microorganism load. The physicochemical composition, aroma and flavor of the processed beverages were found significantly unaltered as compared to the control.

The attempt for standardization of the processing method for preservation of sugarcane juice blends with different fruit juices was made by Sujatha *et al* (2007). The result showed that the addition of potassium metabisulphite as a preservative (125 ppm) was found best as compared to sodium benzoate. The sugarcane juice blended with grape juice and pineapple juice was found highly acceptable on sensory evaluation and could be stored for a period of 120 days in glass bottles as compared to other combinations.

The study conducted by Kumar *et al* (2009) found that the acidity, optical density and TSS of RTS beverage was increased with increase in the sugarcane juice and the storage period. The pH of RTS beverage was decreased with increase in sugarcane juice and the storage period. The samples having juice composition of sugarcane: pineapple (20:80) with sodium benzoate (100 ppm) scored high for colour, flavour, texture and taste followed by samples having juice composition of sugarcane: pineapple (20:80) with potassium metabisulphite (125 ppm), sugarcane: pineapple (80:20) with sodium benzoate (100 ppm) and sugarcane: pineapple (20:80) with sodium benzoate (125 ppm), respectively.

The sugarcane juice was found highly fermentable due to presence of 15-18 per cent sucrose, 0.5 per cent reducing sugars, adequate amount of organic nitrogen and mineral salts essential for microbial growth (Solomon (2009)). It was also observed that its pH was ranged between 5.0-5.5 which is an ideal for the growth of acidophilic microorganism (yeast and lactic acid bacteria). Large population of yeast favors the ethanol production by the utilization of sucrose. The microbial contamination was found higher in the juice. The viable microbial counts of 10⁸-10⁹ cells/ml was reported in juice. The major loss of sugar in raw sugar cane juice was occurred due to the inversion of sucrose and other types of degradation due to bacterial activities, enzymes and other biological factors.

The development of a mild process for sugar cane clarification to obtaining a cloudy, greenish-yellow beverage was studied. The heat treatment at 65 °C for 50 minutes; pH change to 7.0, 7.5 and 8.0; flocculent added to 0, 30 and 60 ppm aluminum polychloride (APC) – Panclar P-1010 while Magnafloc LT-27 was used as clarifier aid at level of 0, 2, or 4 ppm were the different process parameters used. The supernatant liquid was removed using vacuum pump and the decantation time was 45 minutes. The Response Surface Methodology was used to define the treatments and were subjected to physicochemical analysis for turbidity (%), total polysaccharide content (µg/ml), dextran content (µg/ml) and sensory analysis for the attributes of color, appearance and turbidity. This study was conducted by Prati and Moretti (2010) and depicted that the treatment with addition of 60 ppm APC, pH 8 and 0 ppm polyelectrolyte was found the best treatment to obtain a low polysaccharide content and 90 per cent turbidity. The beverage was found acceptable by the consumers on sensory evaluation and scored high for colour, appearance and turbidity.

The study on development of sugarcane juice beverage was conducted by Karmakar *et al* (2011) and during study, the sugarcane juice was collected from the local vendor, pasteurized (at 90 °C for 30 s, 1 min, 2 min, 5 min) and stored (at 4 °C). The juice was analyzed for physicochemical properties and microbiological attributes at every 2 days interval for 25 days. It was observed that the pH and vitamin C were decreased, whereas titrable acidity and microbial count were increased significantly ($P < 0.01$) during storage. The juice which was pasteurized at 90 °C for 5 min and stored at 4 °C showed the 4.7 mg/ml of vitamin C, 50/ ml microbial content, 10 °Brix, 0.072 mol/l acidity and pH 4 after 25 days. It was also reported that sugarcane juice beverage of acceptable quality could be prepared having 25 days storage stability at 4 °C.

The raw sugarcane juice treated with combination treatment of gamma radiation (5 kGy) and permitted preservatives and could stored for more than a month at low temperature (10°C) reported by Mishra *et al* (2011). The preservative used during study were citric acid (0.3 per cent), sodium benzoate (150 ppm), potassium sorbate (250ppm) and sucrose (10 per cent). They also reported that the treatment extended the shelf life of raw sugarcane juice to 15 days at room temperature ($26 \pm 2^{\circ}\text{C}$) and 35 days at 10°C. The microbial load was found to lower than detectable limit within this period. It was found that the phenolics and flavonoids were not affected by addition of these preservatives. The antioxidant activities (free radical scavenging activity, reducing power and nitrite scavenging activity) were also not significantly affected. The juice with this combination of treatment was found highly acceptable on sensory evaluation.

The different proportions of sugarcane juice to curd for the optimization of RTS beverage were used in the study conducted by Singh *et al* (2012). They reported that the average values of the sugarcane juice for total soluble solid, acidity, pH, ascorbic acid, sucrose and reducing sugar were found between the range of 18.3–19.5(°Brix), 0.13–0.18 (per cent), 6.05–6.16 (mg/100 g), 59.14–63.18 (per cent), 5.1–5.4 (per cent) and 4.36–5.43 (per cent), respectively. The RTS beverage of sugarcane juice blended with curd was packed in 200 ml glass bottles and stored for different storage periods (0, 5, 15 and 20 days). The beverages prepared from sugarcane juice and curd (4:1 proportion) were found superior after storage for 15 days.

The study on preservation of sugarcane juice was carried out by Sankhla *et al* (2012). In the study the different treatments viz. pasteurization at 80° C for 10 min along with KMS (150 ppm) and citric acid (0.05 per cent); pasteurization at 80° C for 10 min with KMS (150 ppm), citric acid (0.05 per cent) and pasteurization at 80° C for 20 min were given to sugarcane juice. The product samples were packed in glass bottles, PET bottles and low density polyethylene pouches (LDPE). The packaged samples were then irradiated at 0.25, 0.5 and 1.0 kGy and stored for 90 days at room and low temperature. The non-irradiated

samples was used as control. The viable bacterial count, viable yeast, mold count, moisture content and ascorbic acid content were found decreased significantly ($P > 0.05$) on treatment, where as effect on reducing and total sugars in cane juice was non significant. The glass bottles and PET bottles were found to be best than LDPE pouches. The shelf life of sugarcane juice in both packaging was found at par during storage.

The study on development of sugarcane juice beverage was conducted by Khare *et al* (2012) and reported that the beverage from sugarcane juice of variety Cos 767 having good quality and satisfactory storage stability for 60 days at refrigeration temperature could be prepared by using heat treatment at 75 °C for 10 min, 3 per cent lemon juice as flavour enhancer also a source of citric acid (anti oxidant), 1 per cent salt and 0.6 per cent ginger juice as flavour enhancer. They also reported that the use of KMS (225 ppm) was found to be the best anti microbial agent. The addition of lemon juice lowered the pH of sugarcane juice to 3.01 showed a preservative action and inhibited the growth of microorganisms during storage.

The average values of pH, total soluble solids (°Brix) and titrable acidity of the acidified and processed sugarcane juice beverage which were varied between 3.85-4.29, 15.4-24.5 and 0.145-0.22, respectively depicted by Kunitake *et al* (2013). It was also reported that the poly phenol oxidase (PPO) activity varied between 3.10-40.33 U/ml for the nine lots of acidified sugarcane juice before pasteurization. The heat treatments at 85, 90 and 95 °C for 30 s were used in the processing for the inactivation of the enzyme. POD activity varied between 69.78 and 220.44 U/ml. The processing at 95 °C for 30 s reached complete inactivation. The aerobic mesophilic, fungi and lactic acid bacteria counts in processed lots ranged from 1 to 2 log cfu/ml, 0 to 2 log cfu/ml and 0 to 2 log cfu/ml, respectively. *Coliforms* and *Salmonella* evaluation were in compliance with Brazilian regulations. The beverage scored between 4.9 and 6.5 for all attributes on sensory evaluation.

The quality of sugarcane juice extracted from stored canes, as well as changes in quality of fresh juice stored at different temperatures was studied by Thulasimani *et al* (2013). The stems of sugarcane were stored at 10 and 30°C while the fresh juice at 5 and 30°C temperature. The different physicochemical parameters (juice yield, total soluble solids, total sugar content, titrable acidity, pH, viscosity), total microbial count and sensory evaluation for colour and flavour were studied. It was observed that the sugarcane stems stored at low temperature (10⁰ C) maintained the quality of juice for 10 days whereas low temperature storage at 5⁰C could last for only 4 days. The spoilage of sugarcane occurred faster at 30⁰C than that of stored at 10⁰C temperature. The sugarcane juice was spoiled within a day on storage at 30⁰ C temperature. The microbial count for lactic acid bacteria was increased during storage of sugarcane juice.

The clarification effect of alum on raw sugarcane juice during processing and sucrose loss was investigated by Kimatua *et al* (2015). Significantly lower ($p < 0.05$) sucrose losses

were observed in clarified juice with alum treatment than in the rest of the clarified juices. The pretreated cane juice of intermediate liming showed that values for colour and turbidity were reduced by 36.9 per cent and 98.1 per cent, respectively at 150 mg/lit alum level. The intermediate liming of sugarcane juice at 150 mg/lit alum level resulted in the most compact final mud volume of 10.3 per cent with initial settling rate of 260 ml /min. The residual aluminium concentration in alum treated clarified juices was found lower than the natural aluminium concentration in untreated cane juice and the values reported were 0.025 to 0.048 mg/lit and 0.088 mg/lit, respectively. This study showed that the use of alum in clarification of sugarcane juice improved the clarification efficiency and lowered sucrose loss, so it has a great potential for commercial use.

2.2 Kinnow juice

The study was conducted on preparation of blends of different fruit juices by Bhardwaj and Mukherjee (2011). The different blends viz. kinnow juice: aonla juice: ginger juice (100: 0: 0, 95: 5: 0, 92: 5: 3 ratio) and kinnow juice: pomegranate juice: ginger juice (90: 10: 0, 87: 10: 3 ratio), respectively were prepared for improvement of flavour, palatability, nutritive and medicinal value. The juice blends were pasteurized at 75°C for 15 min and potassium metabisulphite (750 ppm) was added as a preservative. The prepared blends were stored in 200 ml colourless glass bottles at room temperature (28±4°C) for six months. The blends were tested for physicochemical characteristics, sensory evaluation and microbial population regularly after two months interval. It was observed that the blend of juices of kinnow, pomegranate and ginger (87:10:3) was found most effective and showed minimum changes in TSS (12.00 to 14.13°Brix), acidity (0.720 to 0.510 per cent), ascorbic acid (18.38 to 12.90 mg/100 ml) and limonin (0.103 to 0.250 mg/100ml). The blend of kinnow, aonla and ginger juices (92:5:3) was found best in view of non-enzymatic browning (0.081 to 0.104) and minimum population of bacteria, mould and yeast was recorded at the end of storage for six months. The good amount of vitamin C (38.95 mg/100 ml) was recorded in juice blend on storage of six months. The addition of ginger juice in blends improved the quality and reduced microbial growth. The product was found acceptable after six months of storage at room temperature.

The different blends using juices of kinnow, aonla, ginger and pomegranate were prepared to improve flavor and nutritive value by Bhardwaj (2013). The juice blends were prepared by pasteurization (75 °C or 85 °C for 15 min) and addition of potassium metabisulphite (500 or 750 ppm). The blends were packaged in 200 ml colourless glass bottles and stored at 4±1°C temperature. The blends were tested at every three months interval up to six month. He further reported that The individual effect of juice blending ratio, processing temperature and potassium metabisulphite treatment was found to be significant and also increased the storage life with acceptable quality. The processing at 75 °C for 15

min and 750 ppm potassium metabisulphite was found the most effective for the juice blends. Moreover, juice processed at 85 °C temperature showed a minimum microbial population.

The kinnow-aonla RTS beverage with herbal treatments was prepared by Vikram and Prasad (2014). It was observed that the RTS prepared with herbal treatments were found better for TSS, pH, acidity, ascorbic acid content and overall acceptability over control sample. The T5 (ginger powder @100 gram/ lit.) treatment sample recorded highest mean values for TSS (°Brix), pH, ascorbic acid content (%) and overall acceptability score were 15.13, 3.5, 19.4 and 7.78, respectively. It has been also reported that treatment T5 could be used for commercialization of kinnow-aonla RTS beverage. It was revealed that there was an increase in the level of TSS and pH during the storage period of six months. It was also reported that combination of different herbs gave better results for taste as compared to without herbal combinations. The RTS beverage could be recommended for the large scale industrial production.

2.3 Aonla juice

The aonla juice beverage was prepared using juices of adulasa and shatavari leaf, ginger rhizome extract and mint extract by Khapre *et al* (2010). The juices and extracts were mixed in different combinations. The mint extract was added at a rate of 0.4 – 0.6 mg/100 ml. The sugar was added to maintain TSS to 16 °Brix, boiled for 5-7 min, cooled, sealed in bottles and stored at low temperature. The beverage prepared with 90.5 per cent aonla juice, 4 per cent ginger extract, 5 per cent adulasa, 2 per cent shatavari juices and 0.5 mg/100 ml mint was found to be more appropriate. The health beverage showed pH 4.2, deep yellow to yellowish colour and pleasant taste.

In the study conducted by Jain *et al* (2013) reported that aonla is rich source of vitamin C, polypolyphenols and has strong antioxidant activity, therefore becoming popular in recent years for its use as nutraceutical food. It has been also reported that the high heat processing treatments may sometimes cause significant loss of its nutritive and pharmacological qualities. It was also reported that due to highly acidic and astringent in taste, it was mixed with other fruit juices such as lime, ginger, etc. for the preparation of RTS, squash and syrups. Hence it could be exploited along with other fruit juices for development of vitamin C rich fruit juice based beverages.

The various combinations of juices of aonla and ginger along with sugar and artificial sweeteners were tried for development of nutritious RTS beverages. The prepared beverages were evaluated for various physicochemical and sensory qualities during storage by Gaikwad *et al* (2013). The study revealed that the RTS beverage containing aonla and ginger juice with aspartame was scored maximum on sensory evaluation for the all quality attributes such as appearance, colour, flavour, taste and overall acceptability. The ascorbic acid content was found in good amount (180 mg/100 g). The ascorbic acid content was reduced while acidity

was increased during the storage of beverage at room temperature over a period of 60 days.

The study was conducted for the development of therapeutic RTS beverage by blending aloe vera, aonla and ginger juices viz. 50:25:25(A), 60:20:20(B), 70:15:15(C) and 80:10:10(D). The different blends were homogenized at 8000 rpm for 2 min and pasteurized at 85°C for 10 min. The developed therapeutic RTS beverage was complied with Indian standards of RTS fruits beverages by Sasi *et al* (2013). They reported that among different blends of therapeutic RTS beverages, sample C prepared by using blending ratio of 70:15:15, exhibited highest sensory scores for overall acceptability. The developed RTS beverage could be recommended for the commercial production.

2.4 Lemon juice

The variables such as storage prior to processing, juice-extraction pressures (heavy and light) and different methods of juice extraction for lemons were studied by Vandercook *et al* (1966). Lemons on storage for 15 weeks showed a 64 per cent decrease in l-malic acid but 34 per cent increase was observed in the total amino acids. There was no significant change in total polyphenolics. Extremes of extraction pressure showed significant effect which increased the values of amino acid and total polyphenolic content, whereas z-malic acid was decreased. There was no significant effect on the citric acid, sterol, carotenoid and soluble pectin values. The extraction process affected the pectin content. The small changes were observed in some of the constituents with extraction pressure in comparison with the natural range of values. The prediction of citric acid by the multiple-regression approach is independent of commercial fruit storage and processing practices.

The lemon juice powder using spray dryer under optimized conditions of inlet temperature and maltodextrin levels was prepared by Mishra *et al* (2014). The present investigation was undertaken to optimize the amla-lemon based RTS beverage and studied for its storage stability. The effect of inlet temperatures (125, 150, 175 and 200°C) and maltodextrin levels (10, 15 and 20 per cent) on the physicochemical properties, total phenolic content (TPC) and diphenyl picryl hydrazyl (DPPH) scavenging activity of spray dried lemon juice powder was studied. A dry mix for RTS beverage was prepared by mixing spray dried lemon juice powder with spray dried amla juice powder along with citric acid and glucose. The acceptability and nutritive value of amla based RTS fruit beverage was enhanced by the spray dried lemon juice powder. The amla-lemon based RTS beverage showed good nutritional quality and TPC content. The storage at refrigerated temperature with nitrogen flushing prevented colour change and reduced TPC and DPPH losses. The developed product possessed scored good on sensory evaluation. The spray dried lemon powder could be used for further product development to enrich the functional and nutritive properties.

The study undergone through investigation on small scale process for production of blended therapeutic aloe-lemon RTS drink so could be further used for commercial

production by Haque *et al* (2015). The process parameters were optimized in the laboratory while the required machineries were designed and fabricated. The machines were used for producing aloe-lemon drink as per designed process. The blended juices were prepared by using different combinations of aloe-vera and lemon juice i.e., 100:0, 98:2, 96:4, 94:6, 92:8 and 90:10 respectively, filled in bottles and preserved for further nutritional and microbiological study. It was observed that mean values were increased for TSS (13.67-14.04 per cent) and acidity (0.53-0.63 per cent), reducing sugars (3.75 to 4.32 per cent) where as decreased for vitamin C (8.43-7.64mg/100ml), pH (2.74-2.62) and non-reducing sugars (9.53-8.91 per cent) during storage of 90 days. The beverage made from blend of 94:6 was found superior for overall acceptability (score 8.08) followed by blend of 92:8. The developed RTS could be recommended for the large scale production at industrial level.

2.5 Ginger extract

It was studied by Stoilova *et al* (2007) that the ginger oleoresin was used to for microbial decontamination and extension of shelf life of fresh sugarcane juice also for its specific flavor and taste as sugarcane juice gets spoiled due to bacterial fermentation. There were significant differences were observed for the total microbial count. The fresh untreated sugarcane juice had heavy load of bacterial counts and the sample got spoiled during subsequent days of storage. Yeast and *E. coli* were restricted. It was observed that the treated samples had inhibited microbial load and it could be due to gingerol and phenolic compounds. The ginger oleoresin was found effective to control the growth of spoilage microorganisms and hence acts as a natural food preservative for sugarcane juice. It was also noted that ginger oleoresin were found nontoxic and safe for public health. Hence it could be used as a substitute for use of chemical preservatives.

The volatile oils from fresh and dried ginger rhizomes of var. Nedumangadu was extracted and analysed by GC and GC-MS. The study was conducted by Sasidharan and Menon (2010) and reported that zingiberene was the major compound in both the oils. Fresh ginger oil was contained geranial (8.5 per cent) as the second main compound and had more oxygenated compounds (29.2 per cent) as compared to dry ginger oil (14.4 per cent). The dry ginger oil also contained 11 per cent ar-curcumene, 7.2 percent β - bisabolene, 6.6 per cent sesquiphellandrene and 6.6 per cent 2-cadinene. The antimicrobial activity of the oils was assessed and found comparable with reference compounds. The MIC values of the oils ranged from 10 μ g/ml, which was found significant. The study showed its application in treatment of many bacterial and fungal diseases.

The soluble compounds from ginger were extracted by the soxhlet method using methanol and n-hexane as solvents by Hasan *et al* (2012). The extracts of ginger were analyzed by HPLC. Seven components were identified and separated from ginger extracts by HPLC. The two extracts had antimicrobial activity, methanol extract was found superior than

n-hexane extract against the same tested microorganisms. It was revealed that ginger possesses antibiotics and antifungal properties, hence could be used as a potential source of active ingredients for food, pharmaceutical industry as a preservatives.

The ginger oleoresin from ginger rhizomes was extracted using acetone and analyzed by HPLC. Murthy *et al* (2015) revealed that gingerol (12.8 ± 0.5 per cent) is the major compound of the oleoresin. The oleoresin comprised rich fraction of 52.4 ± 0.6 mg GAE/g and exhibited 77.66 ± 0.8 per cent antioxidant activity. The olioresins also exhibited antibacterial and antifungal activity against food born pathogens. The minimum inhibitory concentration (MIC) for bacteria and fungi were 200–300 ppm. The ginger oleoresins has appreciable biological activities due to biomolecules in it and were used as a bio-preservative for fresh sugarcane juice stored at refrigeration (4°C) temperature for 35 days. The samples were analyzed for physicochemical, microbiological and sensory attributes at regular intervals during storage. The ginger oleoresin treated sugarcane juice was found stable at refrigerated temperature with non significant changes in its dietary properties and also aesthetic appeal. The ginger oleoresin attributed biological activity and also illustrated to be effective bio-preservative in sugarcane juice.

The use of ginger juice in the different blends of kinnow, aonla, pomegranate juices for improving flavour and nutritive value was studied by Bhardwaj (2013). The different juice blends were pasteurized (75°C and 85°C for 15 min.) and KMS (500 ppm and 750 ppm) was added. The blends were stored in 200 ml colourless glass bottles at ($4 \pm 1^{\circ}\text{C}$) prolonged the shelf life. The juice blend processed at 75°C for 15 min with 750 ppm KMS was found the most effective treatment. The minimum microbial population was recorded in blend juice processed at 85°C temperature.

The different blends of cashew apple juice with fruit juices of pineapple, passion fruit, papaya and gooseberry were prepared by Sobhana *et al* (2015). The ginger drops were also added to the mixes of different samples. It was revealed that sample prepared by mixing cashew apple juice and pineapple juice in equal proportion with ginger drops, it was found better acceptability on sensory evaluation followed by sample of cashew apple juice blended with pineapple. The blending of cashew apple juice and passion fruit juice (equal proportion) along with and without ginger drops also possessed better acceptability. The squash prepared with blend of cashew apple juice, pineapple juice and ginger had an acidity of 0.24 per cent, vitamin C content of 84.2 mg/100 g and sugar content of 30.1 per cent, whereas, squash containing cashew apple juice, passion fruit juice and ginger drops showed an acidity of 0.72 per cent, vitamin C content of 75.5 mg/100 g and sugar content of 12.53 per cent.

2.6 Mint extract

The study carried on chemical composition of essential oils by by Singh *et al* (2005) found that menthol (77.5–89.3%) was the main constituent of all the cultivars, followed by

menthone (0.3–7.9%) and isomenthone (3.7–6.1%). The highest concentration of menthol (89.3%) and the lowest percentage of menthone (0.3%) was observed in oil of MAS-1 cultivar. The oils were found similar in their compositions but quantitative differences were observed in the concentration of some constituents.

The study conducted by Dhifi *et al* (2012) on chemical composition of essential oils of Tunisian *Mentha spicata* leaves stated that the predominance of oxygenated monoterpenes (92.18%) was found dominant, followed by monoterpene hydrocarbons and sesquiterpenes (2.74 and 3.1 per cent, respectively). The L-menthone (32.74 per cent) and pulegone (26.67 per cent) are the main volatile compound of *M. spicata* EO. The apigenin (38.4 mg/100 g dry weight) was found as a major flavonoid component. *M. spicata* leave extract showed a significant antiradical and superoxide anion scavenging capacity in addition to the great potential for antimicrobial activities against the bacteria tested.

2.7 Blended juice beverages

The review on juice blends for utilization of under-utilized fruits, vegetables and spices was reviewed by Bhardwaj and Pande (2011). In the study they quoted that the utilization of highly nutritive fruits and vegetables is very limited due to high acidity, astringency, bitterness, and some other factors. The underutilized fruits and vegetables such as aonla, mango, papaya, pineapple, citrus, ber, pear, apple, watermelon and medicinal plants like aloe vera and spices can also be used for juice blending for improving flavour, palatability, also nutritive and medicinal value of various fruit juices. All these natural products are valued very highly for their refreshing juice, nutritional value, pleasant flavour, and medicinal properties. The blending of two or more fruit juices along with vegetable juices and spices extract for the preparation of ready to serve (RTS) beverages is thought to be a convenient and economic for utilization of these fruits and vegetables. It was also stated that, one could think of a new product development through blending in the form of a natural health drink, which may also serve as an appetizer. The review was focused on the blending of fruits, under-utilized fruits, vegetables, medicinal plants, and spices in appropriate proportions for the development of natural fruit and vegetable based nutritive beverages.

The study was conducted on health benefits of antioxidant content in fruit juices and increasing trend of fruit juice consumption. The total phenolic contents (TPC) and antioxidant activities of fresh fruit juices, commercial 100 per cent fruit juices and fruit drinks were studied by Wern *et al* (2016). The TPC contents of fresh fruit juices, commercial 100 per cent fruit juices and fruit drinks were found in the range of 13.38-80.40, 21.65-130.39 and 3.32-45.10 mg GAE/100 ml, respectively. The fresh guava juice and commercial guava drinks exhibited the highest antioxidant activities in DPPH assay (205.71-770.12 $\mu\text{mol TE}/100\text{ ml}$) and FRAP assay (320.80-843.13 $\mu\text{mol TE}/100\text{ ml}$). The pomegranate juices demonstrated the highest antioxidant activities among commercial 100 per cent fruit juices with DPPH and

FRAP values of 2705.01 and 2953.85 $\mu\text{mol TE}/100\text{ ml}$, respectively. Fruits drinks group had the lowest TPC and antioxidant activities for all types of fruits. TPC was significantly correlated to FRAP and DPPH assays. The TPC and antioxidant activities of commercial 100 per cent fruit juices and fresh juices were comparable with no significant difference was found between these two groups. It was also reported that commercial fruit drinks were not good source of antioxidants. These findings provide information especially for ageing population in choosing healthy fruit juice or drinks.

2.8 Carbonated fruit juice beverages

The study was undertaken on development of carbonated RTS beverage from pomegranate fruit juice by Mehtre *et al* (2012). The different levels of juices (10, 12 and 15 %) and sugar syrup of 15 $^{\circ}\text{Brix}$ (10, 12 and 14 %) were used. The overall acceptability of 15 per cent juice level and 12 per cent sugar level was found to be superior. The RTS beverage with 15 per cent juice and 12 per cent sugar level was selected to decide the acidity level. They also reported that 0.3 per cent acid level was optimum. It was also observed that carbonation at various pressures had no influence on physicochemical properties of beverage. The beverage prepared with 15 per cent juice, 12 per cent sugar and 0.3 per cent acidity was subjected to carbonation at different CO_2 pressures (60, 80 and 100 psi). The acceptability of beverage at 80 psi carbonation level was found higher.

The investigation was carried out in order to develop a carbonated ready to serve (RTS) beverage from sweet orange juice by Kadam *et al* (2014). The beverage was prepared by using different juice levels (10, 15 and 20%) with sugar syrup (60 $^{\circ}\text{Brix}$ TSS and 0.5% acidity). The beverage was carbonated at 80 and 100 psi pressure levels. The study revealed that the RTS beverage prepared by using the 20 per cent sweet orange juice along with 0.3 per cent acidity, 11.7 $^{\circ}\text{Brix}$ TSS and carbonated at 100 psi pressure was found significantly superior for various quality characteristics over other combinations.

The study was conducted for 10 brands of soft-drinks i.e. coke, pepsi, mirinda, fanta, sprite, mountain dew, 7up, limca, maaza. The samples were analysed for pH, sucrose, phosphate, glucose, fructose, and caffeine contents using standard biochemical procedures by Jain and Yadav (2013). The result showed that the pH of the soft drinks ranged from 2.46 in pepsi to 3.72 in maaza. The most of the soft drinks had alcohol and aldehyde as the flavouring agents. The effect of cold drinks on a tarnished penny or coin was studied to see the effect of soft drinks on the tooth enamel. It was observed that the soft drinks removed the tarnish from the penny and the darker sodas tarnish off the penny faster than the lighter sodas. The amount of ascorbic acid and benzoic acids in drinks inhibited the growth of yeast in soft drinks. It was observed that the amount of benzoic acid was generally low in fanta (29.75 mg/ounce) and highest in mountain dew (55.65 mg/ounce). The amount vitamin C was found low in limca and high in coca cola and pepsi. The caffeine was found only in pepsi, cola, mountain dew

and thumps up.

The importance of storing carbonated sugarcane juice beverages is more practical and use of lighter PET bottles over traditional glass bottles become need. The preservation of beverage quality is a great problem. Mirel *et al* (2005) conducted study for analysis of the variation of carbon dioxide in certain carbonated sugarcane juice beverages stored in PET bottles. The possibilities of obtaining mathematical statistical models had been analysed on basis of the experimental data in order to predict the variation of the CO₂ inside the samples as a function of volume and time. The CO₂ losses starting from the bottling process as well as a comparative average analysis of products proportioning with CO₂ and its loss for three types of concurrent carbonated sugarcane juice beverages existing in the market. The results recorded allowed the analyses of the optimal term of validity in accordance with the bottle's volume. The obtained results was also contributed to the improvement of quality of the carbonated beverages allowing to obtain complete beverage. They reported that the PET bottle of 0.5 litre requires a supplementary dosage because of its small volume. This is the reason for guarantee the 0.5 litre packed product only for 6 months, compared to the other volumes where the validity term can be increased to 1 year. It was also reported that the thickness of the PET bottle walls must be kept uniform on the entire surface to reduce intense CO₂ losses and the increase of mass bottle constituted a barrier for the CO₂ losses. The manufacturing companies of carbonated soft drinks can precisely control the product quality. The elaboration of statistical models in the means of expressing the CO₂ variation in PET bottles, allows the forecast of maintenance period of carbonated soft drinks

The attempt was made to develop a carbonated ready to serve beverage from pomegranate juice by Wagh *et al* (2014). The fruits of matured and freshly harvested cv. Ganesh and Bhagwa were used and the arils were separated mechanically. The juice was extracted by using screw type juice press followed by filtration. The beverage was prepared by using the sugar syrup of 60 °Brix and 0.5% acidity by adding citric acid and then carbonated at 80, 100 and 120 psi pressure respectively. The study depicted that the RTS beverage prepared from Bhagwa cultivar using 60° Brix sugar juice of 0.5% acidity and 80 psi CO₂ pressure in low temperature was found superior for organoleptic characteristics.

CHAPTER III

MATERIAL AND METHODS

The present study on the development of carbonated and non carbonated sugarcane juice beverages (blended with juices of kinnow, aonla, lemon and extract of ginger and mint) was carried out in the department of Food Science and Technology, Punjab Agricultural University, Ludhiana.

3.1 Procurement of materials

3.1.1 Sugarcane

Sugarcane (CoPb 91) was procured from Sugarcane Research Station, Kapurtala, Punjab Agricultural University, Ludhiana.

3.1.2 Kinnow, aonla, lemon, ginger and mint

Fresh fruits of kinnow, aonla and lemon of commonly grown varieties were purchased from the Department of Fruit Science, PAU, Ludhiana and also from local market. Fresh ginger and mint were procured from the local market of Ludhiana.

3.1.3 Chemicals

Chemicals such as DPPH (Aldrich), Tris buffer (Fisher Scientific), Folin-Ciocalteu (S.D.Fine Chem.), Anhydrous Sodium Carbonate (Rankem), Sodium Hydroxide (S.D.Fine Chem.), Anhydrous sodium Sulphate (S.D.Fine Chem.), Std. L-Ascorbic acid (S.D.Fine Chem.), Dichlorophenol-endophenol dye (Thomus Baker Chem.), Oxalic Acid (Qualigens), Tannic Acid (CDH), Methanol (Labor Fine Chem.), Acetone (Rankem), Petroleum Ether (CDH), Alumina (Rankem), Sodium Tungstate (Loba Chem.), Phosphoric acid (S.D.Fine Chem.), Phenolphthalein (CDH), Conc. Hydrochloric Acid (S.D.Fine Chem.), Conc. Sulphuric Acid (S.D.Fine Chem.), Gallic acid Std. (SRL Chem.), Sodium Benzoate (S.D.Fine Chem.), microbial media PDA and PCA (High Media make) were used for estimations and processing.

3.2 Processing

3.2.1 Extraction of sugarcane juice

The sugarcane was first cleaned to remove part of dry leaves, tops and part of roots at the bottom side also inspected for the damaged or infected nodes for their timely removal before processing. Sugarcane is cut into 2.5 feet length then washed by clean tap water using mechanical washer. The skin and nodes were scrapped with special curved blade stainless steel (SS) knife. The clean scrapped sugarcane was again washed in mechanical washer for 2 min. and was surface dried in perforated SS trays using fans. The juice of sugarcane was extracted with double extraction using mechanical crusher (Kalsi make) which is made up of SS. The extracted juice was filtered through the SS screen for the removal of coarse baggage material and then filtered through the four fold clean muslin cloth. The juice was heated to the

optimum temperature for 5 minutes and used for the beverage preparation.

3.2.2 Extraction of kinnow juice

Kinnow were washed in clean tap water if dirt or dust/ mud attached to it. The fruits were then peeled manually using hand gloves and SS knife. Peeled fruits were then made into halves and juice was extracted through juice extractor (Kalsi make). The juice was filtered through the double folded muslin cloth for the removal of course pulp and fibres from the juice. The juice was then heated to the optimum temperature (82⁰C) for 5 minutes, sodium benzoate was added (120ppm), then filled in sterile glass bottles, sealed the bottles with sterile caps. Bottles were then heat processed in boiling water for 30 min ., cooled to room temperature slowly in running tap water, let it for the surface dry and labelled. The bottles were stored at room temperature for the further use of beverage preparation.

3.2.3 Extraction of aonla juice

Fresh aonla fruits were sorted, graded and washed in clean water. Washed fruits were heat treated in hot water (95⁰C) till the fruit colour changes to pale yellow. The seeds were removed manually by pressing the fruit in fingers and segments were separated. The juice was extracted from segments using juice extractor (Kalsi make) with addition of equal quantity of clean water for extraction. Juice and pulp was then filtered through the four fold muslin cloth for the removal of course pulp/ fibres from the juice. The juice was then heated to the optimum temperature (82⁰C) for 5 minutes, sodium benzoate was added (120ppm), then filled in sterile glass bottles, sealed the bottles with sterile caps. Bottles were then heat processed in boiling water for 30 min ., cooled to room temperature slowly in running tap water, let it for the surface dry and labelled. The bottles were stored at room temperature for the further use of beverage preparation.

3.2.4 Extraction of lemon juice

Fresh lemons were purchased from university campus and local market. The fruits were sorted, graded, washed in clean tap water, drained for water removal and then surface dried. The lemons were halved using stainless steel knives on preparation Table (SS) and juice was extracted using manually operated machine (Allwyn manual fruit squeezer). The juice was then filtered through the four fold muslin cloth for the removal of course fibrous material from the juice. The juice was then heated to the optimum temperature (82⁰C) for 5 minutes, sodium benzoate was added (120ppm), then filled in sterile glass bottles, sealed the bottles with sterile caps. Bottles were then heat processed in boiling water for 30 min , cooled to room temperature slowly in running tap water, let it for the surface dry and labelled. The bottles were stored at room temperature for the further use of beverage preparation.

3.2.5 Extraction of ginger extract

Fresh ginger was washed, peeled manually, cut into small pieces and grinded in mixer with double quantity of water. The slurry was then filtered through the double folded muslin

cloth and kept for 2hr in cylindrical transparent plastic container to settle the sediment at bottom. Clear extract is then filtered through 4 fold muslin cloth. The juice was then heated to the optimum temperature (82⁰C) for 5 minutes, sodium benzoate was added (120 ppm) and then filled in sterile glass bottles, sealed the bottles with sterile caps using sealing machine. Bottles were then heat processed in boiling water for 30 min ., cooled to room temperature slowly in running tap water, let it for the surface dry and labelled. The bottles were stored at room temperature for the further use of beverage preparation.

3.2.6 Extraction of mint extract

Fresh mint was washed, cleaned manually to remove roots, stalks and unhealthy leaves, again washed with clean water and drained properly to free from water. The leaves of fresh mint were grinded in mixer with equal quantity of water. The slurry was then filtered through the double folded muslin cloth. The juice was then heated to the optimum temperature (82⁰C) for 5 minutes and extract is then filtered through 4 fold muslin cloth, sodium benzoate was added (120 ppm) and then filled in sterile glass bottles, sealed the bottles with sterile caps using sealing machine. Bottles were then heat processed in boiling water for 30 min , cooled to room temperature slowly in running tap water, let it for the surface dry and labelled. The bottles were stored at room temperature for the further use of beverage preparation. The mint juice showed the problem of coagulation of solids during pasteurization and discolouration (browning) during storage, which hampered the colour of sugarcane juice beverage. Hence was not continued further for its use in sugarcane juice beverages.

3.3 Optimization of processing parameters

3.3.1 Optimization of pasteurization time and temperature

The pasteurization of juices and products at different temperatures viz. 78, 80, 82, 84 and 85 ⁰C were carried out for different periods (1, 2, 3, 4 and 5 min.). Finally the particular temperature and time was selected on the basis of sensory evaluation and storage stability (total plate count for bacteria) of products for 3 months.

3.3.2 Optimization of level of preservative

The sodium benzoate at different levels viz. 100, 110, 120 and 130 ppm were tested for the storage stability of the juices and non carbonated sugarcane juice beverages using sensory evaluation and storage stability (total plate count for bacteria) of products, while for the bottled carbonated beverages, the sodium benzoate levels used were 50, 55, 60 and 65ppm. The selected products of carbonated and non carbonated sugarcane juice beverages after product standardization were further used for the optimization of respective preservative levels for their storage stability and sensory quality..

3.3.3 Optimization of carbonation level of carbonated sugarcane juice beverage

The recipe for the carbonated sugarcane juice beverages were formulated as per the

Table 2 and the beverages were carbonated at different levels of carbonation viz. 70, 80, 90 and 100 psi CO₂ pressure and at 15⁰C temperatures. The carbonation level of beverages was optimized using sensory evaluation and sustainability of bottles to carbonation levels.

3.4 Product formulation

3.4.1 Standardization of recipe for non carbonated sugarcane juice beverages

The different blends of sugarcane juice and fruit juices (kinnow, aonla and lemon) with fresh ginger extract were formulated and recipes were standardized for non carbonated sugarcane juice beverages on the basis of sensory evaluation of products using 9 point hedonic scale by the semi trained panel. The following combination of treatments were used for the standardization of recipe for non carbonated beverages.

Table 3.1: Combination of treatments used for recipe standardization of non carbonated sugarcane juice beverages

Treatment	Juices/ extract (per cent)				
	Sugarcane	Kinnow	Aonla	Lemon	Ginger/mint
T0	96	-	-	2.0	2.0
T1	65	20	11	2.0	2.0
T2	65	15	16	2.0	2.0
T3	70	10	16	2.0	2.0
T4	70	15	11	2.0	2.0
T5	70	20	6.0	2.0	2.0
T6	75	10	10	2.5	2.5
T7	75	15	5.0	2.5	2.5
T8	80	5.0	10	2.5	2.5
T9	80	10	6.0	2.0	2.0
T10	80	9.0	6.0	2.5	2.5
T11	80	10	5.0	2.0	3.0
T12	80	10	5.0	2.5	2.5
T13	75	15	5.0	2.5	2.5
T14	75	15	5.0	2.0	3.0
T15	85	5.0	5.0	2.5	2.5
T16	85	5.0	5.0	2.0	3.0
T17	85	8.0	3.0	2.0	2.0
T18	85	7.0	4.0	2.0	2.0
T19	90	2.5	2.5	2.5	2.5
T20	92	2.0	2.0	2.0	2.0
T21	95	-	-	2.5	2.5
T22	95	-	-	2.0	3.0
T23	95	-	-	3.0	2.0

Method for preparation

Fresh sugarcane juice was extracted using standard method, filtered through the SS sieve and four folded muslin cloth for their clarification. The proportion of juices of sugarcane, kinnow, aonla, lemon and ginger extract was added according to the type of blend. The blends of the products were then heated to the optimum temperature (82⁰C) for 5 min, required sodium benzoate was added (120ppm) and mixed well. The products were filled in sterile glass bottles, sealed the bottles with sterile caps using corking machine. Bottles were then heat processed in boiling water for 30 min , cooled to room temperature slowly in running tap water, kept for the surface dry and labelled. The bottles of the products were stored at room temperature and refrigerated temperature for the further use for sensory evaluation, physicochemical and storage study.

3.4.2 Standardization of recipe for carbonated sugarcane juice beverages

The different blends of sugarcane juice and fruit juices (kinnow, aonla and lemon) with fresh ginger extract were formulated and standardized recipes for carbonated sugarcane juice beverages were selected on the basis of sensory evaluation of products and sustainability of packaging to carbonation levels.

Table 3.2: Combination of treatments used for recipe standardization of carbonated sugarcane juice beverages

Treatment	Juices/extract (per cent)				
	Sugarcane	Kinnow	Aonla	Lemon	Ginger/mint
TC00	95	-	-	2.5	2.5
TC11	90	-	-	5.0	5.0
TC12	90	2.5	2.5	2.5	2.5
TC13	85	2.5	2.5	5.0	5.0
TC14	85	3.5	3.5	4.0	4.0
TC15	85	4.0	4.0	3.5	3.5
TC16	80	5.0	5.0	5.0	5.0
TC17	80	7.0	5.0	4.0	4.0
TC18	80	5.0	5.0	4.0	6.0
TC19	80	5.0	10.0	2.5	2.5
TC20	80	10	5.0	3.0	2.0
TC21	84	5.0	5.0	3.0	3.0
TC22	88	3.0	3.0	3.0	3.0
TC23	86	4.0	4.0	3.0	3.0
TC24	82	6.0	6.0	3.0	3.0
TC25	88	4.0	4.0	2.0	2.0
TC26	92	2.0	2.0	2.0	2.0
TC27	94	-	-	3.0	3.0

Method for preparation

Fresh sugarcane juice was extracted using standard method, filtered through the SS sieve and four folded muslin cloth for their clarification. The sugarcane juice was then heated to the optimum temperature (82⁰C) for 5 minutes and then cooled to room temperature. The proportion of juices of sugarcane, kinnow, aonla, lemon and ginger extract was added according to the type of blend. The required sodium benzoate was added (120ppm) and mixed well. The products were cooled to 15⁰C temperature. Then required quantity of blends was filled in sterile glass bottles. The bottles were kept in the crates according to the code. The carbonation was carried out at different carbonation levels by using manually operated carbonation machine and simultaneously bottles were sealed and kept in coded crates. The bottles of the products were stored at room and refrigerated temperature for the further use to study sensory evaluation, physicochemical parameters and storage stability.

3.5 Sensory evaluation

The samples prepared during product formulation and standardized samples of both carbonated and non carbonated sugarcane juice beverages were evaluated fresh as well as during storage study for sensory evaluation by a panel of semi trained judges (15 No.) for appearance, mouth feel, aroma, taste, consistency and overall acceptability using 9-Point Hedonic Scale (Larmond 1970) as below.

Sensory Score	Rating
9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

3.6 Physicochemical analysis

The samples of juices of sugarcane, kinnow, aonla, lemon, ginger and both carbonated and non carbonated sugarcane juice beverages were analyzed for juice yield, moisture content, total solids, total soluble solids, ash content, titratable acidity, pH, ascorbic acid, antioxidant activity, total phenolics, total sugars, reducing sugars and viscosity.

Preparation of raw materials for physicochemical analysis

The juices of sugarcane, kinnow, aonla, lime, and extract of fresh ginger were extracted using standard methods and physicochemical parameters such as juice yield, percent

waste were calculated simultaneously. The juices were stored in glass bottles. The juices of sugarcane, kinnow, aonla, lemon, ginger and the best samples of developed carbonated and non carbonated sugarcane juice beverages were analysed for moisture content, total solids, total soluble solids, ash content, titratable acidity, pH, ascorbic acid, antioxidant activity, total phenolics, total sugars, reducing sugars and viscosity.

3.6.1 Moisture content

Moisture content was estimated by following the method of Ranganna (2015). 25 gram of each juice and samples of carbonated and non carbonated sugarcane juice beverages were weighed in pre weighed aluminium dishes, dried in an oven at 55°C to a constant weight for the period of four days. The weight of the samples was noted after cooling the moisture dishes in desiccators. The moisture content was calculated by using following formula.

$$\text{Moisture content (per cent)} = \frac{\text{Weight of initial sample} - \text{Weight of dried sample}}{\text{Weight of initial sample}} \times 100$$

3.6.2 Total solids

Total solids content was estimated by following the method of Ranganna (2015). 25 gram of each juice and samples of carbonated and non carbonated sugarcane juice beverages were weighed in pre weighed aluminium dishes, dried in an oven at 55°C to a constant weight for the period of four days. The weights of the respective dried samples were noted after cooling the moisture dishes in desiccators and total solids were calculated by using following formula.

$$\text{Total solids (per cent)} = \frac{\text{Weight of dried sample}}{\text{Weight of initial sample}} \times 100$$

3.6.3 Total soluble solids

Total soluble solids content of juices of sugarcane, kinnow, aonla, lemon, ginger and product samples of carbonated and non carbonated sugarcane juice beverages was determined by using a hand refractometer (Erma, Japan) with scale ranging from 0 to 32° Brix. The drops of juice/beverage samples were placed on the prism/holding stage of refractometer and held against the light. The readings were noted according to the shade of indication on reading scale. The observations were corrected to 20°C and the values were expressed as total soluble solids in °Brix (Ranganna 2015).

3.6.4 Titratable acidity

The titratable acidity was determined following the method of (Ranganna 2015) by titrating a known quantity of sample solution against standard 0.1 N NaOH solution to a faint pink colour in the presence of phenolphthalein indicator. The each juices of sugarcane, kinnow, aonla, lemon, ginger and samples of non carbonated sugarcane juice beverages were mixed thoroughly by shaking the glass bottles while in case of the carbonated beverages, CO₂ was expelled from the beverages by boiling, cool and then used for dilution . 10 ml of each

sample except lemon juice (2 ml) is mixed with distilled water and volume was made to 100 ml in volumetric flask and filtered through Whatman filter paper no. 4. Then 10 ml of aliquot was taken in the titrating flask and after adding 1-2 drops of phenolphthalein indicator, it was titrated against 0.1 N NaOH to faint pink end point. The acidity was expressed as citric acid (equivalent weight- 64) per cent.

$$\text{Acidity (per cent citric acid)} = \frac{\text{Titre value} \times 64 \times \text{normality of NaOH} \times \text{volume made up} \times 100}{\text{Weight of sample} \times \text{volume of aliquot} \times 1000}$$

3.6.5 pH

The pH of the samples was determined by using digital pH meter (Elico India) which was calibrated with standard buffer solutions of pH 4 and 7 according to the method of AOAC (2000).

3.6.6 Ascorbic acid

Ascorbic acid was extracted from the sample with 0.4 per cent oxalic acid and determined by titrimetric method using 2, 6-dichlorophenol indophenol dye solution (0.04 per cent) which was standardized against standard L-ascorbic acid (0.1 mg/ml of 0.4 per cent oxalic acid). The each juices of sugarcane, kinnow, aonla, lemon, ginger and samples of carbonated and non carbonated sugarcane juice beverages were mixed thoroughly by shaking the glass bottles. 10 ml of sample was taken and mixed with 0.4 per cent oxalic acid solution and volume was made to 100 ml. It was filtered through Whatman filter paper no. 4. Then 10 ml aliquot was titrated with standardized dye. The end point was recorded as pink colour, which persisted for at least 15sec. The results were calculated and expressed as ascorbic acid mg per 100 g of sample (Ranganna 2015) using following formula.

$$\text{Ascorbic acid (mg/100 g)} = \frac{\text{Titre value} \times \text{dye factor} \times \text{volume made up}}{\text{Aliquot of extract taken} \times \text{weight of sample}} \times 100$$

3.6.7 Sugars

Dubois *et al* (1956) for total sugar, Nelson (1944) and Somogyi (1952) method for reducing sugar were followed. 5ml sample was refluxed with 80 per cent isopropyl alcohol into round bottom flask. After 2 hours refluxing and cooling, samples were filtered with Whatman no. 4 filter paper and final volume was made up to 100ml with distilled water.

3.6.7.1 Total Sugars

0.1 ml at aliquot was diluted with distilled water up to 1 ml. 1 ml of 5 per cent phenol reagent and 5 ml concentrated H₂SO₄ was added to aliquot into different test tubes. The intensity of colour was noted at 490nm against a reagent blank on spectrophotometer. The total sugars were calculated by using standard curve of glucose.

$$\text{Total sugars (mg/100ml)} = \frac{\text{Conc. of glucose} \times \text{OD of sample} \times \text{volume made up} \times 100}{\text{Corresponding OD of glucose} \times \text{aliquot taken} \times \text{weight of sample}}$$

3.6.7.2 Reducing sugars

One ml of the aliquot was taken into different test tubes and 1 ml of copper reagent C was added. After adding reagent, test tubes were kept in boiling water bath for 20 minutes. After cooling, 1 ml of Nelson reagent was added to each test tube and final volume was made up to 13 ml with distilled water. The intensity of developed colour was noted at 525nm against reagent blank on spectrophotometer. The reducing sugars were calculated by using standard curve of glucose.

Total reducing sugars (mg/100ml) =

$$\frac{\text{Conc. of glucose} \times \text{OD of sample} \times \text{Volume made up} \times 100}{\text{Correspond OD of glucose} \times \text{aliquot taken} \times \text{weight of sample}}$$

3.6.8 Phenolic content

Total polyphenols were determined by Folin-Ciocalteu method given by Wolfe *et al* (2003). 5ml sample was taken and 50 ml of 80 per cent methanol was added and refluxed for 2 hours at 40-45°C in round bottom flask. It was filtered and residue was again refluxed with 80 per cent methanol for one hour. After filtration of extract, final volume was made up to 100 ml with 80 per cent methanol. 0.2 ml filtrate was taken along with 0.8 ml water. 5 ml Folin-Ciocalteu reagent was added. After 5 minutes, 4 ml saturated sodium carbonate solution was mixed. Absorbance of the developed colour after 15 minutes was measured at 765 nm using a spectrophotometer. A standard curve was plotted by taking Gallic acid as reference standard.

$$\text{Total polyphenols (mg GAE/100ml)} = \frac{\text{Conc. of phenols from graph} \times \text{Final volume}}{\text{Aliquot used} \times \text{Volume of sample}} \times 100$$

3.6.9 Total antioxidant activity

Free radical scavenging activity was determined by DPPH (2, 2-diphenyl-1 picryl-hydrazyl) method. A method according to Brand-Williams *et al* (1995) was followed with some modification. 5 g of sample was taken. 80 per cent methanol was added and refluxed for 2 hours at 40-45°C in round bottom flask. It was filtered and residue was again refluxed with 80 per cent methanol for one hour. After filtration of extract final volume was made up to 100 ml with 80 per cent methanol. To 1 ml of methanolic extract of sample, 2ml of 1mM freshly prepared DPPH and 1 ml of 50 mM tris buffer was added and absorbance was determined at 517nm (80 per cent methanol and tris buffer as blank) after 30 minutes in dark. The free radical scavenging activity was evaluated by comparing the absorbance of the sample solution to which distilled water was added instead of sample, 2ml of 1mM of DPPH and 1ml of 50mM tris buffer.

$$\text{Antioxidant activity (\%)} = \frac{(A_0 - A)}{A_0} \times 100$$

Where

A_o = Absorbance of control

A = Absorbance of sample

3.6.10 Total flavonoid content

Total flavonoids were measured using aluminium chloride colorimetric assay, as described by Marinova *et al* (2005). One ml of methanolic extract or standard solution of quercetin prepared using distilled water (10-100 µg/ ml) was added to test tubes containing 4 ml of double - distilled water. To the mixture was added 0.3 ml 5% NaNO₂. After 5 min, 0.3 ml 10 % AlCl₃ was added. After 6 min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with double-distilled water. The solution was mixed thoroughly and the absorbance of the samples and standard against reagent blank were read at 510 nm using Double beam UV–VIS spectrophotometer. Total flavonoid content was expressed as mg of quercetin equivalent (QE) per 100 g on fresh weight basis (FWB).

3.6.11 Minerals

The samples for the minerals estimation was prepared by measuring the 2 ml of each juice/ beverage sample in the 100ml flask. Then 10 ml of diacid (3 parts conc. nitric acid: 1 part perchloric acid) was added in the each sample flask and left it overnight. The samples were digested on hot plate at 245⁰C for 30-45 min till the sample becomes colourless. Make volume to 25 ml with double distilled water and filter it through ashless filter paper. Then the samples tested for the mineral estimation by Inductively Coupled Plasma- Atomic Emission Spectrophotometer (ICP-AES). The results are noted directly for the different minerals content (ppm) in diluted samples. The dilution factor was used to multiply the readings to get the actual quantity of minerals in the undiluted samples and the actual results were quoted in mg/100ml.

3.6.12 Viscosity

The viscosity of the juices and the carbonate and non carbonated beverage samples were determined by using RV model viscometer. The No.1 spindle was used for the estimation of viscosity. The dial readings were noted. The viscosity was calculated in cP by multiplying the dial reading with respective spindle factor.

3.6.13 Colour

The colour of the samples was measured by using Lovibond Tintometer as described in Ranganna (2015). Assure that all the standard slides of red, yellow and blue colour in racks were at zero position. The instrument was made on. The liquid sample was filled in the clean and dry covet and placed in the sample place provided inside the Tintometer. The opening of box was closed. By a system of prism and mirrors, the sample was made to occupy half the field of view, while other half received reflected light from standard white surface which passes through coloured glass slides mounted in the racks. The sample colour is matched by

adjusting the standard slides of red, yellow and blue colour. The values of slides of red, yellow and blue were noted for the quantification of colour value of sample. The results were interpreted as per the standard method described by Ranganna (2015).

3.6.14 Carbon dioxide gas volume in bottled beverage

To measure the CO₂ gas pressure and gas volume in the carbonated drink, CO₂ gas pressure tester was used by using the method described by Ranganna (2015). The bottle containing carbonated drink was placed in the slot provided into the neck of the CO₂ gas pressure tester and tightly secured with a threaded stem. Sniff valve on the pressure gauge stem was closed followed by inserting the needle point of gauge through the crown cork. The reading on the gauge was noted and to allow the escape of head space pressure the sniff valve was opened until the first bubble rises in the liquid. The CO₂ gas dissolved in drink was then measured by closing the sniff valve and shaking the bottle until the gauge reached maximum pressure. The pressure was recorded and the CO₂ pressure tester from the crown cork was immediately removed. Then the temperature of the drink was measured with the help of thermometer. The volume of CO₂ gas dissolved was then calculated by referring to gas volume test chart (Jacobs 1951). The CO₂ gas volume was defined as the amount of gas in ml that a given volume of water will absorb at atmospheric pressure (760 mm Hg at a temperature of 15.5⁰C i.e. 60 ⁰F).

3.6.15 Carbon dioxide

CO₂ (w/v) in the carbonated sugarcane juice beverages was determined according to the AOAC (2005) with slight modifications. To 25 ml N/10 NaOH, 10 ml chilled carbonated beverage ($\leq 0^{\circ}\text{C}$) was pipette by keeping the tip submerged just below the surface of NaOH in the flask followed by the titration with N/10 HCl to a colourless end point (A). The presence of acids other than H₂CO₃ was corrected by degassing the beverage at room temperature in heavy walled filter flask and agitating under vacuum of 27” Hg for one min. 10 ml of decarbonated beverage was titrated against N/10 NaOH using phenolphthalein as indicator (B). Net ml NaOH used was calculated by subtracting the NaOH titre in ‘B’ from 25ml. The CO₂ content in the beverage was then calculated as under:

$$\text{mg CO}_2 / 100 \text{ ml} = \frac{(\text{Net ml NaOH} \times \text{Normality} - \text{ml HCl} \times \text{Normality}) \times 44}{\text{Volume of sample}} \times 100$$

3.7 Storage studies

3.7.1 Non carbonated sugarcane juice beverages

The best samples of non carbonated sugarcane juice beverages packed in 200 ml glass bottles were stored at room (30+4⁰C) and refrigeration (4⁰C) temperature for six months. The effect of storage on TSS, titratable acidity, pH, ascorbic acid, total sugars, reducing sugars and viscosity was studied at fixed interval of one month.

3.7.2 Carbonated sugarcane juice beverages

The best samples of carbonated sugarcane juice beverages packed in 200 ml glass bottles were stored at room (30+4⁰C) and refrigeration (4⁰C) temperature for six months. The effect of storage on TSS, titratable acidity, pH, ascorbic acid, total sugars, reducing sugars and viscosity was studied at fixed interval of one month.

3.8 Microbial examination

3.8.1 Total plate count (Aerobic mesophilic plate count)

The total plate count for bacteria was counted by using method of AOAC (1995). The homogenate of beverage was prepared by making 1:10 dilution of the well mixed sample by aseptically transferring sample to the desired volume of diluents (10 ml in 90 ml sterile distilled water). Serial dilutions in sterile test tubes were prepared by using sterile pipette and shaking each dilution 25 times in 30 cm arc. All petriplate were labelled with the sample number, dilution, date and any other desired information. Pour the 1 ml of undiluted and diluted samples in sterile petriplates using sterile pipette. Then poured 10-12 ml of molten plate count agar (PCA) at temperature 42-45⁰C in each petriplate within 15 min from the time of preparation of original dilution. The media and dilutions were mixed by swirling gently clockwise, anticlockwise, to and fro thrice and taking care that the content do not touch the lid. Allowed to set and incubated the petriplates inverted position at 35 0C for 48±2 h. Number of colonies were counted using following formula and result expressed as CFU/ml.

$$N = \frac{\sum C}{(N1 + 0.1N2)D}$$

Where

N= Number of colonies

$\sum C$ = Sum of colonies counted on all petriplates retained

N1= Number of petriplates retained in the Ist dilution

N2= Number of petriplates retained in the IInd dilution

D= Dilution factor corresponding to Ist dilution

3.8.2 Yeast and mould count

The yeast and mould count was counted by using dilution plate method using potato dextrose agar (Krishnakumar and Devadas, 2006a). The homogenate of beverage was prepared by making 1:10 dilution of the well mixed sample by aseptically transferring sample to the desired volume of diluents (10 ml in 90 ml sterile distilled water). Serial dilutions in sterile test tubes were prepared by using sterile pipette and shaking each dilution 25 times in 30 cm arc. All petriplate were labelled with the sample number, dilution, date and any other desired information. Pour the 1 ml of undiluted and diluted samples in sterile petriplates using sterile pipette. Each petriplate was made in duplicate. Potato dextrose agar (PDA) of high

media was acidified with sterile 10 per cent tartaric acid to pH 3.5 ± 0.1 . Then poured 10-12 ml of the PDA (tempered to 45°C) in each petriplate and mixed by swirling and allowed to solidify. The petriplates were inverted and incubated at $20-25^{\circ}\text{C}$ for 2 to 5 or 7 days. The plates were observed daily and marked the colonies. The plates after 7 days were discarded if its growth was not observed. Colonies were counted and multiplied by the inverse of the corresponding dilutions and reported as yeast or mould count per ml.

3.9 Statistical analysis

The data related to sensory and physico-chemical attributes of fresh and stored samples of carbonated and non carbonated sugarcane juice beverages were statistically analyzed to find out the effect of storage period and temperature on the sensory and physico-chemical characteristics of the products with the help of Analysis of Variance (ANOVA) as described by Gomez and Gomez (2010).

CHAPTER IV

RESULTS AND DISCUSSION

Research experiments undertaken to study the development of carbonated and non carbonated sugarcane juice beverages using juices of kinnow, aonla, lemon and ginger have been discussed under the following headings:

- 4.1 Optimization of processing parameters
 - 4.1.1. Optimization of pasteurization time and temperature
 - 4.1.2. Optimization of level of preservative
- 4.2 Physicochemical and phytochemical constituents of juices
- 4.3 Development of non carbonated sugarcane juice beverages
 - 4.3.1 Standardization of recipe for non carbonated sugarcane juice beverages
 - 4.3.2 Sensory evaluation of non carbonated sugarcane juice beverages
 - 4.3.3 Physico-chemical and phytochemical constituents of non carbonated sugarcane juice beverages
 - 4.3.4 Effect of blending on phytochemicals of non carbonated sugarcane juice beverages
 - 4.3.5 Effect of storage period on sensory qualities of non carbonated sugarcane juice beverages
 - 4.3.6 Effect of storage temperature on sensory qualities of non carbonated sugarcane juice beverages
 - 4.3.7 Effect of storage period on physicochemical and phytochemical constituents of non carbonated sugarcane juice beverages
 - 4.3.8 Effect of storage temperature on physicochemical and phytochemical constituents of non carbonated sugarcane juice beverages
 - 4.3.9 Effect of storage period on color (Tintometer color units) of non carbonated sugarcane juice beverages
 - 4.3.10 Effect storage period and storage temperature on microbial quality of non carbonated sugarcane juice beverages
- 4.4 Development of carbonated sugarcane juice beverages
 - 4.4.1 Standardization of recipe for carbonated sugarcane juice beverages
 - 4.4.2 Sensory evaluation of carbonated beverages
 - 4.4.3 Effect of carbonation levels on sensory qualities of carbonated sugarcane juice beverages

- 4.4.4 Physicochemical and phytochemical constituents of carbonated sugarcane juice beverages
- 4.4.5 Effect of carbonation levels on phytochemicals of carbonated sugarcane juice beverages
- 4.4.6 Effect of storage period and temperature on phytochemicals of carbonated sugarcane juice beverages
- 4.4.7 Effect of storage period on sensory qualities of carbonated sugarcane juice beverages
- 4.4.8 Effect of storage temperature on sensory qualities of carbonated sugarcane juice beverages
- 4.4.9 Effect of storage period on physicochemical and phytochemical constituents of carbonated sugarcane juice beverages
- 4.4.10 Effect of storage temperature on physicochemical and phytochemical constituents of carbonated sugarcane juice beverages
- 4.4.11 Effect of storage period on colour (Tintometer colour units) of carbonated sugarcane juice beverages
- 4.4.12 Effect of storage temperature on colour (Tintometer colour units) of carbonated sugarcane juice beverages
- 4.4.13 Effect of storage period on CO₂ gas volume of carbonated sugarcane juice beverages
- 4.4.14 Effect of storage period on CO₂ gas absorption of carbonated sugarcane juice beverages
- 4.4.15 Effect storage period and storage temperature on microbial quality of carbonated sugarcane juice beverages

4.1 Optimization of processing parameters

The pasteurization of juices and products at different temperatures viz. 78, 80, 82, 84 and 85 °C were carried out for different periods (2, 3, 4, 5 and 6 min.). Finally the particular temperature and time was selected on the basis of sensory evaluation and storage stability (Total plate count for bacteria) of products.

4.1.1. Optimization of pasteurization temperature and time of sugarcane juice beverages

The juices and products pasteurized at 82 °C temperature for 5 min. were found suitable on their sensory evaluation as score was superior over the other pasteurization temperatures and times. The products were found stable for 6 months but there was difference in sensory scores as shown in Table 4.3.

Table 4.3: Optimization of pasteurization temperature and time of sugarcane juice beverages

Pasteurization Temperature (°C)	Pasteurization Time (min)	Overall acceptability	Total plate count (cfu/ml)
78	2	7.5	-
	3	7.5	-
	4	7.5	-
	5	7.6	-
	6	7.7	-
80	2	7.5	-
	3	7.5	-
	4	7.5	-
	5	7.7	-
	6	7.6	-
82	2	7.6	-
	3	7.7	-
	4	7.8	-
	5	8.0	-
	6	7.6	-
84	2	7.5	-
	3	7.5	-
	4	7.6	-
	5	7.7	-
	6	7.7	-
86	2	7.4	-
	3	7.4	-
	4	7.3	-
	5	7.3	-
	6	7.1	-
CD at 5%	0.01	0.02	-

4.1.2 Optimization of level of preservative for non carbonated sugarcane juice beverages pasteurized at 82°C for 5 min

The non carbonated sugarcane juice beverages were standardized and then the best samples of non carbonated sugarcane juice beverages were selected for the optimization of level of sodium benzoate as a preservative. The preservative sodium benzoate at different levels viz. 100, 110, 120 and 130 ppm were tested for the sensory quality and storage stability (Total plate count for bacteria) of the bottled products. The products pasteurized at 82°C temperature for 5 min and 120ppm of sodium benzoate was found superior on sensory evaluation and storage stability (total plate count) for 6 months as depicted in Table 4.4.

Table 4.4: Optimization of level of preservative for non carbonated sugarcane juice beverages pasteurized at 82°C for 5 min.

Samples	Sodium benzoate (ppm)	Overall quality	Total plate countt (cfu/ml)
T23	100	7.7	-
	110	7.8	-
	120	8.0	-
	130	7.6	-
T11	100	7.6	-
	110	7.8	-
	120	8.0	-
	130	7.6	-
T12	100	7.7	-
	110	7.7	-
	120	8.0	-
	130	7.6	-
T14	100	7.6	-
	110	7.8	-
	120	8.0	-
	130	7.6	-
CD at 5%	0.01	0.01	-

4.1.3 Optimization of level of preservative for carbonated sugarcane juice beverages (juice base pasteurized at 82°C for 5 min and cooled to 15°C)

Firstly the carbonated sugarcane juice beverages were developed. The best samples of carbonated sugarcane juice beverages after product standardization were selected for the optimization of preservative level of sodium benzoate. The carbonated sugarcane juice beverages viz. Control (TC27), TC21, TC23 and TC24 were selected for the optimization of the sodium benzoate at different levels viz. 50, 55, 60 and 65 ppm were tested for the storage stability (Total plate count for bacteria) of the bottled products. The juice base of products pasteurized at 82°C temperature for 5 min and 60 ppm of sodium benzoate in the bottled carbonated sugarcane juice beverages was found superior on sensory evaluation and storage stability (total plate count) for 6 months as shown in Table 4.5.

Table 4.5: Optimization of level of preservative for carbonated sugarcane juice beverages (juice base pasteurized at 82⁰C for 5 min and cooled to 15⁰C)

Samples	Sodium benzoate (ppm)	Overall quality	Total plate count (cfu/ml)
TC27	50	7.6	-
	55	7.7	-
	60	8.0	-
	65	7.6	-
TC21	50	7.6	-
	55	7.8	-
	60	8.0	-
	65	7.6	-
TC23	50	7.7	-
	55	7.7	-
	60	8.0	-
	65	7.6	-
TC24	50	7.6	-
	55	7.8	-
	60	8.0	-
	65	7.6	-
CD at 5%	NS	0.02	-

4.2. Physico-chemical and phytochemical constituents of juices

Physicochemical composition of pasteurized juices of sugarcane, kinnow, aonla, lemon and ginger are depicted in the Table 4.6. The juices of sugarcane, kinnow, aonla, lemon and ginger were extracted following the standard methods. The juices of sugarcane, kinnow, aonla, lemon and ginger were analyzed for the physicochemical and phytochemical constituents and antioxidant activity. The juices were analyzed for the proximate composition viz. moisture content, total solids, ash, content, titratable acidity, pH, ascorbic acid, antioxidant activity, total polyphenols, total sugars, reducing sugars and viscosity.

4.2.1 Sugarcane juice

The average value for per cent juice yields of sugarcane was found 54.61. The juice yields for different varieties of sugarcane were found different and values reported by Chauhan *et al* (2002) for sugarcane juice yield were in accordance (48 to 56.4 %). The moisture content of sugarcane juice was observed as 79.26 per cent. The values reported by

Chauhan *et al* (2002) for the moisture content in sugarcane juices of different varieties were slightly higher those ranged between 80 to 81.7 per cent. The values for total solids (%), ash content (%) and total soluble solids (^oB) recorded were 20.73, 0.29 and 20.54, respectively. The total sugar and reducing sugar content values in per cent reported in sugarcane juice were 18.57 and 0.42, respectively. The above results are in agreement with result reported by Chauhan *et al* (2002). The acidity (%) and pH values recorded as 0.21 and 5.35 which showed less value for acidity and more in case of pH than reported by Chauhan *et al* (2002). The viscosity values (cp) observed as was 3.66. The ascorbic acid (mg/100ml), antioxidant activity (% inhibition), total phenolic content (mg GAE/100ml) and total flavonoids (mg QE/100ml) values were reported as 3.65, 43.23, 487.3 and 2.56, respectively. The minerals content in the juice of sugarcane data depicted in Table 4.7 showed that the good source of calcium, iron, potassium, sodium, magnesium, phosphorus, sulphur, copper and zinc with the respective values (mg/100ml) observed were 13.87, 1.78, 49.52, 13.89, 13.42, 25.27, 62.123, 0.52, and 0.39, respectively. These values are at par with the values reported by de Souza *et al* (2015) where higher values were reported for potassium and zinc content. Hence it could be used as a best source for natural drinks used to rehydrate the body.

Table 4.6: Physicochemical and phytochemical constituents of juices of sugarcane, kinnow, aonla, lemon and ginger

Parameters	Fresh juices					CD at 5%
	Sugarcane	Kinnow	Aonla	Lemon	Ginger	
Juice yield (%)	54.61	47.15	129.46	40.7	182	NS
Moisture content (%)	79.26	88.20	90.90	90.22	97.30	0.71
Total solids (%)	20.73	11.80	9.10	9.77	2.70	0.04
TSS (^o Brix)	20.54	10.30	8.60	9.16	2.10	3.10
Ash (%)	0.29	0.35	0.23	0.33	0.27	0.01
Titrateable acidity (%)	0.21	1.16	1.38	4.95	0.27	0.03
pH	5.35	3.24	2.77	2.44	5.68	0.11
Ascorbic acid (mg/100ml)	3.65	23.60	253.42	28.86	5.60	3.41
Antioxidant activity (% inhibition)	43.23	69.30	88.32	67.41	78.20	0.57
Total polyphenols (mg GAE/100ml)	487.3	1266.61	1536.6	1021.3	505	0.99
Total flavonoids (mg QE/100ml)	2.56	16.42	167.3	17.63	150	0.87
Total sugars (%)	18.57	9.59	1.48	4.39	1.75	0.77
Reducing sugars (%)	0.42	2.13	0.93	1.16	0.06	0.13
Viscosity (cp at 25 ^o C)	3.66	5.90	4.80	3.33	3.20	0.36

Table 4.7: Mineral content of the of juices of sugarcane, kinnow, aonla, lemon and ginger (mg/100ml)

Mineral	Sugarcane	Kinnow	Aonla	Lemon	Ginger	CD at 5%
Calcium	13.87	11.95	11.82	11.29	10.07	0.05
Iron	1.78	0.17	1.81	0.18	0.17	0.15
Potassium	49.52	145.00	29.16	103.27	70.73	0.41
Sodium	13.89	4.26	7.52	2.48	3.47	0.02
Magnesium	13.42	6.42	5.08	5.66	6.82	0.01
Copper	0.52	0.11	0.10	0.12	0.13	0.01
Phosphorous	25.27	21.93	9.32	19.73	17.60	0.01
Manganese	0.40	0.33	0.34	0.33	0.59	0.01
Zinc	0.39	0.32	0.30	0.17	0.32	0.01
Sulphur	62.13	11.12	8.12	8.12	14.63	NS
Nickel	0.01	0.01	0.01	0.01	0.02	NS

4.2.2 Kinnow juice

From the Table 4.6, the average value for the juice yield of kinnow during processing was observed as 47.15 per cent and for the total soluble solids was reported 10.3 °Brix. The average values for physicochemical parameters such as moisture content, total solids, ash content, acidity, total sugars and reducing sugars were found 88.20, 11.80, 0.35, 1.16, 9.59 and 2.13 per cent, respectively. The average values for ascorbic acid(mg/100ml), antioxidant activity (% inhibition), total phenolics (mg GAE/100ml) and total flavonoids (mgQE/100ml) in kinnow juice were reported as 23.6, 69.3, 1266.6 and 16.42, respectively. The similar results for ascorbic acid content from squeezed juice samples in Verna species was 262±18.8 mg L⁻¹ and in Fino species was 532±20.2 mg L⁻¹ (Marin *et al* 2002). The minerals content in the juice of kinnow data depicted in Table 4.7 showed that the good source of calcium, iron, potassium, sodium, magnesium, phosphorus, sulphur, copper and zinc with the respective values (mg/100ml) observed were 11.95, 0.17, 145, 4.26, 6.42, 21.93, 11.12, 0.11 and 0.32, respectively. Savic *et al* (2015) reported the same results for the mineral content in orange juices. The values for potassium and magnesium were found at par with value reported for orange juice by Demir *et al* (2015). Hence it could be used as a best source for natural drinks used to rehydrate the body.

4.2.3 Aonla juice

It was observed from Table 4.6 that, the average value for the juice yield of aonla during processing was observed as 129.46 per cent as during juice extraction the equal

proportion of water was used for the proper extraction of juice from the aonla fruits and for the total soluble solids was reported 8.6 °Brix. The average values for physico-chemical parameters such as moisture content, total solids, ash content, acidity, total sugars and reducing sugars were found 90.90, 9.1, 0.23, 1.38, 1.48 and 0.93 per cent, respectively. The average values for ascorbic acid (mg/100ml), antioxidant activity (% inhibition), total phenolics (mg GAE/100ml) and total flavonoids (mgQE/100ml) content in aonla juice were reported as 253.42, 88.32, 1536.6 and 167.3, respectively. These values were found lower as compared to fruit content as the equal proportion of water was used during extraction of juice. The minerals content in the juice of aonla data depicted in Table 4.7 showed that the good source of calcium, iron, potassium, sodium, magnesium, phosphorus, sulphur, copper and zinc with the respective values (mg/100ml) observed were 11.82, 1.81, 29.16, 7.52, 5.08, 9.32, 8.12, 0.1 and 0.3, respectively. The higher values were reported by Parvin and Khatkar (2015). Hence it could be used as a best source for natural drinks used to rehydrate the body.

4.2.4 Lemon juice

The average juice yield of lemons was observed as 40.7 per cent during study. Mookerjee *et al* (1964) reported that juice content of different varieties of lemons varied from 21.26 to 42.86 per cent, while Mahajan (1989) reported higher values (48.9 to 49.33 per cent) for lemon juice content from Himachal Galgals. According to Ranganna (2015) the juice content in the lemons should not be less than 30 percent as per the USDA standards and not less than 28 per cent for green lemon fruits meant for export. The moisture content in lemon was 90.22 per cent; similar level of moisture content was reported by Verma and Sastry (1969). The 90.7 per cent moisture content was reported in lemon by Gebhardt *et al* (1982). The TSS 9.16°B was observed while similar results were reported by Verma and Sastry (1969), Jain *et al* (1984) and Khurdiya (1988). The titratable acidity recorded for the lemon juice was 4.95 per cent. The values of titratable acidity were in the range of acidity reported by Ranken (1984). The ash content was observed as 0.43 per cent which found conformity with the value reported by Ranken (1984). The pH value of lemon juice was observed as 2.44. The ascorbic acid content (mg/100ml) was 28.86 which was found lower than value reported by Ranken (1984) and Marin *et al* (2002). The antioxidant activity (% inhibition), total phenolics (mg GAE/100ml) and total flavonoids (mgQE/100ml) were observed as 67.41, 1021.3 and 17.63, respectively. Hajimahmoodi *et al* (2012) reported the values of antioxidant capacity (235.47 to 888.59 mg VEE L⁻¹), ascorbic acid (18.7 to 22.9mg/100ml) and total phenolics (11 to 27.8 mg GAE/100ml) natural lemon juice samples while Guimaraes *et al* (2010) reported 11.7±17 mg GAE/g for crude juice of lemon. The per cent values for total sugars and reducing sugars observed in juice were 4.39 and 1.16, respectively. The range for reducing sugar content reported by Mahajan and Lal, (1991) was 1.23-1.36 while Jain *et al* reported 1.08 per cent of reducing sugar.

4.2.5 Ginger juice

It was observed from Table 4.6 that, the average value for the juice yield of ginger rhizomes during processing was observed as 182 per cent as during juice extraction the double proportion of water was used for the proper extraction of juice from the ginger rhizomes and for the total soluble solids was reported 2.1 °Brix. The average values for physicochemical parameters such as moisture content, total solids, ash content, acidity, total sugars and reducing sugars were found 97.3, 2.7, 0.27, 0.27, 1.75 and 0.06 per cent, respectively. The average values for ascorbic acid (mg/100ml), antioxidant activity (% inhibition), total polyphenols (mg GAE/100ml) and total flavonoids (mgQE/100ml) content in ginger juice were reported as 5.6, 78.2, 505 and 150, respectively. These values were found lower as compare to fruit content as the equal proportion of water was used during extraction of juice. The average value for pH and viscosity (cp) was observed as 5.68 and 3.2, respectively. The minerals content in the juice of ginger, data depicted in Table 4.7 showed that the good source of calcium, iron, potassium, sodium, magnesium, phosphorus, sulphur, copper and zinc with the respective values (mg/100ml) observed were 10.07, 0.17, 70.73, 3.47, 6.82, 17.6, 14.63, 0.13 and 0.32, respectively. The values (mg/100g) of Ca, P, Fe, Zn, Cu, and Mn content for dry ginger were reported by Shirin and Prakash (2010) as 88.4, 174, 8, 0.92, 0.54 and 9.15, respectively. Hence it could be used as a best source for natural drinks used to rehydrate the body.

4.3 Development of non carbonated sugarcane juice beverages

The development of non carbonated sugarcane juice beverages were carried out by blending the different proportions of juices of sugarcane, kinnow, aonla, lemon and ginger as stated in Table 3.1.

4.3.1 Standardization of recipe for non carbonated sugarcane juice beverages

The samples of non carbonated sugarcane juice beverages were made using different combination treatments viz. T1 to T23 as shown in Table 3.1 for the standardization of recipe. The combination of treatments were made in a such a way that there was no need to add external sugars in the beverages. The sugarcane juice was considered as a natural source for sweetening of the beverages. The required quantity of sugar level was maintained for the better acceptability of the beverages. The fresh juice from fresh sugarcane was extracted using stainless steel made juice extractor (Kalsi make) with double extraction, filtered through the four fold muslin cloths, the different combination treatments for juice blends for the non carbonated sugarcane juice beverages were made using the juices of kinnow, aonla, lemon and ginger along with the sugarcane juice. The juice mixes of different treatments were pasteurized at 82°C for 5 min and the preservative was adjusted to 120ppm level in each non carbonated beverage samples which was kept within the permissible limit of FSSAI. The beverages were hot filled in the sterile bottles of 200 ml capacity, sealed and heat processed for 30 min in boiling water. The bottled samples were stored in refrigerated storage.

Table 4.8: Sensory evaluation of non carbonated sugarcane juice beverages

Treatment	Appearance	Mouth feel	Aroma	Taste	Consistency	Overall acceptability
T0	7.66	7.00	7.16	7.16	7.33	7.16
T1	7.66	6.33	7.50	7.00	8.00	6.33
T2	7.83	7.16	7.30	7.16	7.66	7.00
T3	7.16	7.73	7.50	6.83	7.50	7.50
T4	7.56	6.66	6.33	6.83	7.33	6.66
T5	7.50	6.33	7.50	6.66	7.33	6.66
T6	7.46	7.50	7.33	7.50	7.00	7.33
T7	7.63	7.50	7.33	7.50	7.33	7.33
T8	7.16	6.83	7.33	7.33	7.33	6.83
T9	7.50	6.83	7.16	6.50	7.53	7.16
T10	6.50	7.00	7.00	7.00	7.50	7.00
T11	8.16	8.00	8.00	8.33	8.00	8.16
T12	8.00	8.16	8.16	8.00	8.16	8.00
T13	7.83	7.83	7.33	7.33	8.16	7.83
T14	8.50	8.16	8.33	8.33	8.00	8.50
T15	7.83	7.33	8.00	7.83	7.66	7.66
T16	7.83	6.66	7.66	7.16	7.33	7.33
T17	6.83	6.50	7.50	6.66	6.83	7.33
T18	6.83	6.66	6.50	6.66	6.50	6.66
T19	7.33	6.50	6.66	6.83	6.66	6.66
T20	6.83	7.16	6.66	6.33	7.33	6.66
T21	7.83	7.00	6.83	7.66	7.60	7.33
T22	7.66	6.66	7.00	7.33	7.00	7.33
T23	8.00	8.00	7.83	8.00	7.33	8.00
CD at 5%	0.63	0.62	0.58	0.68	0.47	0.61



Plate 1: Non-carbonated sugarcane juice beverages

4.3.2 Sensory evaluation of non carbonated sugarcane juice beverages

The were samples prepared by using different treatments as shown in Table 3.1 and then chilled. The chilled samples were tested for sensory quality parameters viz. appearance, mouth feel, aroma, taste, consistency and overall acceptability by the semi trained panel using 9 point hedonic scale. The non carbonated sugarcane juice beverage prepared by using treatment T23 (sugarcane juice 95%, lemon juice 3%, ginger juice 2 %) was found superior on sensory evaluation and scored 8 for overall acceptability and was further used as a control sample, while the non carbonated sugarcane juice beverage samples blended with juices of kinnow, aonla, lemon and ginger by using treatments of T11 (80% sugarcane juice, 10 % kinnow juice, 5 % aonla juice, 2 % lemon juice and 3 % ginger juice), T12 (80% sugarcane juice, 10 % kinnow juice, 5 % aonla juice, 2.5 % lemon juice and 2.5 % ginger juice) and T14 (75% sugarcane juice, 15 % kinnow juice, 5 % aonla juice, 2 % lemon juice and 3 % ginger juice) were scored high over the other treatments for all the parameters of sensory quality viz. appearance, mouth feel, aroma, taste, consistency and overall acceptability on sensory evaluation by judges of semi trained panel using 9 point hedonic scale. It was observed from Table 8 that the non carbonated sugarcane juice beverages were found highly acceptable due to the use of natural juices of sugarcane, kinnow, aonla, lemon and ginger and limited preservative. The sample of treatment T14 was found superior for all parameters of sensory quality on sensory evaluation by the semi trained panel using 9 point hedonic scale followed by the T11, T12 and T23 treatments. The treatment T23 is used as control for the further study of non carbonated sugarcane juice beverages as it contains only lemon and ginger juices with sugarcane juice. The other blends of T11, T12 and T14 contained sugarcane juice along with the kinnow, aonla, lemon and ginger juices. The best samples of non carbonated sugarcane juice beverages of treatments T23 (control), T11, T12 and T14 were chosen for further study. The samples of these treatments were prepared in bulk and stored for its further study of physico-chemical characteristics, storage stability and microbial quality.

The non carbonated sugarcane juice beverages viz Control (T23), T11, T12 and T14 were prepared by using fruit juice blends along with the sugarcane juice in a such manner that no external sugar, acid was added except the limited preservative level of sodium benzoate (120ppm) which was within the permissible limit of FSSAI law. Hence found best source for the fruit beverage.

4.3.3 Physicochemical and phytochemical constituents of non carbonated sugarcane juice beverages

The non carbonated sugarcane juice beverages T23, T11, T12 and T14 were analyzed for physicochemical characteristics as depicted in Table 4.9 showed that total soluble solids were found higher (18.7 °B) in control sample than T11, T12 and T14. The per cent values for ash content, acidity, total sugars and reducing sugars of non carbonated sugarcane juice

beverages T23, T11, T12 and T14 were 0.16, 0.52, 17.73, 0.44; 0.12, 0.47, 16.25, 0.54; 0.16, 0.65, 16.35, 0.56 and 0.17, 0.61, 15.78, 0.63, respectively. Also the values for the ascorbic acid (mg/100ml), antioxidant activity (% inhibition), total polyphenols (mg GAE/100ml) and total flavonoids (mgQE/100ml) for respective beverages were observed as 4.3, 16.53, 16.62, 17.3; 53.2, 80.21, 80.7, 81.9; 496.6, 621.3, 624.2, 660.3; 5.22, 15.42, 15.3, 16.2, respectively. The viscosities (cp) were ranged between 3.55 to 3.84 which showed no significant difference. The mineral content values (mg/100ml) for the non carbonated sugarcane juice beverage samples viz. T23, T11, T12 and T14 were found at par and showed good amount of minerals in the beverages shown in Table 4.10. The mineral content values (mg/100ml) for all beverages were found in good amount as calcium (13.18 – 13.73), iron (1.45-1.52), potassium (43.23 – 51.77), sodium (4.37 – 4.97), phosphorus (24.01 – 24.17), sulphur (37.52 – 38.65), zinc (0.36- 0.39), magnesium (6.58 – 7.73), copper (0.09 – 0.11) and nickel (0.01).

The high quality and quantity nutrients in the non carbonated sugarcane juice beverages viz. T23, T11, T12 and T14, with natural sugar, limited preservative use within permissible limit along with ginger as a natural preservative with use of natural source of ascorbic acid (kinnow, lemon, aonla) and no acid added made it highly acceptable. The taste was found highly acceptable during sensory evaluation.

Table 4.9: Physicochemical and phytochemical constituents of non carbonated sugarcane juice beverages

Parameters	Non carbonated beverages				CD at 5%
	Control (T23)	T11	T12	T13	
Moisture content (%)	80.96	81.64	81.69	81.68	0.14
Total solids (%)	19.03	18.36	18.30	18.31	0.14
TSS (°Brix)	18.70	17.30	17.30	16.76	0.42
Ash (%)	0.16	0.12	0.16	0.17	0.01
Titratable acidity (%)	0.52	0.47	0.65	0.61	0.30
pH	2.42	2.56	2.37	2.39	0.09
Ascorbic acid (mg/100ml)	4.30	16.53	16.62	17.30	0.86
Antioxidant activity (% inhibition)	53.20	80.21	80.70	81.90	0.53
Total phenol (mg GAE/100ml)	496.6	621.3	624.2	660.30	0.16
Total flavonoids (mg QE/100ml)	5.22	15.42	15.30	16.20	0.14
Total sugars (%)	17.73	16.25	16.35	15.78	0.07
Reducing sugars (%)	0.44	0.54	0.56	0.63	0.02
Viscosity (cp)	3.60	3.62	3.84	3.55	NS

Table 4.10: Mineral content of the of non carbonated sugarcane juice beverages

(mg/100ml)

Mineral	Non carbonated beverages				CD at 5%
	Control (T23)	T11	T12	T14	
Calcium	13.72	13.18	13.59	13.73	0.02
Iron	1.52	1.45	1.49	1.51	0.01
Potassium	43.23	45.14	49.5	51.77	0.47
Sodium	4.97	4.85	4.37	4.91	0.01
Magnesium	7.73	6.58	7.72	7.15	0.05
Copper	0.11	0.09	0.11	0.10	0.01
Phosphorous	24.17	24.01	24.11	24.14	0.02
Manganese	0.33	0.34	0.35	0.33	0.01
Zinc	0.36	0.39	0.37	0.38	0.01
Sulphur	38.65	37.64	38.53	37.52	0.05
Nickel	0.01	0.01	0.01	0.01	NS

4.3.4 Effect of blending on phytochemicals in non carbonated sugarcane juice beverages

The effect of blending of sugarcane juice with fruit juices depicted in Fig.1 showed that there was increase in ascorbic acid (4 times), antioxidant activity was doubled, total phenolics were increase by 27 % while total flavonoids increased to 3 times than the sugarcane juice. The blend T14 was found superior for the content of phytochemicals followed by T12 and T11. Hence the blending of sugarcane juice showed the significant increase in the phytochemical content of blended beverages and improved the nutritional quality of non carbonated beverages.

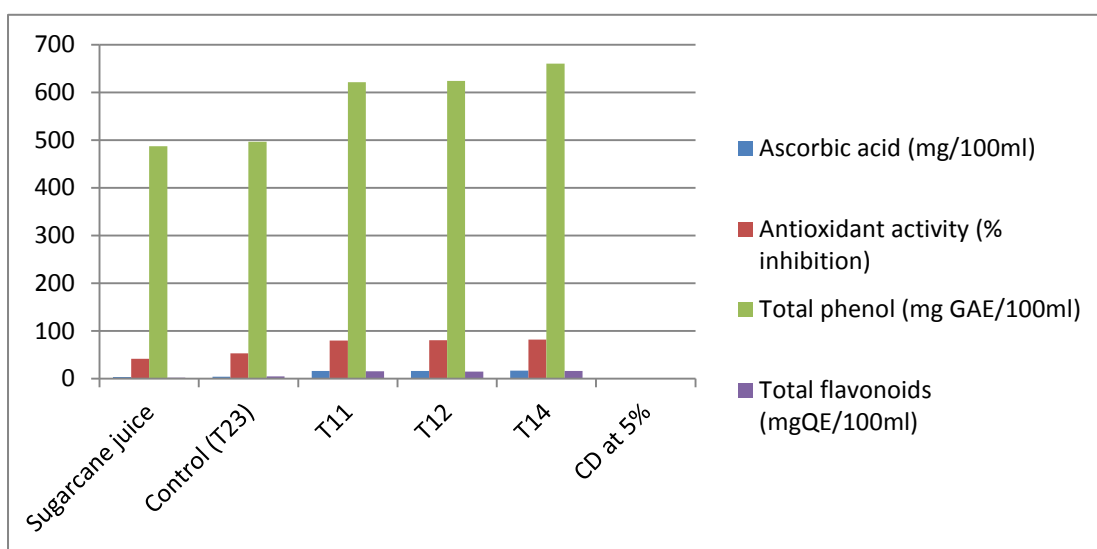
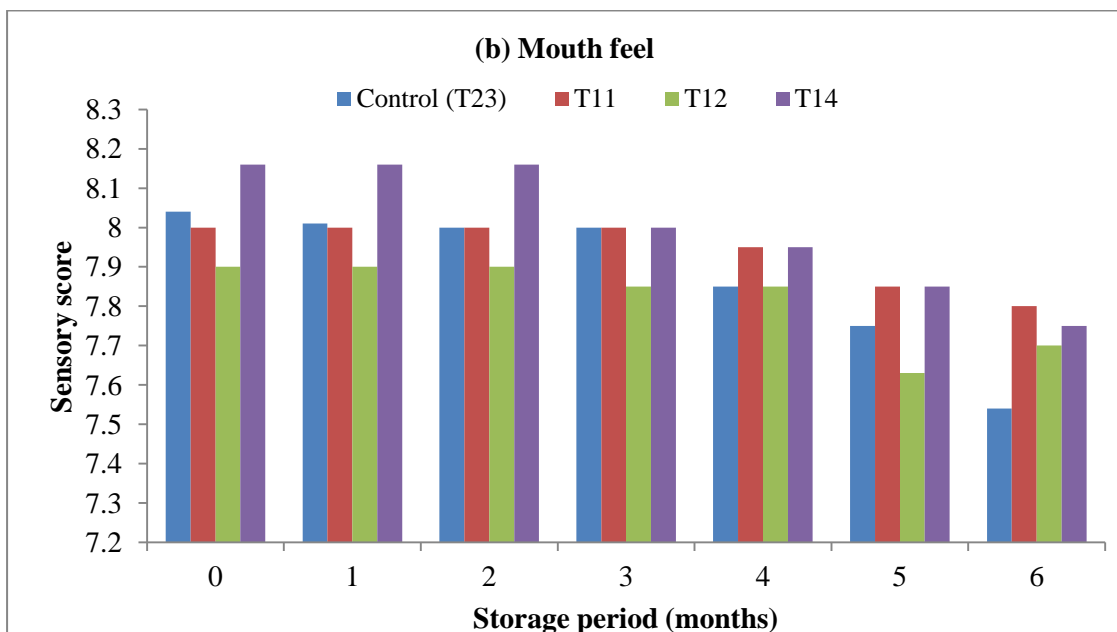
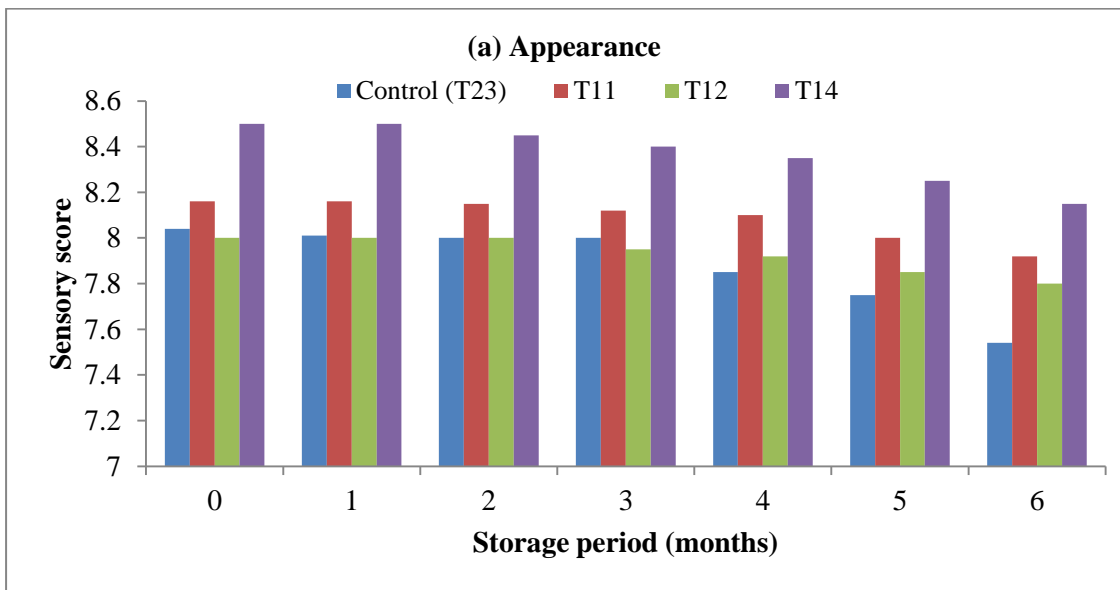


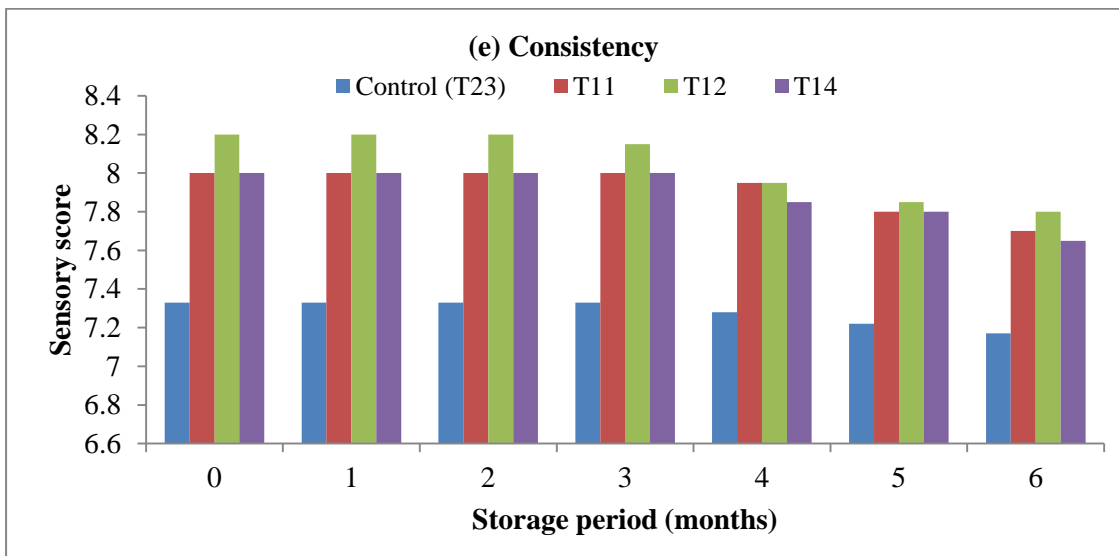
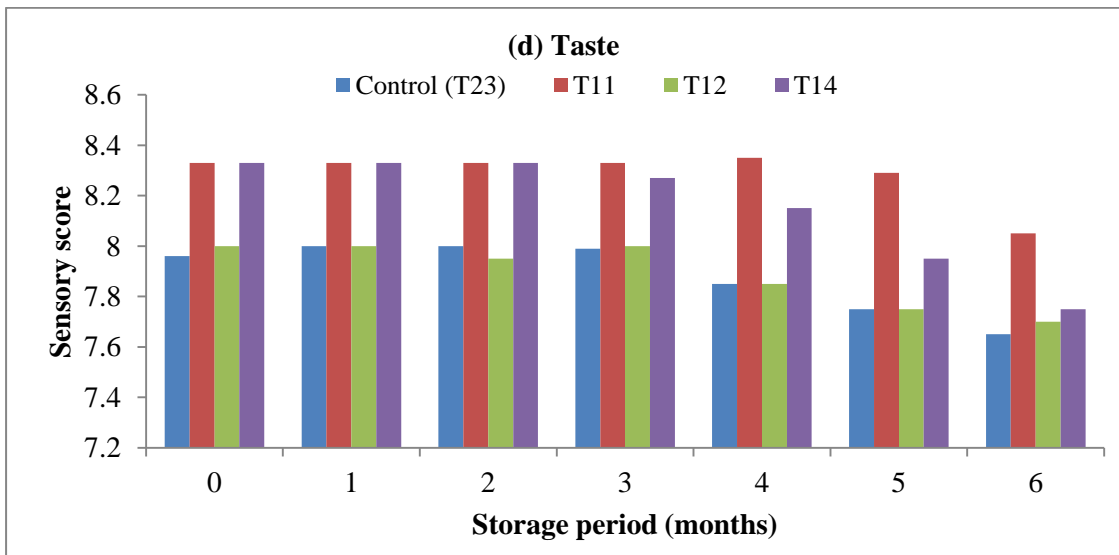
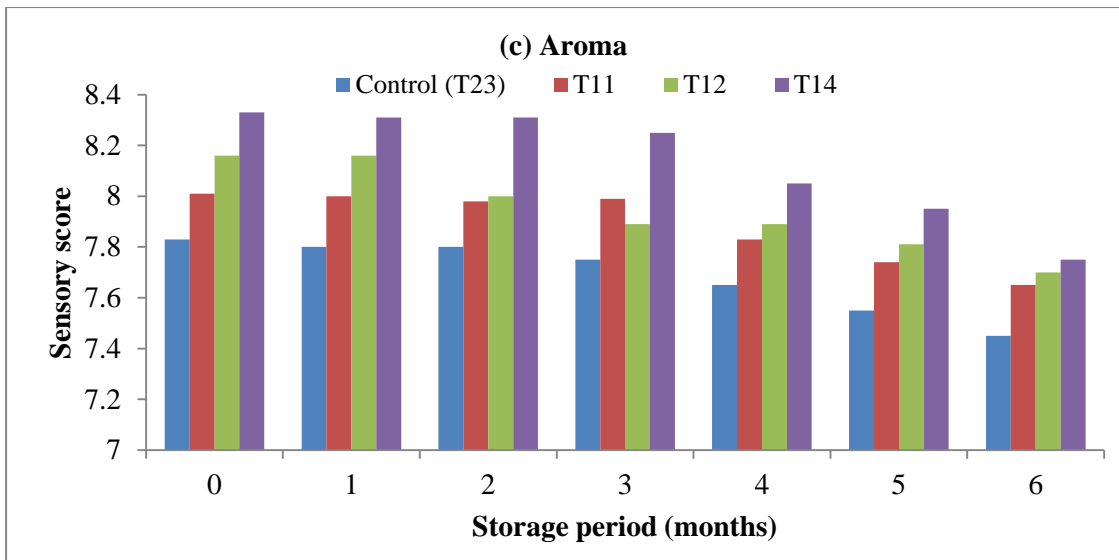
Fig. 1: Effect of blending on the phytochemicals of non carbonated sugarcane juice beverages

4.3.5 Effect of storage period on sensory qualities of non carbonated sugarcane juice

beverages

The effect of storage period on sensory qualities of non carbonated sugarcane juice beverages (T23, T11, T12 and T14) were studied for the 6 months at room ($30\pm 4^{\circ}\text{C}$) and refrigeration (4°C) temperature and the periodic observations were noted after each month. The data presented in Fig. 2 showed that all the samples of non carbonated sugarcane juice beverages were found acceptable with slight decreased sensory score by semi trained panel during the storage of 6 months for all parameters viz. appearance, mouth feel, aroma, taste, consistency and overall acceptability though the beverages were found stable during the storage period.





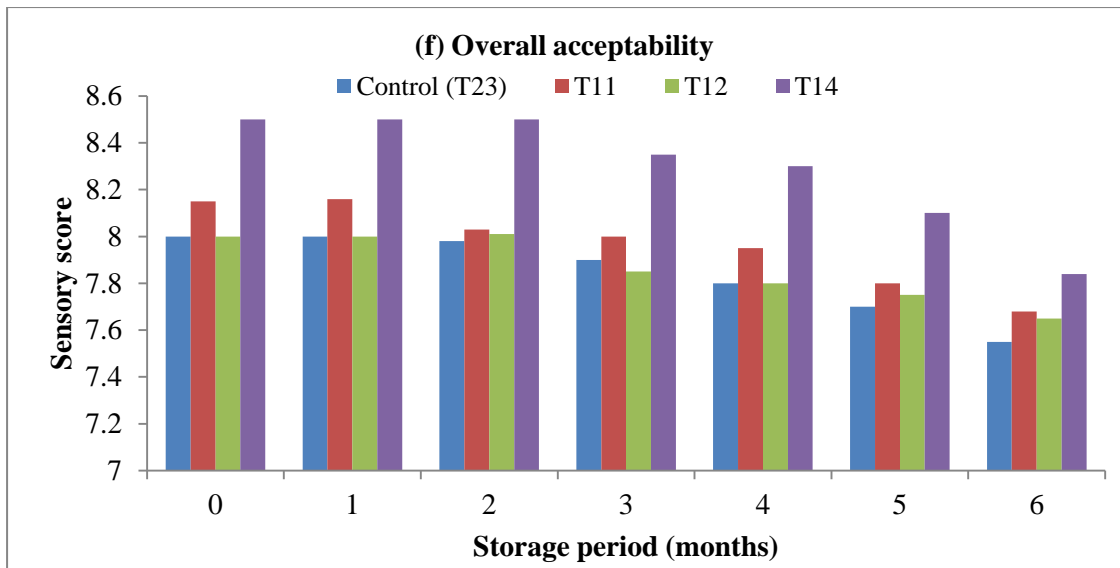
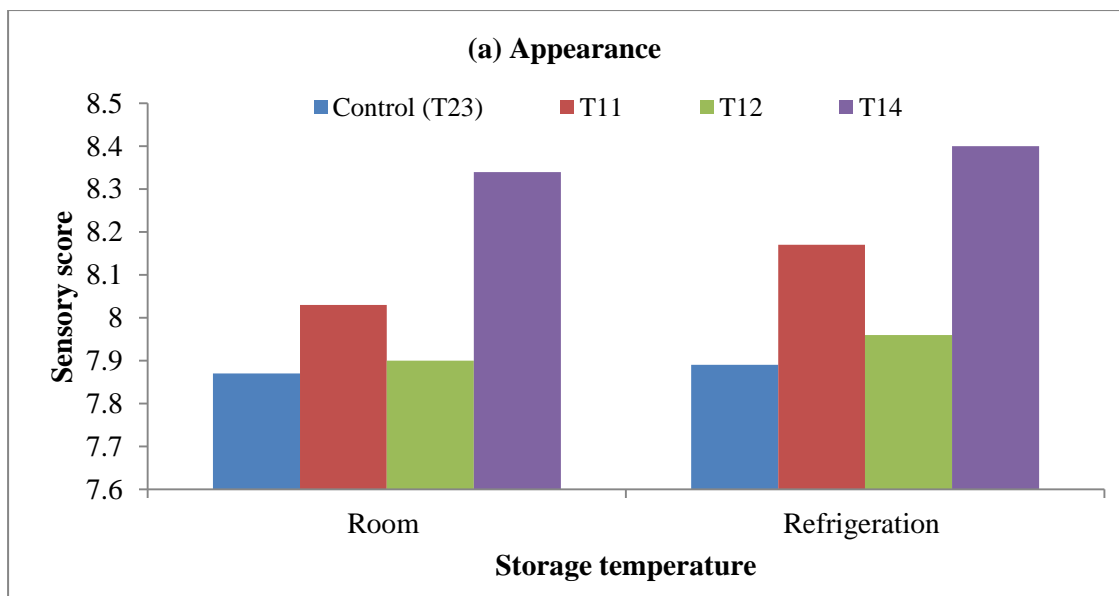
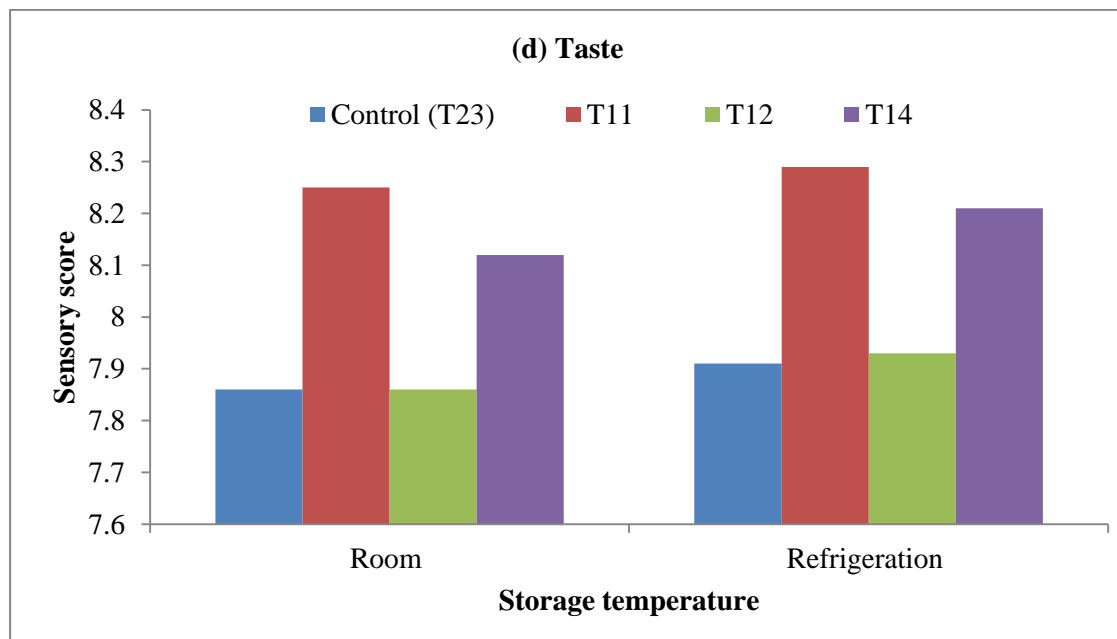
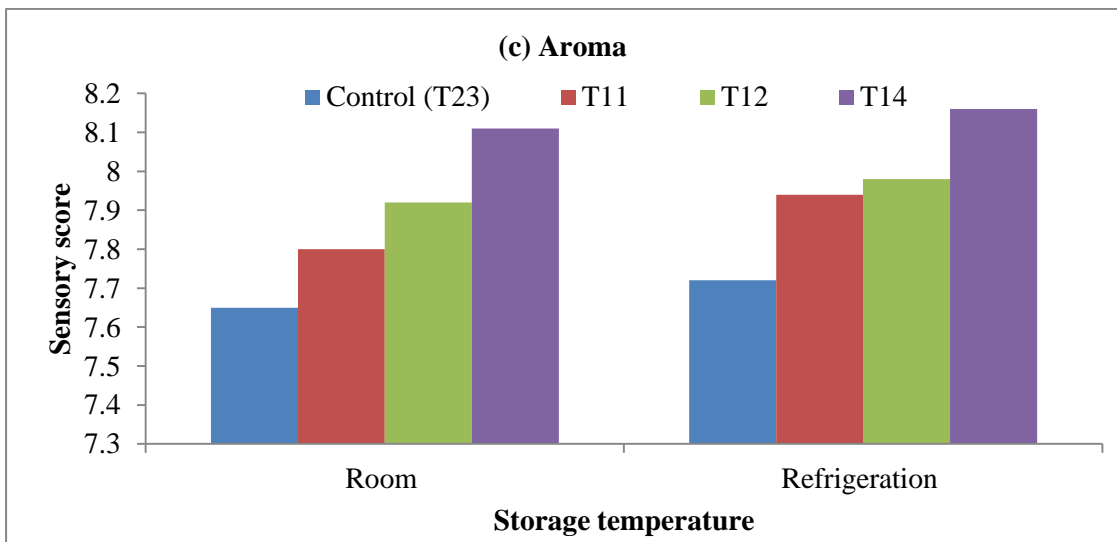
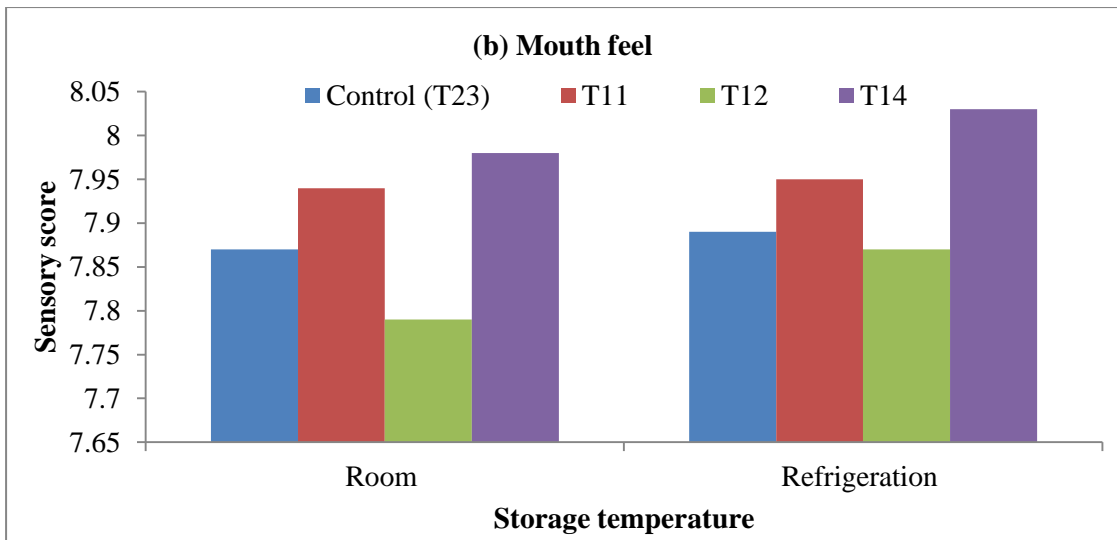


Fig. 2 : Effect of storage period on sensory qualities of non carbonated sugarcane juice beverages

4.3.6 Effect of storage temperature on sensory qualities of non carbonated sugarcane juice beverages

The effect of storage temperature both at room ($30\pm 4^{\circ}\text{C}$) and refrigeration (4°C) on sensory qualities of non carbonated sugarcane juice beverages depicted in Fig. 3 showed that the beverages stored at refrigerated temperature scored slightly high for all the sensory parameters than the stored at room temperature. The non carbonated sugarcane juice beverages were found slightly superior in quality at refrigerated storage for 6 months over the storage at room temperature but both beverages were scored well on sensory evaluation.





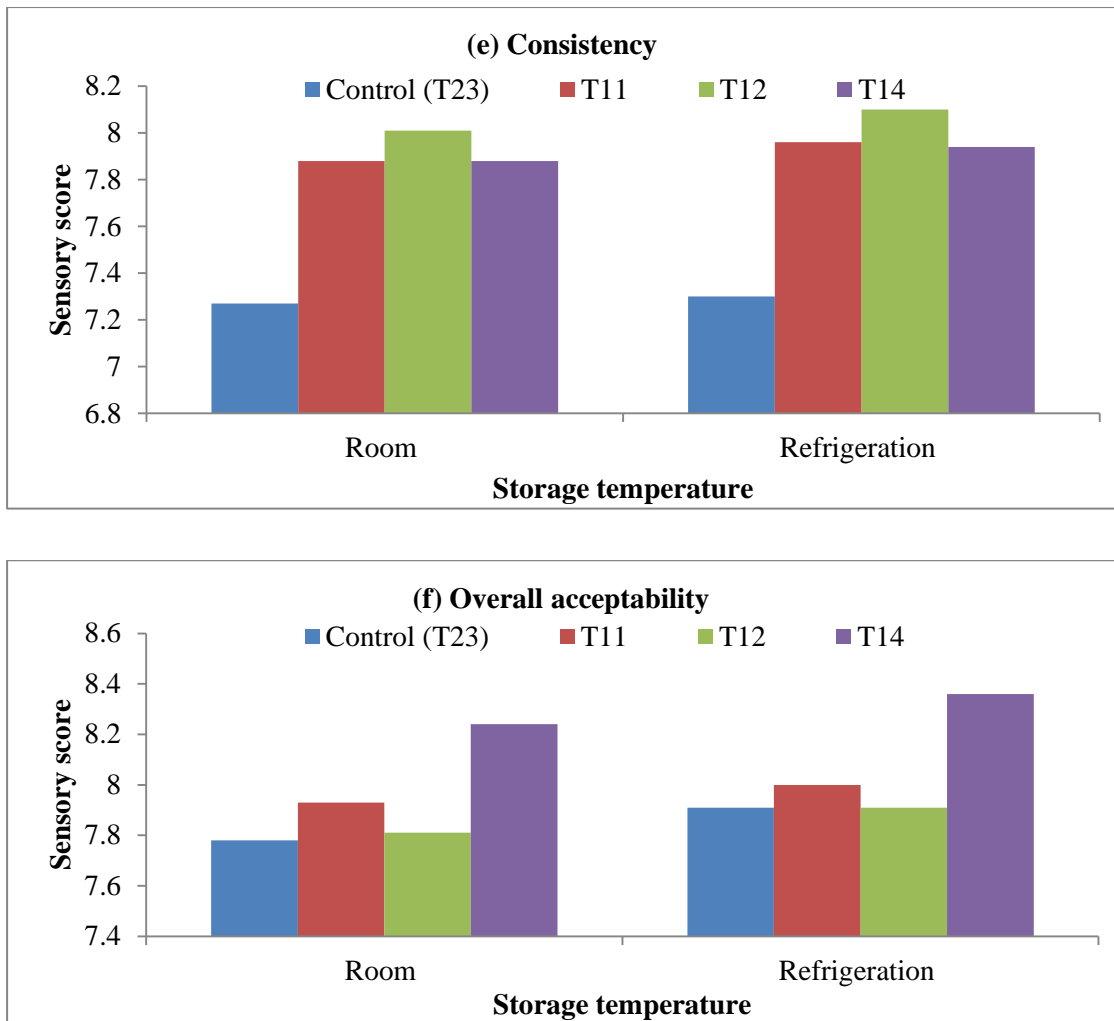


Fig. 3 : Effect of storage temperature on sensory qualities of non carbonated sugarcane juice beverages (6 months storage)

4.3.7 Effect of storage period on physicochemical and phytochemical constituents of non carbonated sugarcane juice beverages

The effect of storage period on physicochemical characteristics of non carbonated sugarcane juice beverages (T23, T11, T12 and T14) during 6 months storage at room ($30\pm 4^{\circ}\text{C}$) and refrigeration (4°C) temperature is noted in Table 4.11. The observations showed that there was slight decrease in values of total soluble solids (1.6%), pH (3.63%), ascorbic acid (1.35%), total sugars (2.38%) and viscosity (1.71%) for all the beverages after 3 months during storage of 6 months. The values for the phytochemical viz. total polyphenol and total flavonoids were decreased by 20 per cent while the antioxidant activity was decreased by 25 per cent during the storage of six months. The same trend has been reported by Karpagavalli and Amutha (2015). The values for titratable acidity and reducing sugars were increased 4.91% and 7.9%, respectively during the storage of 6 months; however the beverages showed stability till 3 months while further storage slightly decreased the values. The products were found acceptable after six months storage.

Table 4.11: Effect of storage period on physicochemical and phytochemical constituents of non carbonated sugarcane juice beverages

Treatment	Storage period (months)						
	0	1	2	3	4	5	6
	Total soluble solids (^o B)						
Control (T23)	18.70	18.70	18.70	18.70	18.69	18.65	18.61
T11	17.30	17.30	17.30	17.25	17.20	17.15	17.00
T12	17.30	17.30	17.30	17.28	17.25	17.23	17.00
T14	16.76	16.76	16.72	16.62	16.40	16.37	16.35
CD at 5%	0.13						
	Titratable acidity (%)						
Control (T23)	0.52	0.52	0.52	0.52	0.53	0.55	0.56
T11	0.47	0.47	0.47	0.47	0.48	0.49	0.51
T12	0.65	0.65	0.65	0.65	0.66	0.67	0.68
T14	0.61	0.61	0.61	0.61	0.62	0.63	0.64
CD at 5%	NS						
	pH						
Control (T23)	2.42	2.42	2.42	2.41	2.40	2.39	2.37
T11	2.56	2.56	2.56	2.55	2.54	2.52	2.51
T12	2.37	2.37	2.37	2.36	2.35	2.22	2.00
T14	2.39	2.39	2.38	2.37	2.37	2.36	2.36
CD at 5%	NS						
	Ascorbic acid (mg/100ml)						
Control (T23)	4.30	4.22	4.00	3.38	3.36	3.34	3.32
T11	16.53	16.00	14.72	13.500	13.00.	12.60	12.35
T12	16.62	16.20	15.60	14.80	14.01	13.20	12.44
T14	17.30	16.60	16.00	15.20	14.50	13.90	12.78
CD at 5%	0.02						
	Total polyphenols (mgGAE/100ml)						
Control (T23)	496.6	473.22	458.31	442.00	426.22	413.20	396.21
T11	621.3	604.23	585.52	569.32	548.00	526.30	492.00
T12	624.2	605.23	585.52	564.22	543.33	519.65	498.32
T14	660.30	640.00	618.34	595.11	574.42	552.10	531.21
CD at 5%	0.08						

Treatment	Storage period (months)						
	0	1	2	3	4	5	6
	Total flavonoids (mgQE/100ml)						
Control (T23)	5.22	5.02	4.82	4.63	4.55	4.36	4.20
T11	15.42	14.90	14.41	13.95	13.82	12.63	12.41
T12	15.30	14.81	14.31	13.82	13.32	12.81	12.38
T14	16.20	15.50	14.20	14.13	13.40	13.24	12.98
CD at 5%	0.03						
	Antioxidant activity (% inhibition of DPPH)						
Control (T23)	53.20	50.10	47.32	45.25	43.20	41.10	38.99
T11	80.21	77.20	74.21	71.65	69.54	64.35	60.88
T12	80.70	77.80	74.56	71.00	68.24	64.50	61.20
T14	81.90	78.52	75.31	71.50	68.75	64.37	61.50
CD at 5%	0.01						
	Total sugars(%)						
Control (T23)	17.73	17.73	17.73	17.72	17.71	17.67	17.63
T11	16.25	16.25	16.25	16.20	16.10	15.85	15.80
T12	16.35	16.35	16.35	16.25	16.20	15.93	15.80
T14	15.78	15.78	15.78	15.65	15.50	15.40	15.30
CD at 5%	0.04						
	Reducing sugars(%)						
Control (T23)	0.44	0.44	0.44	0.44	0.45	0.47	0.49
T11	0.54	0.54	0.54	0.55	0.56	0.57	0.58
T12	0.56	0.56	0.56	0.57	0.58	0.59	0.61
T14	0.63	0.63	0.63	0.64	0.65	0.66	0.68
CD at 5%	NS						
	Viscosity(cp)						
Control (T23)	3.60	3.60	3.60	3.59	3.58	3.54	3.52
T11	3.62	3.62	3.62	3.61	3.60	3.59	3.57
T12	3.84	3.84	3.84	3.83	3.82	3.81	3.80
T14	3.55	3.55	3.55	3.53	3.52	3.50	3.47
CD at 5%	0.02						

4.3.8 Effect of storage temperature on physicochemical and phytochemical constituents of non carbonated sugarcane juice beverages

The effect of storage temperature on physicochemical characteristics of non carbonated sugarcane juice beverages (T23, T11, T12 and T14) during 6 months storage at room ($30\pm 4^{\circ}\text{C}$) and refrigeration (4°C) temperature is noted in Table 4.12. It was observed that, the all treatments (T23, T11, T12 and T14) beverages stored at refrigerated temperature depicted slightly high values for all physico-chemical parameters than the samples stored at room temperature. The non carbonated sugarcane juice beverages were found slightly superior in quality those stored at refrigerated storage for 6 months over the storage at room temperature but both beverages showed statistically non significant changes in the values of all parameters. The values for physic chemical parameters of T23, T11, T12 and T14 beverages stored at room and refrigerated temperature were found as total soluble solids (18.99, 17.2, 17.23, 16.5 and 19.05, 17.27, 17.28, 16.63), titratable acidity (0.53, 0.49, 0.66, 0.63 and 0.52, 0.48, 0.65, 0.62), pH (3.4, 3.53, 3.27, 3.37 and 3.41, 3.55, 3.32, 3.38), ascorbic acid (3.2, 12.35, 12.44, 12.38 and 3.53, 12.54, 12.63, 12.81), total sugars (17.69, 16.02, 16.12, 15.51 and 17.71, 16.13, 16.21, 15.69), reducing sugars (0.46, 0.56, 0.57, 0.65 and 0.45, 0.54, 0.56, 0.54) and viscosity (3.57, 3.6, 3.82, 3.52 and 3.58, 3.61, 3.83, 3.53), respectively.

The values for the phytochemical viz. total polyphenol showed 20 % loss of polyphenols at room temperature while 18 per cent loss was observed at refrigeration storage for six months. The total flavonoids were decreased by 20 per cent both at room and refrigerated storage while, while the antioxidant activity was decreased by 25 per cen at room temperature and 20 per cent at refrigerated storage of six months. The same trend has been reported by Karpagavalli and Amutha (2015).

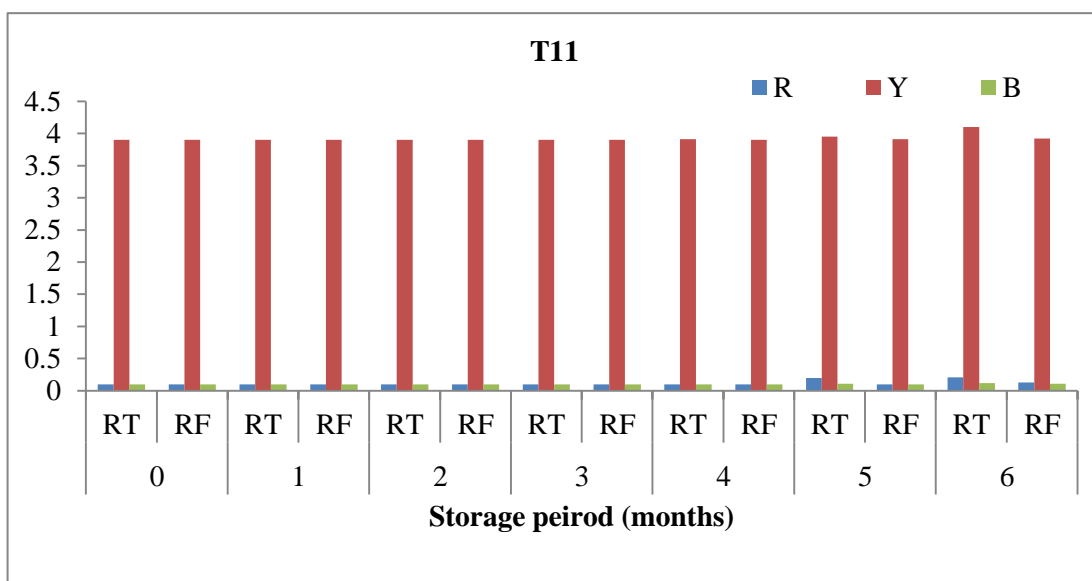
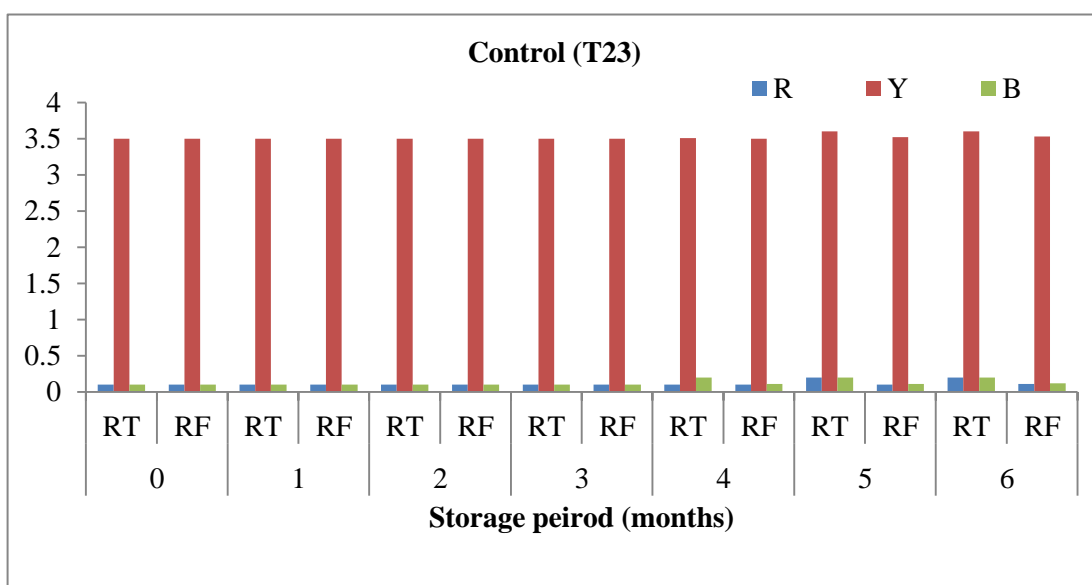
Table 4.12: Effect of storage temperature on physicochemical and phytochemical constituents of non carbonated sugarcane juice beverages (6 months storage)

Storage temperature	Non carbonated beverages			
	Control (T23)	T11	T12	T14
	Total soluble solids ($^{\circ}\text{B}$)			
Room	18.99	17.20	17.23	16.50
Refrigeration	19.05	17.27	17.28	16.63
CD at 5%	0.06			
	Titratable acidity (%)			
Room	0.53	0.49	0.66	0.63
Refrigeration	0.52	0.48	0.65	0.62
CD at 5%	NS			

Storage temperature	Non carbonated beverages			
	Control (T23)	T11	T12	T14
	pH			
Room	3.40	3.53	3.27	3.37
Refrigeration	3.41	3.55	3.32	3.38
CD at 5%	NS			
	Ascorbic acid (mg/100ml)			
Room	3.32	12.35	12.44	12.78
Refrigeration	3.53	12.54	12.63	12.81
CD at 5%	NS			
	Total polyphenols (mgGAE/100ml)			
Room	396.21	492.00	498.32	531.21
Refrigeration	404.10	502.40	509.52	540.35
CD at 5%	0.02			
	Total flavonoids (mgQE/100ml)			
Room	4.20	12.41	12.38	12.98
Refrigeration	4.21	12.43	12.40	12.99
CD at 5%	NS			
	Antioxidant activity (% inhibition of DPPH)			
Room	38.99	60.88	61.20	61.50
Refrigeration	40.88	64.32	64.55	64.67
CD at 5%	0.01			
	Total sugars(%)			
Room	17.69	16.02	16.12	15.51
Refrigeration	17.71	16.13	16.21	15.69
CD at 5%	NS			
	Reducing sugars(%)			
Room	0.46	0.56	0.57	0.65
Refrigeration	0.45	0.54	0.56	0.54
CD at 5%	NS			
	Viscosity(cp)			
Room	3.57	3.60	3.82	3.52
Refrigeration	3.58	3.61	3.83	3.53
CD at 5%	0.01			

4.3.9 Effect of storage period and storage temperature on colour (Tintometer colour units) of non carbonated sugarcane juice beverages

The effect of storage period on colours (Tintometer colour units) of non carbonated sugarcane juice beverages (T23, T11, T12 and T14) data depicted in Fig. 4 showed that there was a slight increase in values of tintometer colour units of R, Y and B values during storage of 6 months for all treatments, the Y values were found major followed by R for all treatments. The storage period had significant effect on colour values during storage of six months though all the samples were found stable for 3 months. But these changes are acceptable on sensory evaluation of products. The samples stored at refrigerated temperature showed no significant effect on colour values.



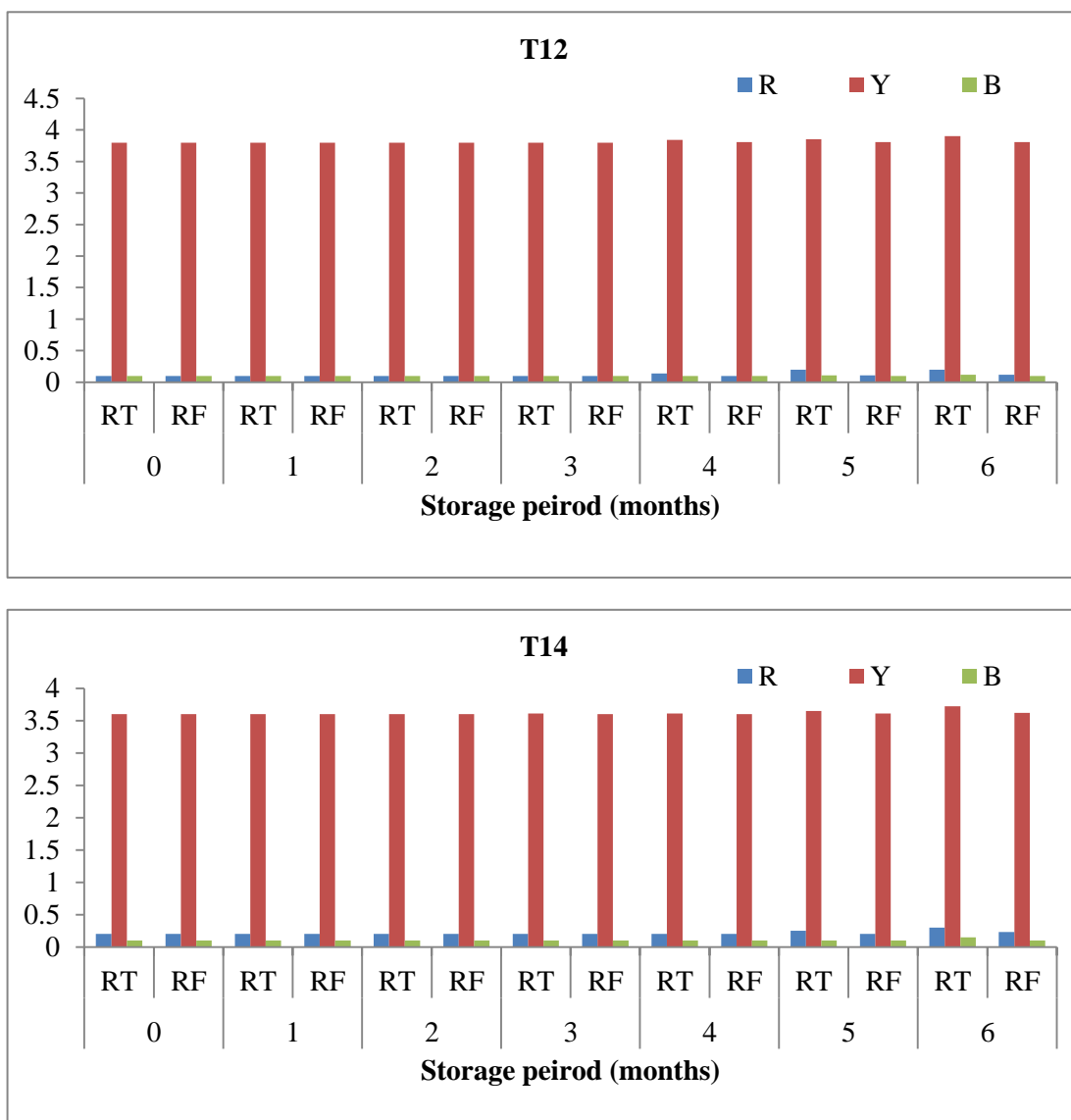


Fig. 4: Effect of storage period and storage temperature on colours (Tintometer colour units) of non carbonated sugarcane juice beverages

4.3.10 Effect of storage period and storage temperature on microbiological quality of non carbonated sugarcane juice beverages.

It was observed from Table 4.13 that the total plate count was absent in the non carbonated sugarcane juice beverages upto 5 months storage both at room and refrigerated temperatures., while the treatment T23 and T11 showed the value for total plate count (cfu/ml) of 5 and 3 after the storage of five month, while 12 and 7 after six months storage at room temperature. The treatment T12 showed 3 cfu/ml after six months storage at room temperature. The total plate count was found absent in all the treatments stored at refrigerated temperature. The total plate count in the samples of all treatments was found far below the prescribed limit of FSSAI law. The yeast and mould count was found absent in all the treatments at both the temperatures of storage. It is observed that all the treatments were

found storage stable during the storage of 6 months as the ascorbic acid content and acidity due to lemon and citrus fruit juices, also the presence of ginger juice in the beverages. The beverage also possessed stability due to the presence of prescribed limit of preservative (sodium benzoate 120ppm). The samples stored at low temperature had better stability than stored at room temperature.

Table 4.13: Effect of storage period and storage temperature on microbiological quality of non carbonated sugarcane juice beverages

Treatment	Storage period (months)													
	0		1		2		3		4		5		6	
	RT	RF	RT	RF	RT	RF	RT	RF	RT	RF	RT	RF	RT	RF
	Total plate count (cfu/ml)													
T23(Control)	-	-	-	-	-	-	-	-	-	-	5	-	12	-
T11	-	-	-	-	-	-	-	-	-	-	3	-	7	-
T12	-	-	-	-	-	-	-	-	-	-	-	-	3	-
T14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Yeast and Mould count (cfu/ml)													
T23(Control)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T11	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T12	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T14	-	-	-	-	-	-	-	-	-	-	-	-	-	-

4.4 Development of carbonated sugarcane juice beverages

4.4.1 Standardization of recipe for carbonated sugarcane juice beverages

The juices of sugarcane, kinnow, aonla, lemon and ginger were extracted using standard processes as described in materials and methods. The different blends of juices for combination treatments (TC00 to TC27) were formulated according to the combinations shown in the Table 3.2. The blends of juices were pasteurized at 82⁰C for 5min and then cooled to 15⁰C temperature. The ratio of juice volume: carbonated water used for the carbonated sugarcane juice beverages was 1:1 (v/v). The 100 ml of blended juice was filled in the each sterile bottles of 200 ml capacity for each treatment and carbonation was carried out with carbonated water at different levels of carbonation viz. 70, 80, 90 and 100psi using manually operated carbonation machine. The 15⁰C temperature was used for the carbonation of beverage with carbonated water. The sealed bottles were chilled and evaluated for the sensory qualities by semi trained panel using nine point hedonic scales. It was observed from the data depicted in Table 4.14 that the treatments TC27 (94% sugarcane juice, 3 % lemon

juice and 3 % ginger juice), TC21(84% sugarcane juice, 5 % kinnow juice, 5 % aonla juice, 3 % lemon juice and 3 % ginger juice), TC23(86% sugarcane juice, 4 % kinnow juice, 4 % aonla juice, 3 % lemon juice and 3 % ginger juice) and TC24 (82% sugarcane juice, 6 % kinnow juice, 6 % aonla juice, 3 % lemon juice and 3 % ginger juice), prepared at 80 psi carbonation level scored high for overall acceptability on sensory evaluation as compared to the other levels of carbonation (70, 90 and 100 psi). The treatment TC24 scored high values for the overall acceptability followed by TC21, TC23 and TC27. The score observed for overall acceptability of the treatments TC27 (control), TC21, TC23, TC24 were 7.66, 8, 8, 8.33, respectively. The overall acceptability score for other treatments was observed less and statistically significance difference was observed among all treatments. The sample prepared of treatment TC27 was considered as control for further study of the best treatments viz. TC21, TC23 and TC24. Kadam *et al* (2014) reported that RTS beverage prepared by using the 20 % sweet orange juice along with 0.3% acidity, 11.7 °Brix TSS and carbonated at 100 psi pressure was found significantly superior while Wagh *et al* (2014) reported that the RTS beverage prepared from Bhagwa cultivar of pomegranate by using 60° Brix sugar juice of 0.5% acidity and 80 psi CO₂ pressure in low temperature was found superior in organoleptic characteristics. Mehtre *et al* (2012) reported best results at 15 % pomegranate juice, 12 % sugar, 0.3 per cent acidity and 80 psi carbonation level..

4.4.2 Sensory evaluation of carbonated sugarcane juice beverages

The samples of treatments (TC00 to TC27) of carbonated sugarcane juice beverages were prepared at 80 psi carbonation level, chilled and tested for all parameters of sensory quality viz. appearance, mouth feel, aroma, taste, consistency and overall acceptability which depicted in Table 4.14 and 4.15. It was observed that the treatment TC24 was found superior over all the treatments for all the parameters of sensory qualities followed by TC21, TC23 and TC27. The sensory score for treatment TC24 for appearance, mouth feel, aroma, taste, consistency and overall acceptability was 8.33, 8.33, 8, 8.16, 7.83 and 8.33, respectively. There was a significant difference in sensory score among the other treatments. The treatments TC21, TC23 and TC27 were found superior on sensory evaluation of the products for the appearance, mouth feel, aroma, taste, consistency and overall acceptability scored 8., 8.16, 7.83, 8.16, 7.83, 8; 7.83, 7.83, 7.83, 7.83, 7.66, 8; 7.33, 7.67, 7.6, 7.4, 7.16, 7.66, respectively. The sample TC27 was considered as a control sample for the further study of carbonated sugarcane juice beverages blended with the juices of kinnow, aonla, lemon and ginger. The treatments TC21, TC23 and TC24 were continued for the further study along with TC27 as a control.

Table 4.14: Sensory evaluation (overall acceptability) for optimization of carbonation level and standardization of recipe for the carbonated sugarcane juice beverages

Treatment	Carbonation levels (psi)			
	70	80	90	100
TC00	7.12	7.66	7.63	7.62
TC11	6.82	6.83	6.80	6.70
TC12	6.10	6.33	6.30	6.12
TC13	7.30	7.33	7.30	7.12
TC14	6.80	6.83	6.80	6.70
TC15	7.23	7.33	7.31	7.28
TC16	7.21	7.33	7.31	7.27
TC17	7.20	7.33	7.31	7.29
TC18	7.23	7.40	7.20	7.19
TC19	6.60	6.66	6.60	6.55
TC20	7.00	7.33	7.32	7.29
TC21	7.80	8.00	7.78	7.70
TC22	7.00	7.16	7.00	6.90
TC23	7.80	8.00	7.88	7.60
TC24	8.10	8.33	7.90	7.70
TC25	6.80	6.83	6.80	6.50
TC26	6.00	6.33	6.30	6.10
TC27	7.13	7.66	7.55	7.14
CD at 5%	A-Treatment = 0.06, B-Carbonation levels = 0.03, A×B = 0.11			

Table 4.15: Sensory evaluation of carbonated sugarcane juice beverages at 80 psi carbonation level

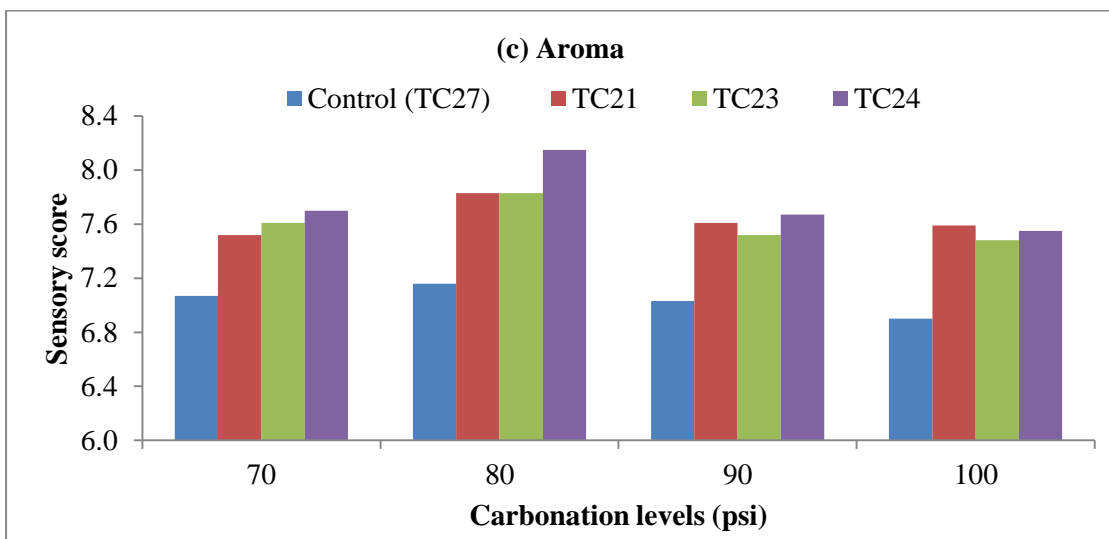
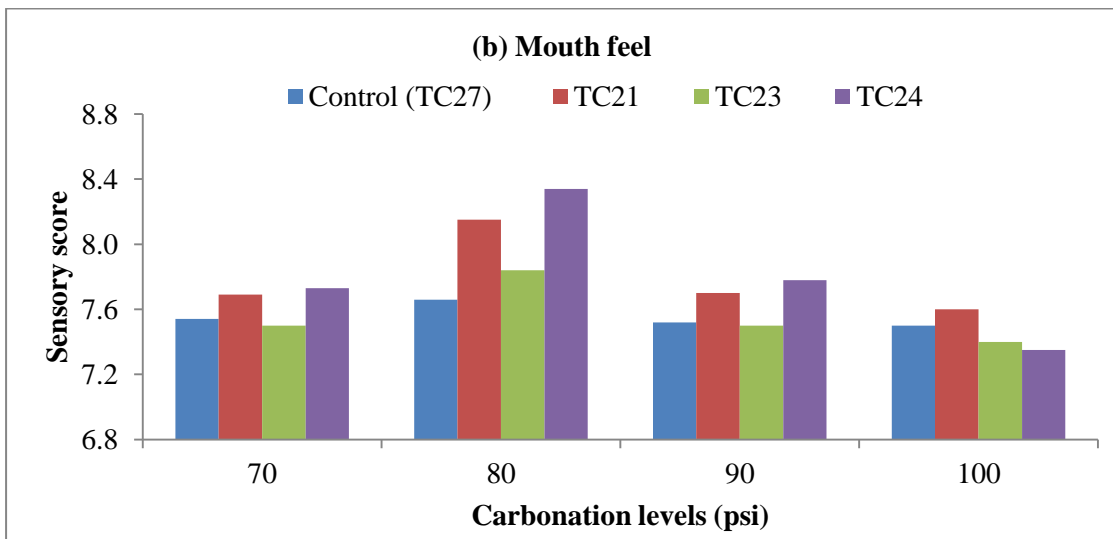
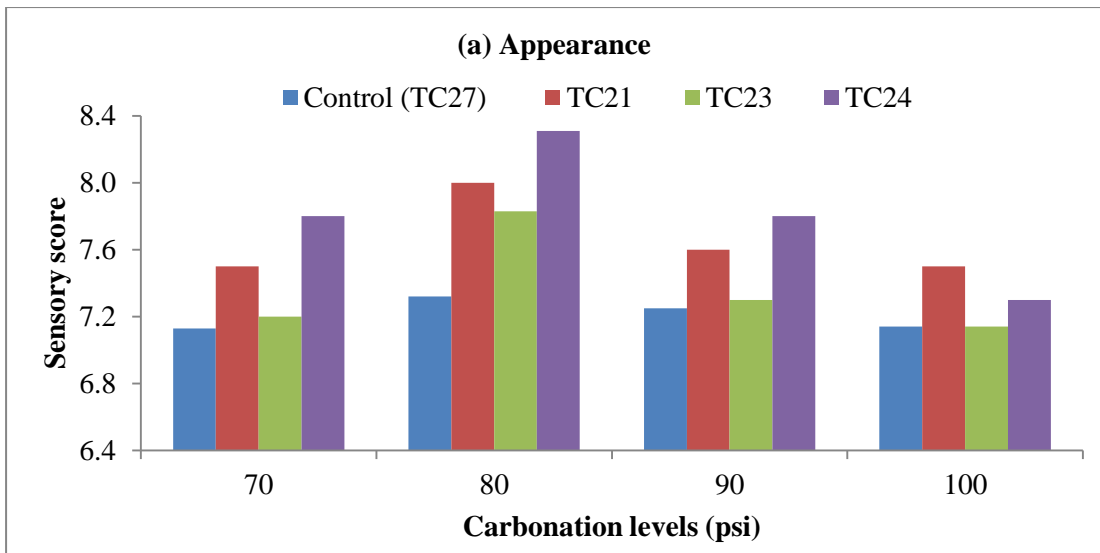
Treatment	Appearance	Mouth feel	Aroma	Taste	Consistency	Overall acceptability
TC00	7.66	7.66	7.50	7.56	7.66	7.66
TC11	7.50	7.16	7.66	6.83	7.50	6.83
TC12	7.50	7.50	7.66	6.33	6.66	6.33
TC13	7.16	7.00	7.33	7.50	7.33	7.33
TC14	7.33	6.83	6.83	7.16	7.16	6.83
TC15	7.16	7.16	6.83	7.16	7.16	7.33
TC16	7.50	6.83	7.53	7.16	7.53	7.33
TC17	7.33	7.00	7.43	7.33	7.60	7.33
TC18	7.33	7.16	7.50	7.16	7.53	7.40
TC19	7.33	6.33	7.16	6.66	7.33	6.66
TC20	7.33	7.33	7.43	7.16	7.16	7.33
TC21	8.00	8.16	7.83	8.16	7.83	8.00
TC22	6.50	6.33	6.66	7.00	7.16	7.16
TC23	7.83	7.83	7.83	7.83	7.66	8.00
TC24	8.33	8.33	8.00	8.16	7.83	8.33
TC25	7.66	7.00	7.16	6.66	7.33	6.83
TC26	6.66	6.33	6.50	6.33	6.66	6.33
TC27	7.33	7.67	7.60	7.40	7.16	7.66
CD at 5%	0.56	0.72	0.51	0.50	0.50	0.56

4.4.3. Effect of carbonation levels on sensory qualities of carbonated sugarcane juice Beverages

It was observed from Fig. 5 that, the beverages TC27, TC21, TC23 and TC24 formulated at 80 psi carbonation level showed higher sensory score on 9 point Hedonic scale for all attributes viz. appearance, mouth feel, aroma, taste, consistency and overall acceptability of sensory quality over 70, 90 and 100 psi levels of carbonation. Statistically significant differences were observed for the sensory score of all the treatments of carbonation levels. The treatment TC24 and TC21 were found superior for the mouth feel and taste than other treatments but all carbonated beverages were found acceptable on sensory quality evaluation by semi trained panel members.



Plate 2: Carbonated sugarcane juice beverages



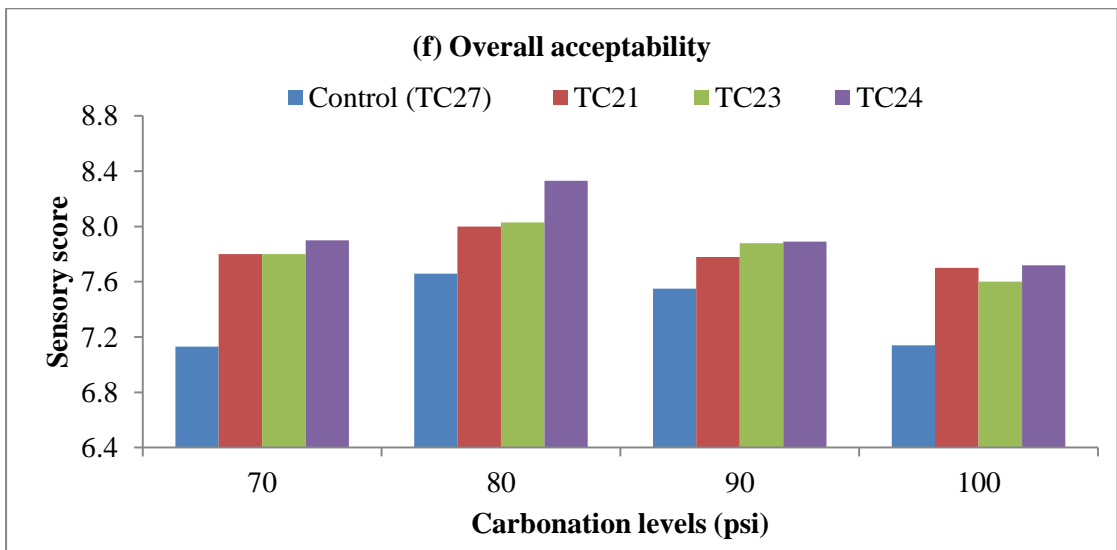
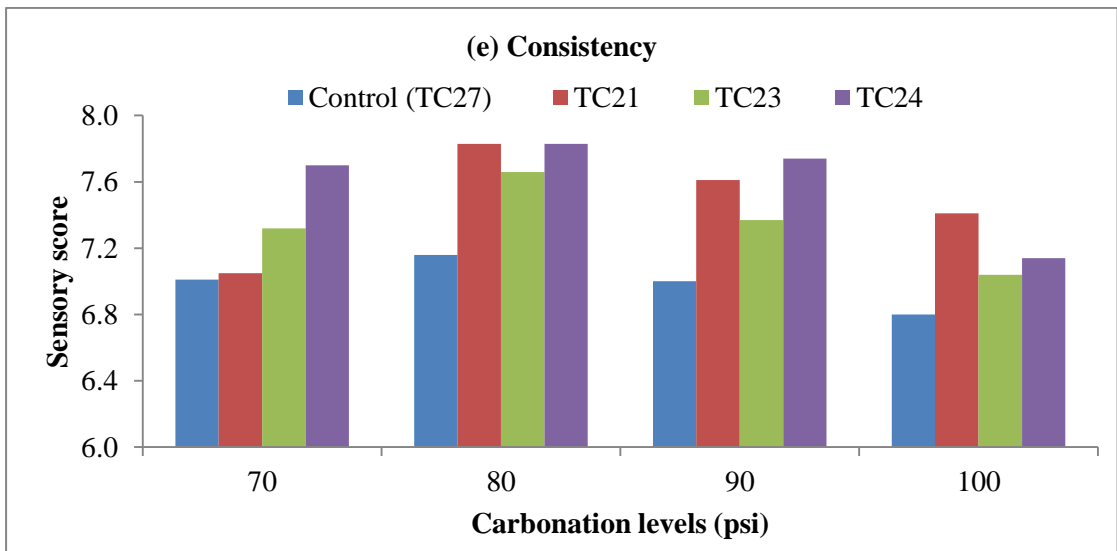
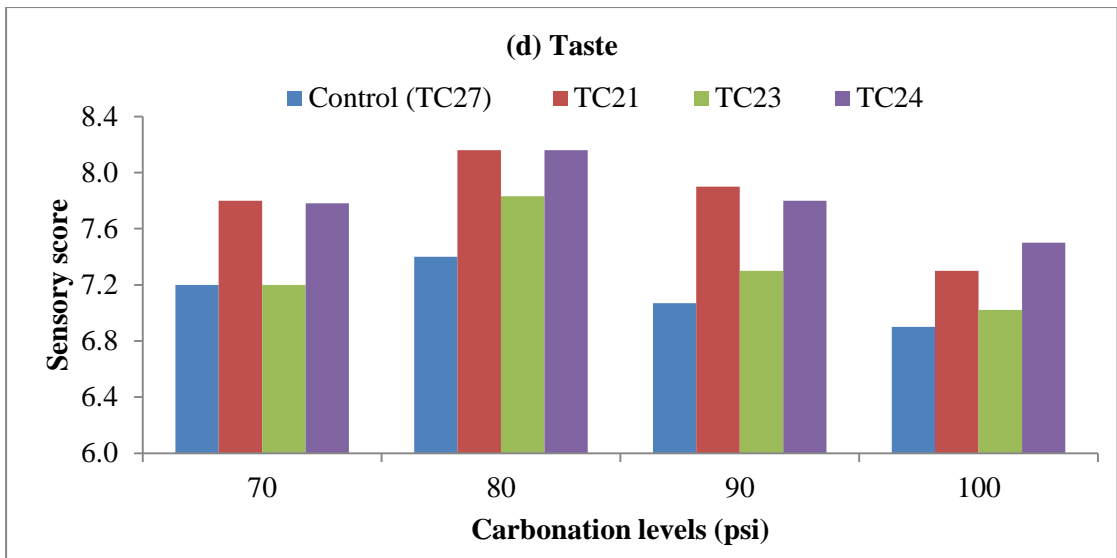


Fig. 5: Effect of carbonation levels on sensory qualities of carbonated sugarcane juice beverages

4.4.4 Physicochemical characteristics of carbonated sugarcane juice beverages

The samples of carbonated sugarcane juice beverages of treatments TC27 (control), TC21, TC23 and TC24 were chosen for further study, prepared in bulk and further studied for its physicochemical characteristics, storage stability and microbial quality. The bottled samples of carbonated sugarcane juice beverages were stored at room ($30\pm 4^{\circ}\text{C}$) and refrigeration (4°C) temperature. The carbonated sugarcane juice beverages TC27 (control), TC21, TC23 and TC24 were analyzed for physico-chemical characteristics as depicted in Table 4.16 showed that total soluble solids were found higher (14.6°B) in control sample than TC21, TC23 and TC24. The per cent values for ash content, acidity, total sugars and reducing sugars of carbonated sugarcane juice beverages TC27 (control), TC21, TC23 and TC24 were 0.13, 0.33, 11.73, 0.3; 0.13, 0.34, 11.7, 0.3; 0.11, 0.34, 11.72, 0.28 and 0.13, 0.38, 11.6, 0.31, respectively.

Table 4.16: Physicochemical and phytochemical constituents of carbonated sugarcane juice beverages (80psi carbonation).

Parameters	Carbonated beverages				CD at 5%
	Control (TC27)	TC21	TC23	TC24	
Moisture content (%)	85.39	86.76	86.72	86.79	0.01
Total solids (%)	14.60	13.23	13.28	13.21	0.06
TSS($^{\circ}\text{Brix}$)	12.74	12.73	12.76	12.66	NS
Ash (%)	0.13	0.13	0.11	0.13	0.01
Titrateable acidity(%)	0.33	0.34	.034	0.38	0.01
pH	3.56	3.53	3.54	3.62	0.03
Ascorbic acid (mg/100ml)	2.17	8.80	7.40	9.72	0.13
Antioxidant activity (% inhibition)	32.30	74.10	73.20	74.00	0.41
Total polyphenols (mg GAE/100ml)	247.20	291.40	287.30	300.10	0.11
Total flavonoids (mg QE/100ml)	3.60	7.80	7.10	8.72	0.06
Total sugars (%)	11.73	11.70	11.72	11.60	0.05
Reducing sugars (%)	0.30	0.30	0.28	0.31	0.01
Viscosity (cp)	4.03	3.85	4.01	3.16	0.43

Also the values for the ascorbic acid (mg/100ml), antioxidant activity (% inhibition), total polyphenols (mg GAE/100ml and total flavonoids (mgQE/100ml) for respective beverages were observed as 2.17, 32.3, 247.2, 3.6; 8.8, 74.1, 291.4, 7.8; 7.4, 73.2, 287.3, 7.1;

9.72, 74, 300.1, 8.72, respectively. The viscosities (cp) of the treatments were ranged between 3.16 to 4.03 showed significant differences. The mineral content values (mg/100ml) for the carbonated sugarcane juice beverage samples viz. TC27, TC21, TC23 and TC24 were found at par and showed good amount of minerals in the beverages as depicted in Table 4.17. The mineral content values (mg/100ml) were found in the range as calcium (10.35-12.45), iron (1.39-1.43), potassium (36.7 - 42.55), sodium (2.16 - 2.44), phosphorus (14.90 - 16.26), sulphur (20.87-23.3), zinc (0.31- 0.37), magnesium (4.37- 5.62), copper (0.13 - 0.18) and nickel (0.01). Mehtre *et al* (2012) reported that that carbonation at various pressures had no influence on physicochemical properties of beverage.

Table 4.17: Mineral content of the of carbonated sugarcane juice beverages (mg/100ml).

Mineral	Carbonated beverages				CD at 5%
	Control (TC27)	TC21	TC23	TC24	
Calcium	12.45	10.35	12.43	11.96	0.01
Iron	1.43	1.42	1.39	1.39	0.09
Potassium	36.70	42.55	39.37	39.10	0.10
Sodium	2.38	2.28	2.16	2.44	0.04
Magnesium	5.62	5.02	4.92	4.57	0.01
Copper	0.13	0.13	0.14	0.18	0.01
Phosphorous	15.50	15.32	16.26	14.90	0.02
Manganese	0.35	0.35	0.34	0.35	0.01
Zinc	0.34	0.33	0.31	0.37	0.01
Sulphur	23.30	21.37	21.33	20.87	0.07
Nickel	0.01	0.01	0.01	0.01	NS

4.4.5 Effect of blending on phytochemicals of carbonated sugarcane juice beverages

The effect of blending of sugarcane juice with fruit juices depicted in Fig. 6 showed that there was increase in ascorbic acid (2 times), antioxidant activity was approximately doubled, total phenolics were decreased by 40 per cent, while total flavonoids increased to 3 times than the sugarcane juice, even though the juice base for the carbonated beverages used was 1:1 (juice: carbonated water). The blend TC24 was found superior for the content of phytochemicals followed by TC21 and TC23. The carbonated beverages contained 41 to 43 per cent sugarcane juice and 7 to 9 per cent other juices. Thus these carbonated sugarcane juice beverages prepared from sugarcane juice by blending with fruit juices showed the significant increase in the phytochemical content in blended beverages.

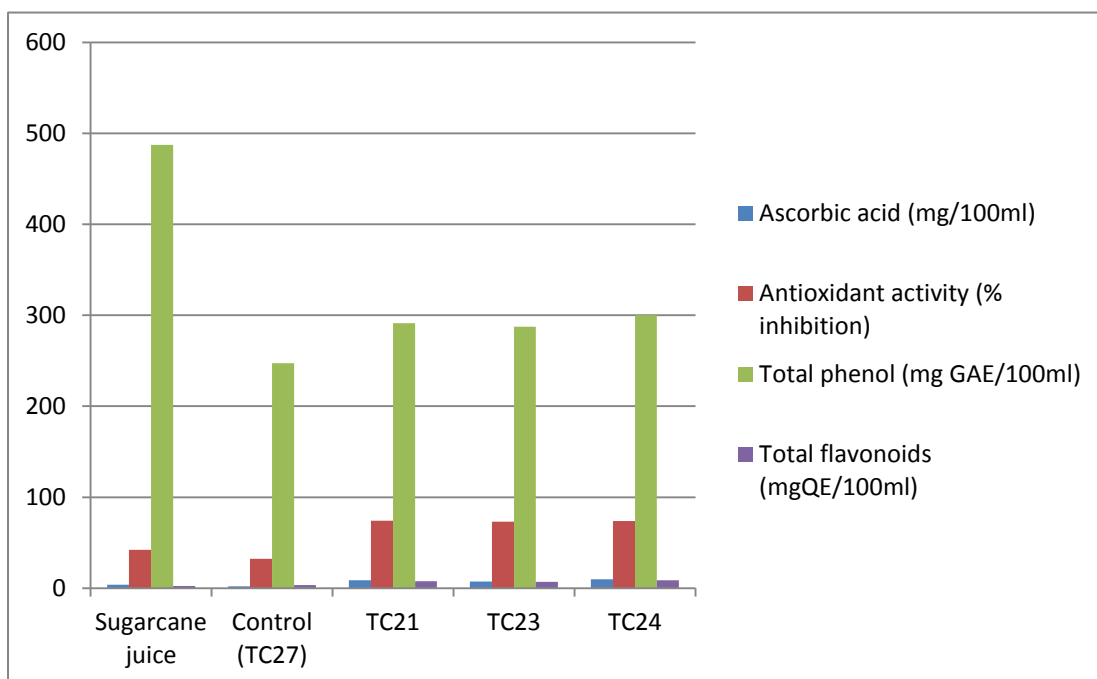
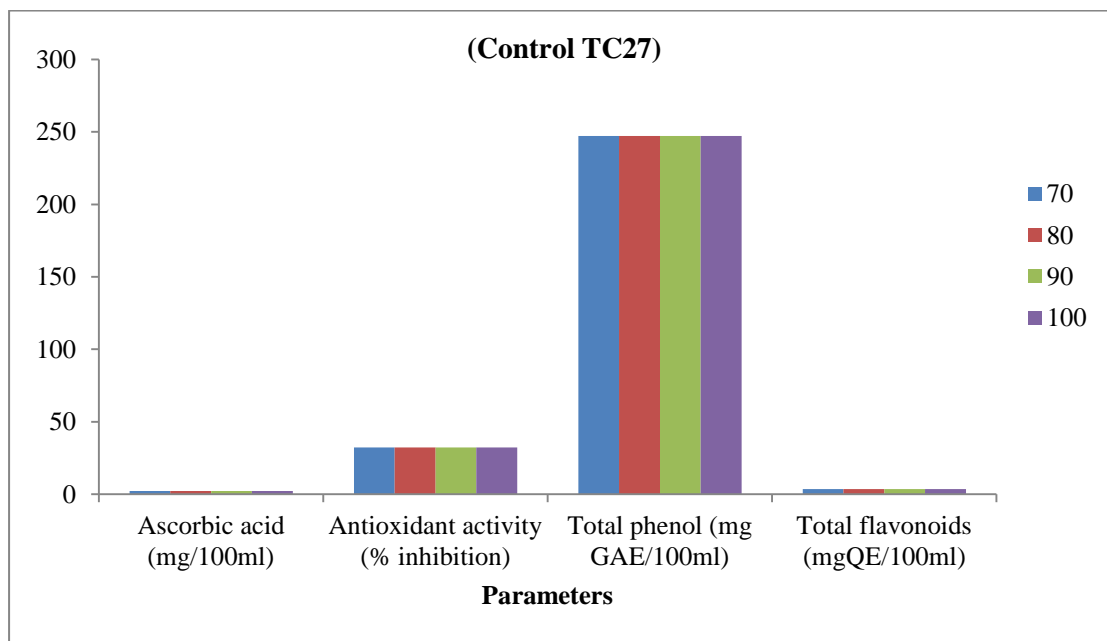


Fig. 6: Effect of blending on phytochemicals of carbonated sugarcane juice beverages

4.4.6 Effect of carbonation levels on phytochemicals of carbonated sugarcane juice beverages

The Fig. 7 showed that there is no significant effect of carbonation levels on the phytochemicals of all samples of carbonated sugarcane juice beverages. The level for the carbonation of beverages were used on the basis of sensory evaluation as depicted previously.



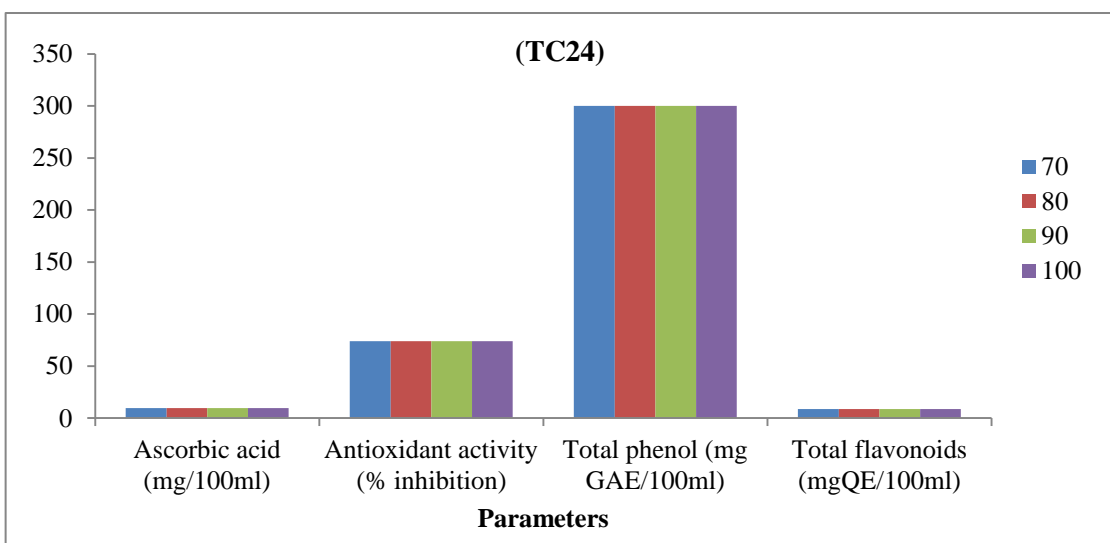
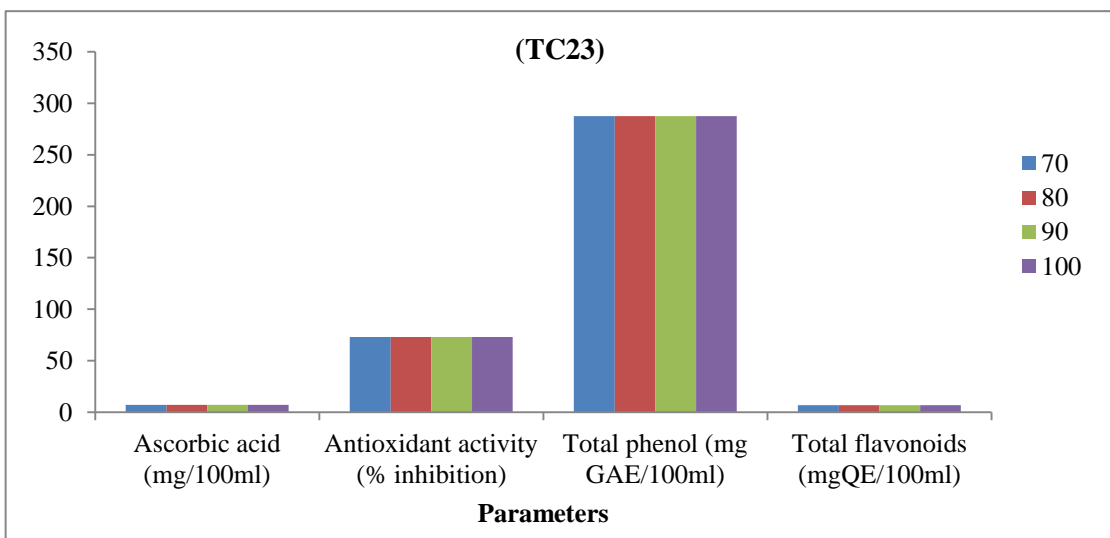
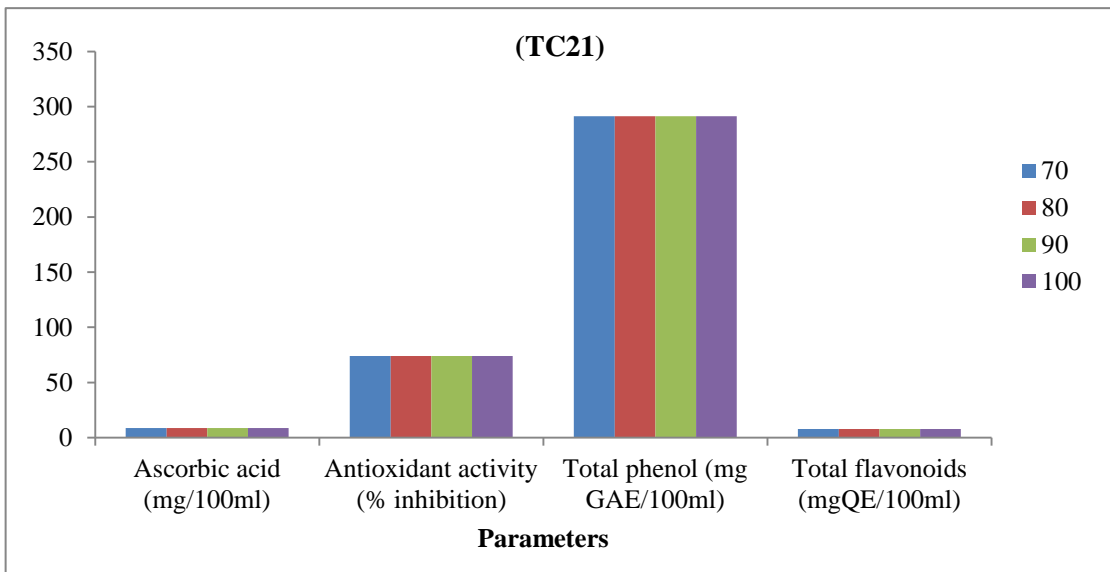
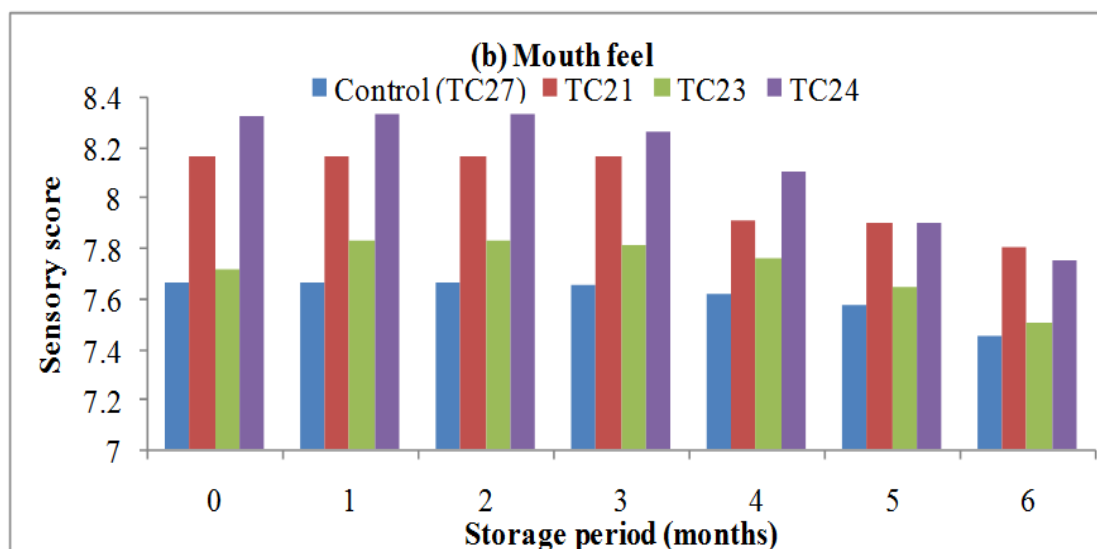
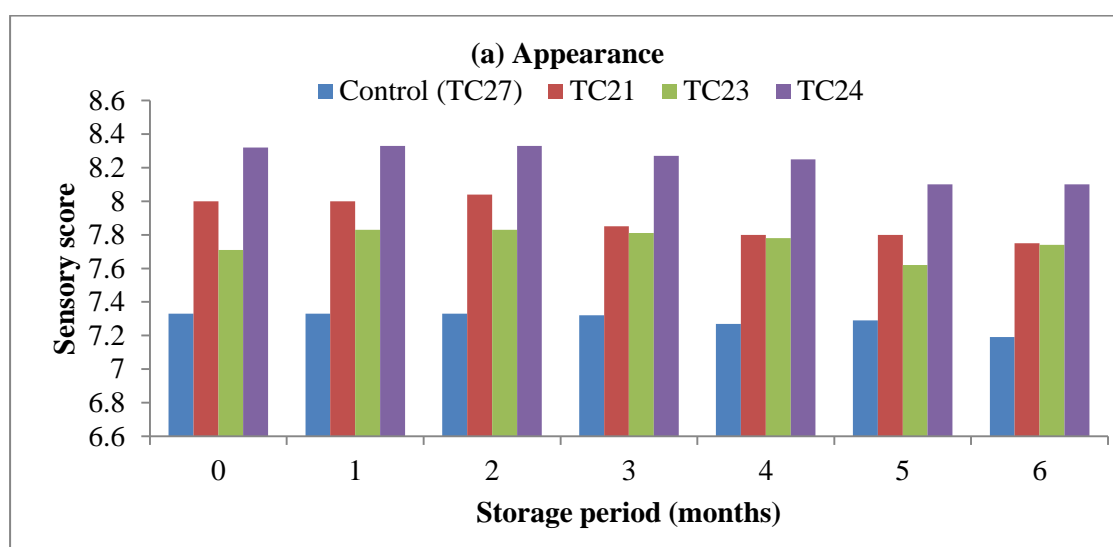
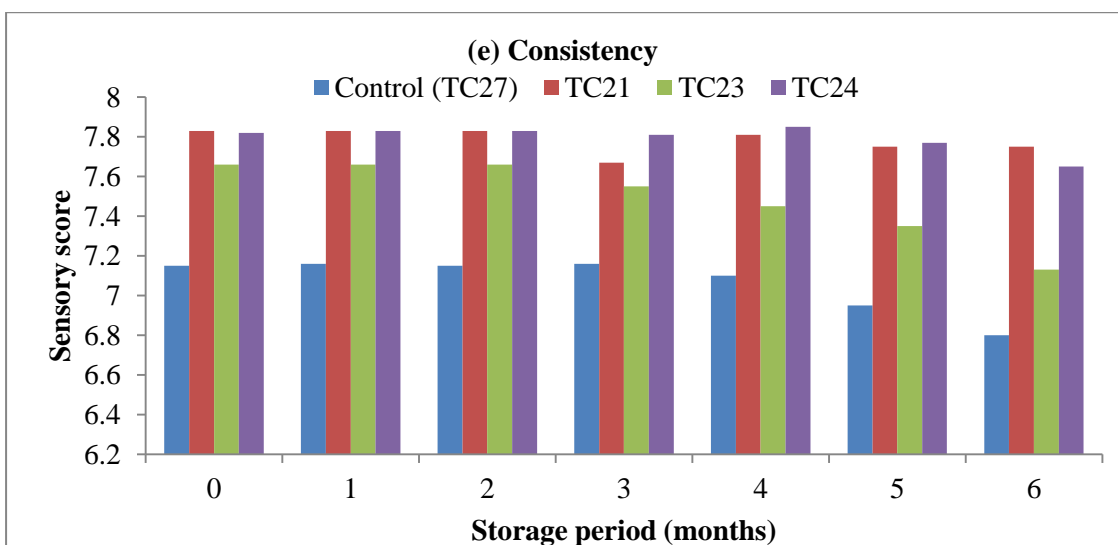
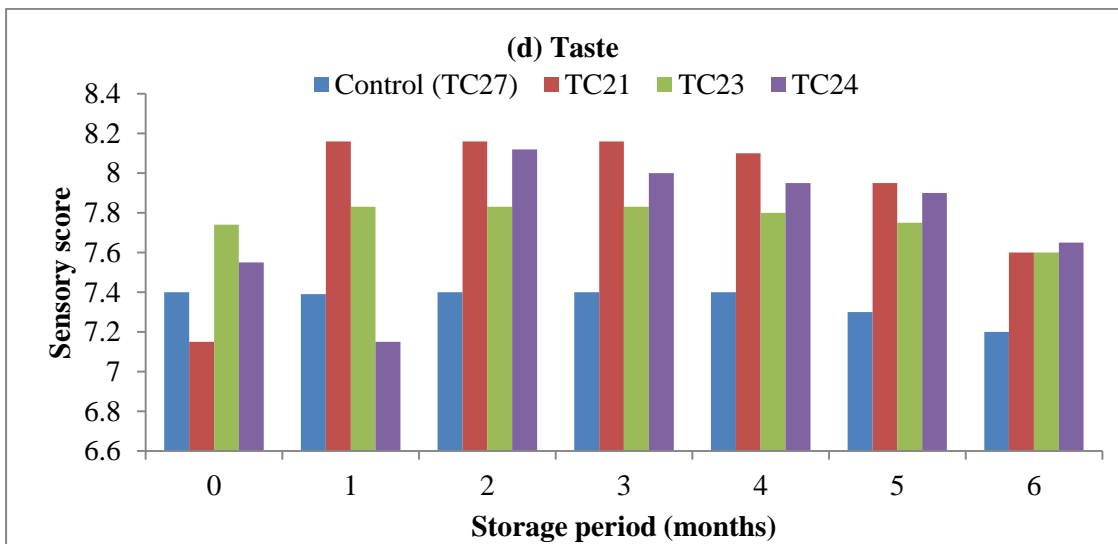
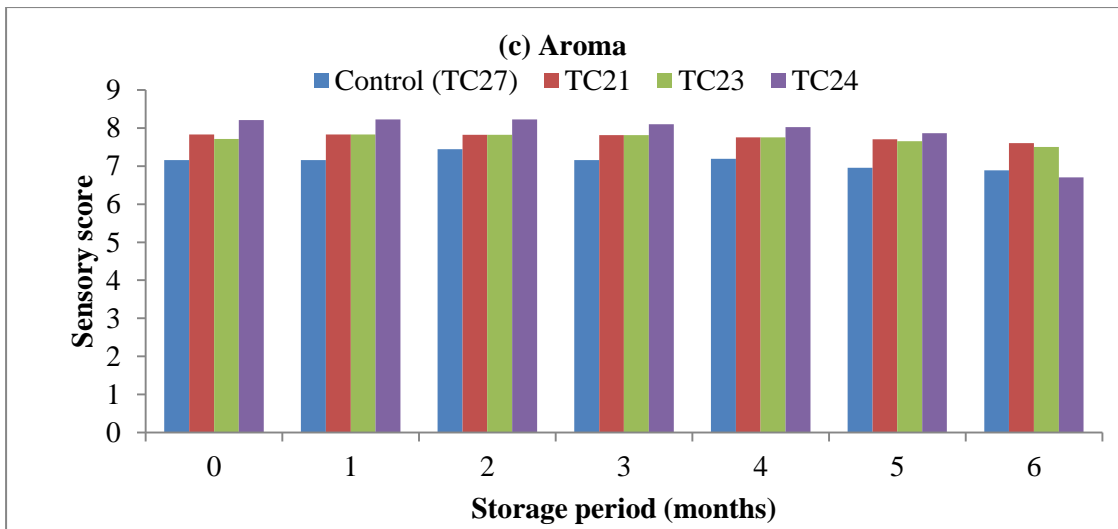


Fig. 7: Effect of carbonation levels on phytochemicals of carbonated sugarcane juice beverages

4.4.8 Effect of storage period on sensory qualities of carbonated sugarcane juice beverages

The effect of storage period on sensory qualities of carbonated sugarcane juice beverages (TC27 (control), TC21, TC23 and TC24) were studied for the 6 months at room ($30\pm 4^{\circ}\text{C}$) and refrigeration (4°C) temperature and the periodic observations were noted after each month. The results depicted in Fig. 8 showed that all the samples of carbonated sugarcane juice beverages were found acceptable with slight decreased sensory score by semi trained panel during the storage of 6 months for all parameters viz. appearance, mouth feel, aroma, taste, consistency and overall acceptability though the beverages were found stable during the storage period.





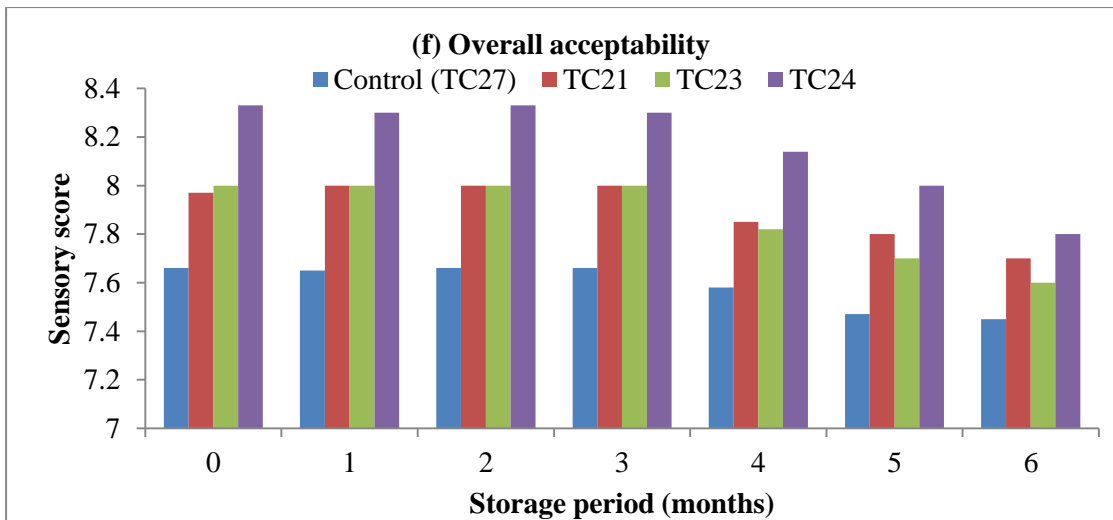
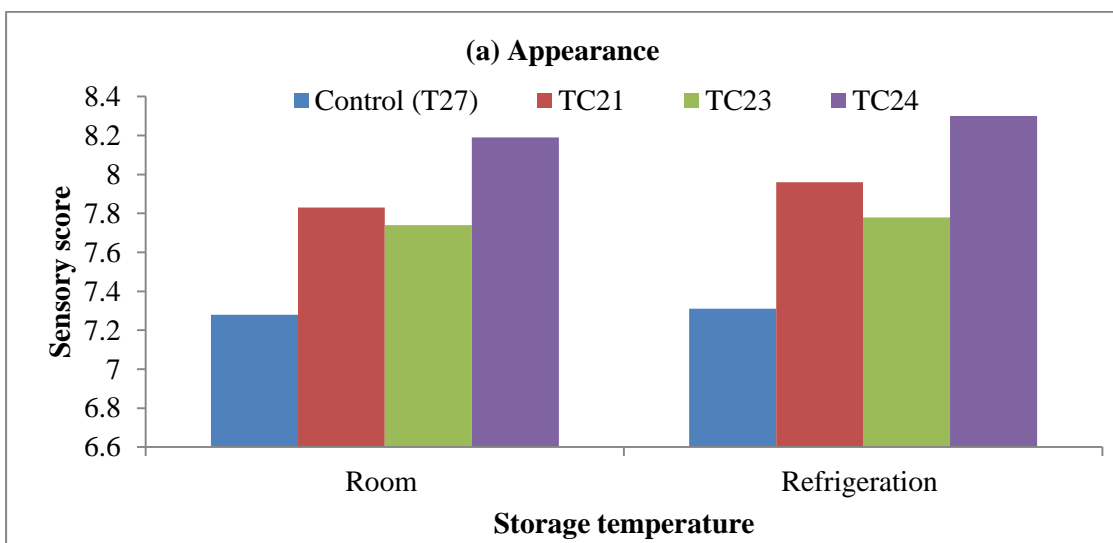
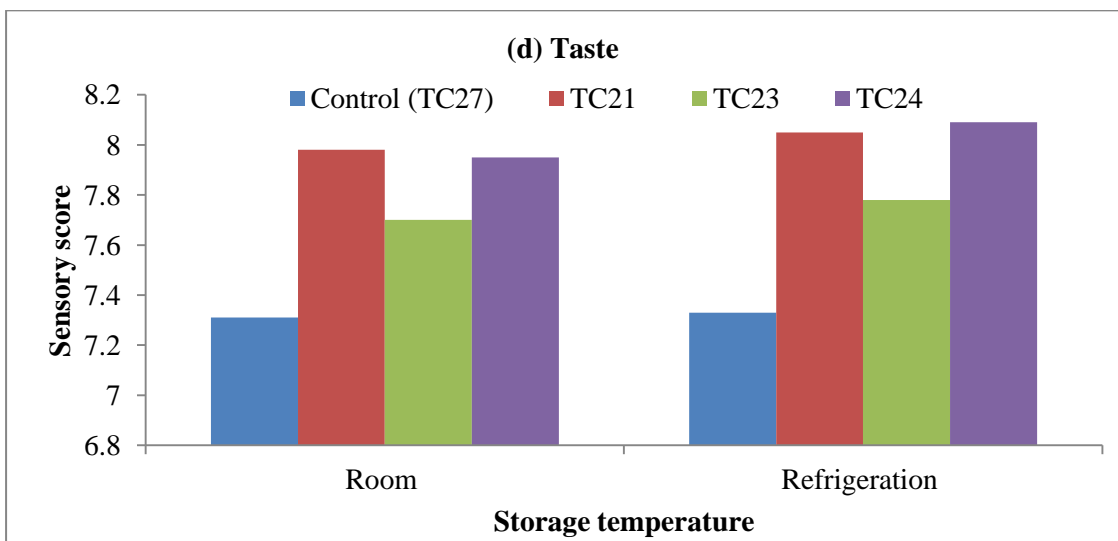
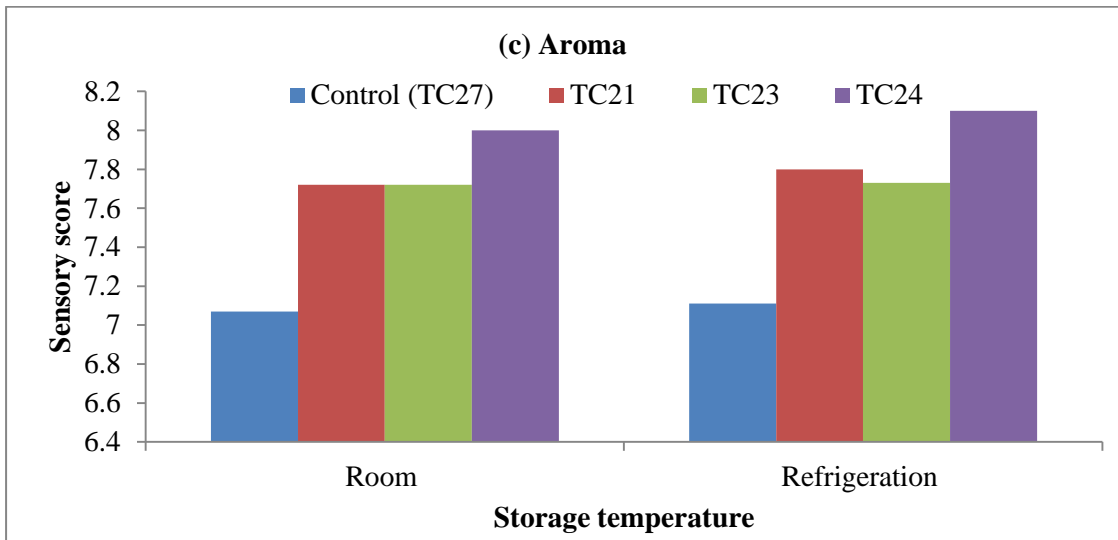
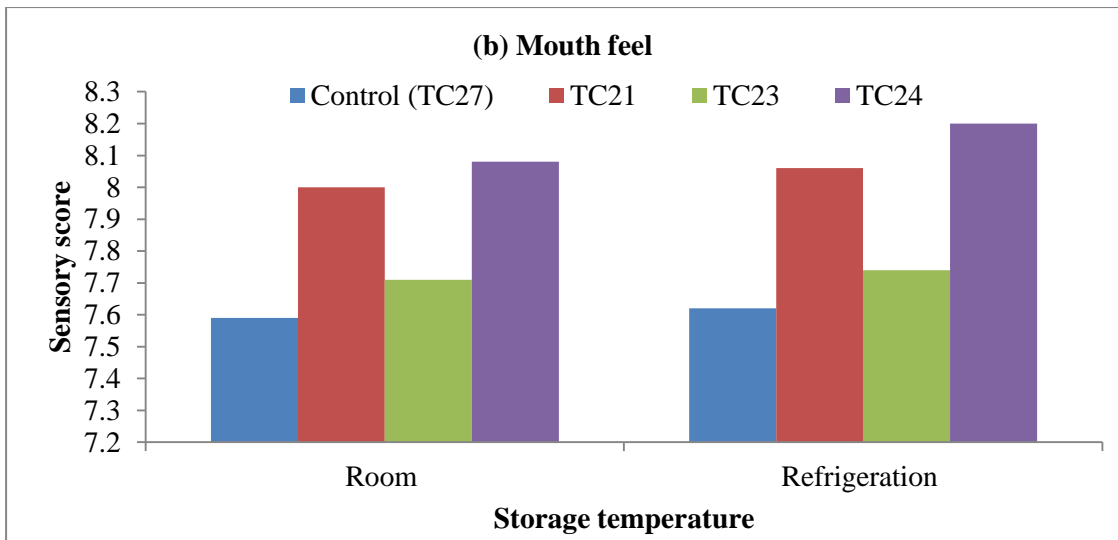


Fig 8: Effect of storage period on sensory qualities of carbonated sugarcane juice beverages

4.4.9 Effect of storage temperature on sensory qualities of carbonated sugarcane juice beverages

The effect of storage temperature both at room ($30\pm 4^{\circ}\text{C}$) and refrigeration (4°C) on sensory qualities of carbonated sugarcane juice beverages depicted in Fig. 9 showed that the beverages stored at refrigerated temperature scored slightly high for all the sensory parameters than the stored at room temperature. The carbonated sugarcane juice beverages were found slightly superior in quality at refrigerated storage for 6 months over the storage at room temperature but both beverages were scored well on sensory evaluation.





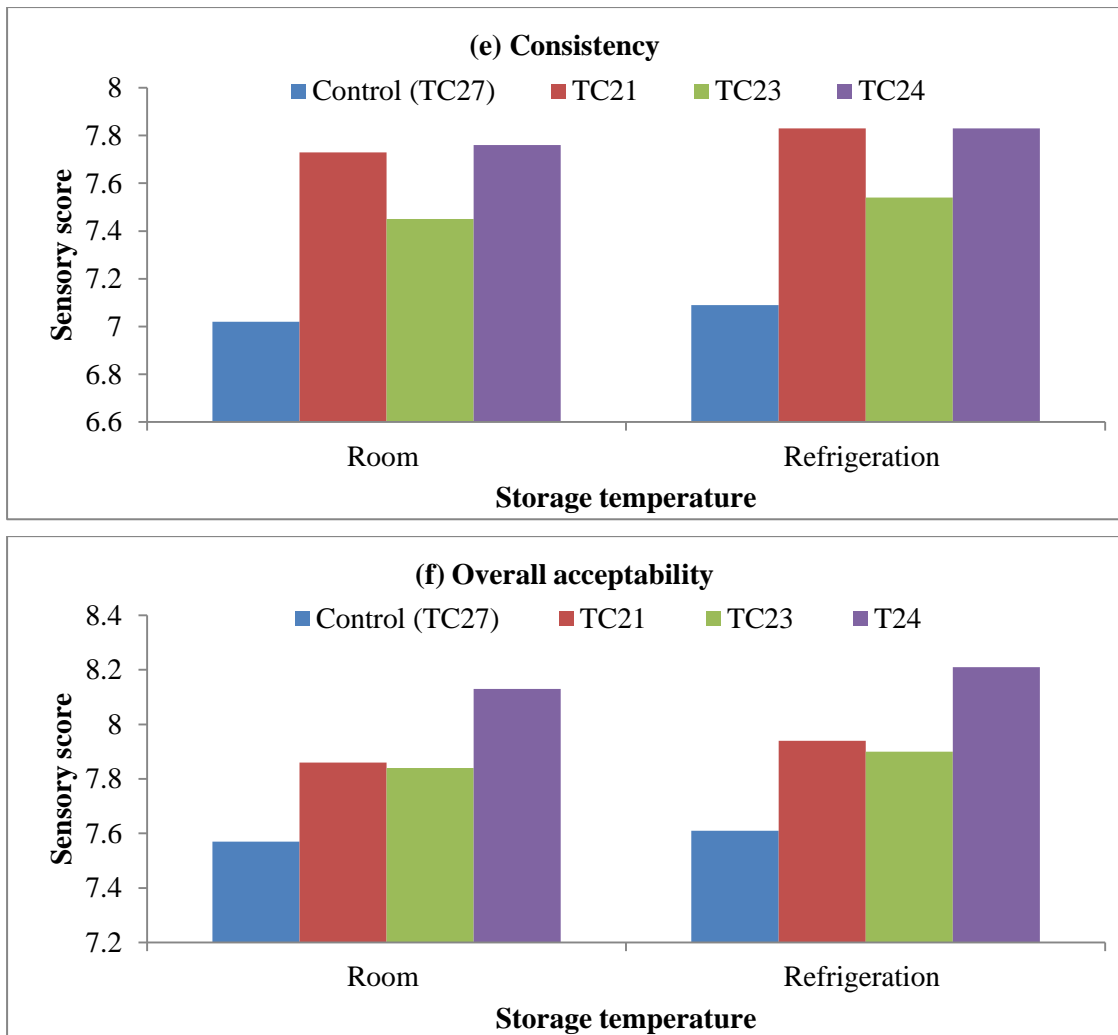


Fig 9: Effect of storage temperature on sensory qualities of carbonated (80psi) sugarcane juice beverages (6 months storage)

4.4.10 Effect of storage period on physicochemical and phytochemical constituents of carbonated sugarcane juice beverages

The effect of storage period on physicochemical characteristics of carbonated sugarcane juice beverages ((TC27, TC21, TC23 and TC24)) during 6 months storage at room ($30\pm 4^{\circ}\text{C}$) and refrigeration (4°C) temperature is noted in Table 4.18. The observations showed that there was slight decrease in values of total soluble solids (1.27%), pH (2.73%), ascorbic acid (5.11%), total sugars (2.11%) and viscosity (1.85%) for all the beverages after 3 months during storage of 6 months. The values for the phytochemical viz. total polyphenol and total flavonoids were decreased by 20 per cent while the antioxidant activity was decreased by 25 per cent during the storage of six months. The same trend has been reported by Karpagavalli and Amutha (2015). The values for titratable acidity and reducing sugars were increased 18% and 14.9%, respectively during the storage of 6 months; however the beverages showed stability till 3 months while further storage slightly decreased the values. The products were found acceptable after six months storage.

Table 4.18: Effect of storage period on physicochemical and phytochemical constituents of carbonated (80 psi) sugarcane juice beverages

Treatment	Storage period (months)						
	0	1	2	3	4	5	6
	Total soluble solids (⁰ B)						
Control (TC27)	12.74	12.74	12.74	12.73	12.71	12.65	12.56
TC21	12.73	12.73	12.73	12.73	12.65	12.63	12.56
TC23	12.76	12.76	12.76	12.15	12.72	12.65	12.57
TC24	12.66	12.66	12.66	12.61	12.55	12.55	12.55
CD at 5%	NS						
	Titratable acidity (%)						
Control (TC27)	0.33	0.33	0.33	0.33	0.36	0.39	0.44
TC21	0.34	0.34	0.34	0.34	0.39	0.41	0.43
TC23	0.34	0.34	0.34	0.34	0.39	0.41	0.43
TC24	0.38	0.38	0.38	0.38	0.41	0.42	0.45
CD at 5%	0.01						
	pH						
Control (TC27)	3.56	3.56	3.55	3.55	3.53	3.51	3.47
TC21	3.53	3.53	3.53	3.53	3.51	3.50	3.42
TC23	3.54	3.54	3.54	3.53	3.50	3.47	3.43
TC24	3.62	3.62	3.62	3.62	3.60	3.59	3.54
CD at 5%	NS						
	Ascorbic acid (mg/100ml)						
Control (TC27)	2.17	2.17	2.17	2.16	2.16	2.15	2.14
TC21	8.80	8.80	8.80	8.80	8.75	8.70	8.67
TC23	7.40	7.40	7.40	7.40	7.37	7.36	7.34
TC24	9.80	9.80	9.80	9.79	9.75	9.73	9.71
CD at 5%	0.01						
	Total polypolyphenols (mgGAE/100ml)						
Control (TC27)	247.20	240.00	233.30	225.50	218.40	209.60	200.50
TC21	291.40	285.50	280.45	269.84	260.42	249.30	240.65
TC23	287.30	280.33	277.65	265.44	258.32	247.45	238.74
TC24	300.20	293.45	286.35	278.50	267.55	254.36	242.68
CD at 5%	0.11						

Treatment	Storage period (months)						
	0	1	2	3	4	5	6
	Total flavonoids (mg QE/100ml)						
Control (TC27)	3.60	3.41	3.20	3.00	2.94	2.92	2.90
TC21	7.80	7.61	7.43	7.22	6.89	6.62	6.32
TC23	7.10	7.00	6.91	6.81	6.72	6.65	6.60
TC24	8.72	8.65	8.52	8.45	8.36	8.25	7.12
CD at 5%	0.06						
	Antioxidant activity (% inhibition of DPPH)						
Control (TC27)	32.30	31.20	29.10	28.30	26.2	25.42	24.30
TC21	74.10	72.5	70.25	67.65	64.35	60.32	56.80
TC23	73.20	70.32	67.35	64.55	61.25	57.77	54.30
TC24	74.00	71.65	69.24	66.20	62.63	58.36	55.79
CD at 5%	0.41						
	Total sugars (%)						
Control (TC27)	11.73	11.73	11.73	11.70	11.67	11.63	11.53
TC21	11.70	11.70	11.70	11.67	11.57	11.53	11.46
TC23	11.72	11.72	11.72	11.70	11.65	11.56	11.49
TC24	11.60	11.60	11.60	11.60	11.50	11.46	11.35
CD at 5%	NS						
	Reducing sugars (%)						
Control (TC27)	0.39	0.39	0.39	0.39	0.42	0.45	0.49
TC21	0.30	0.30	0.30	0.30	0.31	0.32	0.33
TC23	0.28	0.28	0.28	0.28	0.29	0.30	0.31
TC24	0.30	0.30	0.30	0.30	0.31	0.32	0.33
CD at 5%	0.01						
	Viscosity(cp)						
Control (TC27)	4.04	4.04	4.04	4.04	4.01	3.96	3.93
TC21	3.85	3.85	3.85	3.84	3.83	3.82	3.82
TC23	4.01	4.01	4.00	3.98	3.96	3.93	3.91
TC24	3.16	3.16	3.16	3.15	3.14	3.13	3.12
CD at 5%	0.01						

4.4.11 Effect of storage temperature on physicochemical and phytochemical constituents of carbonated sugarcane juice beverages

The effect of storage temperature on physico-chemical characteristics of carbonated sugarcane juice beverages (TC27, TC21, TC23 and TC24) during 6 months storage at room ($30\pm 4^{\circ}\text{C}$) and refrigeration (4°C) temperature is noted in Table 4.19. It was observed that, the all treatments (TC27, TC21, TC23 and TC24) beverages stored at refrigerated temperature depicted slightly high values for all physico-chemical parameters than the samples stored at room temperature. The values for the phytochemical viz. total polyphenol showed 20 % loss of polyphenols at room temperature while 18 per cent loss was observed at refrigeration storage for six months. The total flavonoids were decreased by 20 per cent both at room and refrigerated storage while, while the antioxidant activity was decreased by 25 per cent at room temperature and 20 per cent at refrigerated storage of six months. The same trend has been reported by Karpagavalli and Amutha (2015). The carbonated sugarcane juice beverages were found slightly superior in quality those stored at refrigerated storage for 6 months over the storage at room temperature but both beverages showed non significant changes in the values of all parameters.

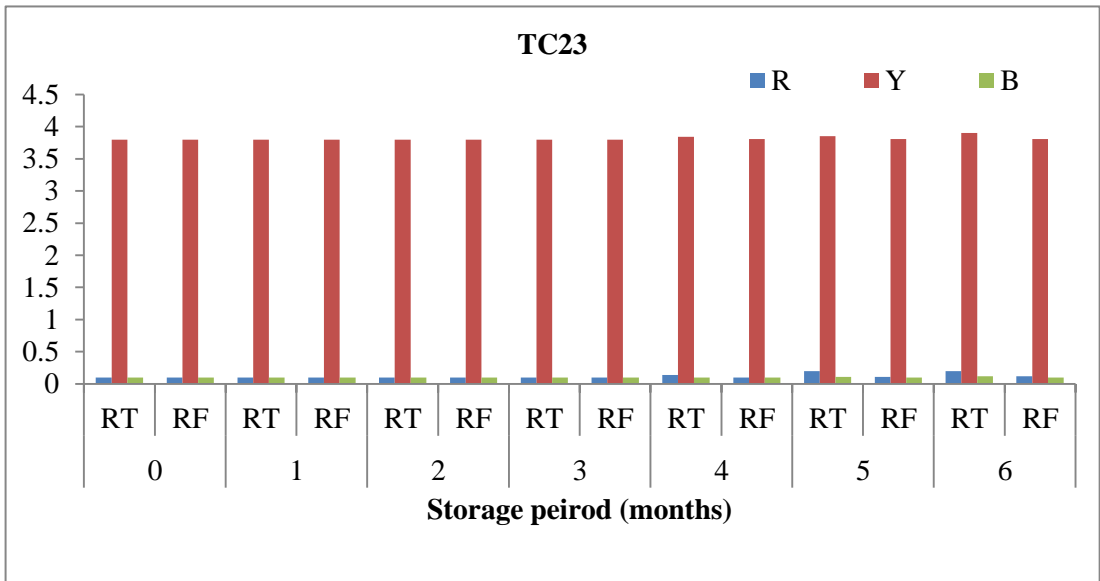
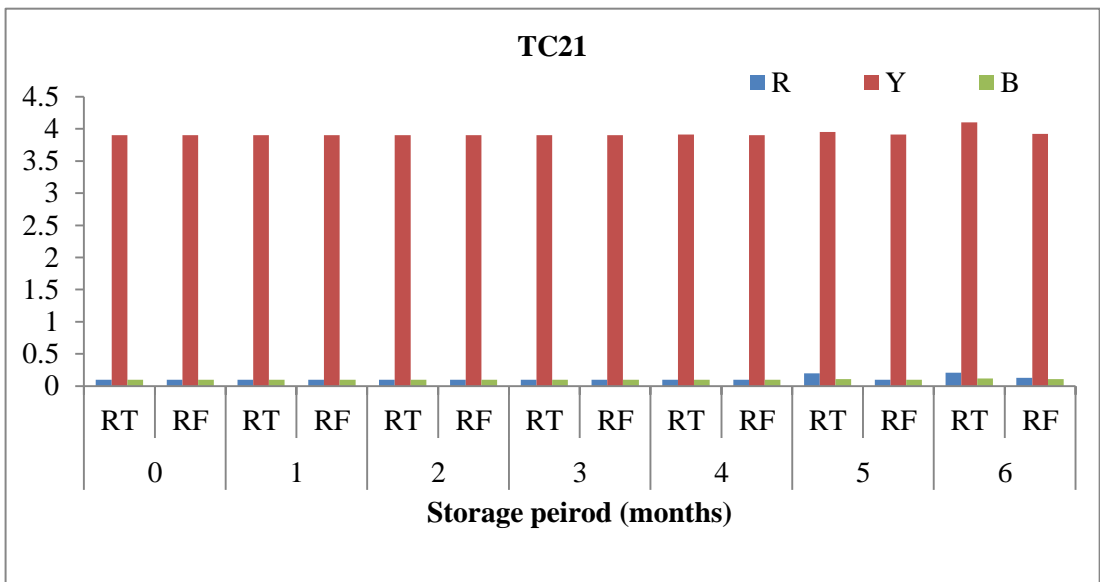
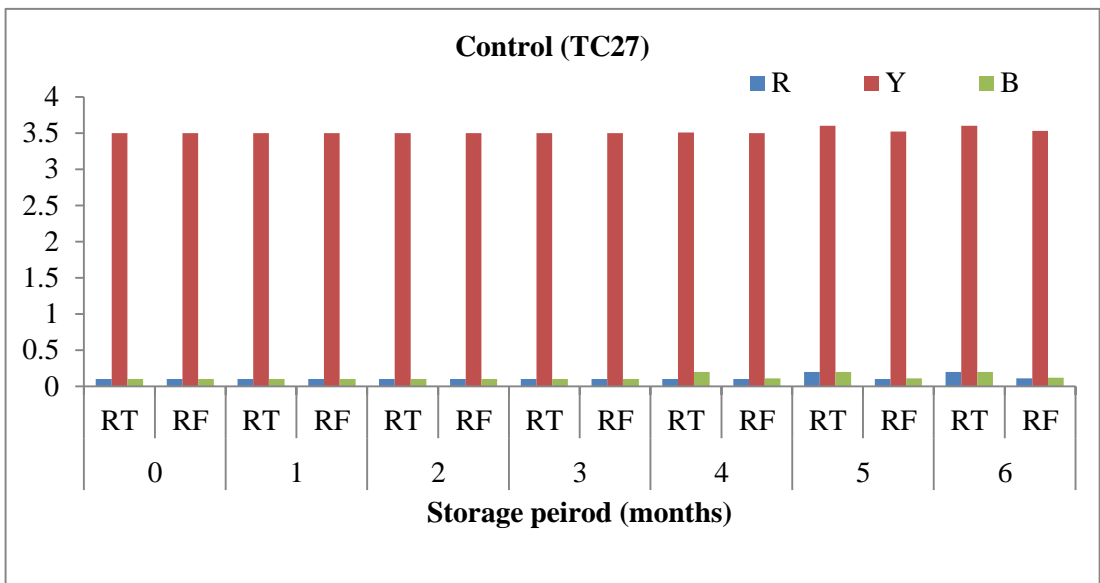
Table 4.19: Effect of storage temperature on physicochemical and phytochemical constituents of carbonated sugarcane juice beverages (6 months storage)

Storage temperature	Carbonated beverage			
	Control (TC27)	TC21	TC23	TC24
	Total soluble solids ($^{\circ}\text{B}$)			
Room	12.68	12.65	12.68	12.60
Refrigeration	12.71	12.70	12.71	12.63
CD at 5%	0.005			
	Titratable acidity (%)			
Room	0.37	0.38	0.37	0.40
Refrigeration	0.35	0.36	0.35	0.39
CD at 5%	0.004			
	pH			
Room	3.53	3.49	3.49	3.59
Refrigeration	3.54	3.51	3.51	3.61
CD at 5%	NS			
	Ascorbic acid (mg/100ml)			
Room	2.14	8.67	7.34	9.71
Refrigeration	2.19	8.74	7.41	9.78
CD at 5%	NS			

Storage temperature	Carbonated beverage			
	Control (TC27)	TC21	TC23	TC24
	Total polypolyphenols (mg GAE/100ml)			
Room	200.50	240.65	238.74	242.68
Refrigeration	203.10	244.25	241.36	246.24
CD at 5%	NS			
	Total flavonoids (mg QE/100ml)			
Room	2.90	6.32	6.60	7.12
Refrigeration	2.89	6.32	6.59	7.12
CD at 5%	NS			
	Antioxidant activity (% inhibition of DPPH)			
Room	24.30	56.80	54.30	55.79
Refrigeration	25.56	59.02	57.20	58.66
CD at 5%	0.01			
	Total sugars (%)			
Room	11.57	11.57	11.64	11.52
Refrigeration	11.65	11.654	11.67	11.54
CD at 5%	0.01			
	Reducing sugars (%)			
Room	0.43	0.30	0.29	0.32
Refrigeration	0.41	0.30	0.28	0.31
CD at 5%	0.001			
	Viscosity(cp)			
Room	4.00	3.83	3.98	3.14
Refrigeration	4.01	3.85	3.99	3.15
CD at 5%	NS			

4.4.12 Effect of storage period and storage temperature on colour (Tintometer colour units) of carbonated sugarcane juice beverages

The effect of storage period on colours (Tintometer colour units) of carbonated sugarcane juice beverages (TC27, TC21, TC23 and TC24) data depicted in Fig.10 showed that there was a slight increase in values of (Tintometer colour units) of R, Y and B values during storage of 6 months for all treatments, the Y values were found major followed by R for all treatments. The storage period had significant effect on colour values during storage of six months though all the samples were found stable for 3 months. But these changes are acceptable on sensory evaluation of products. The samples stored at refrigerated temperature showed no significant effect on colour values.



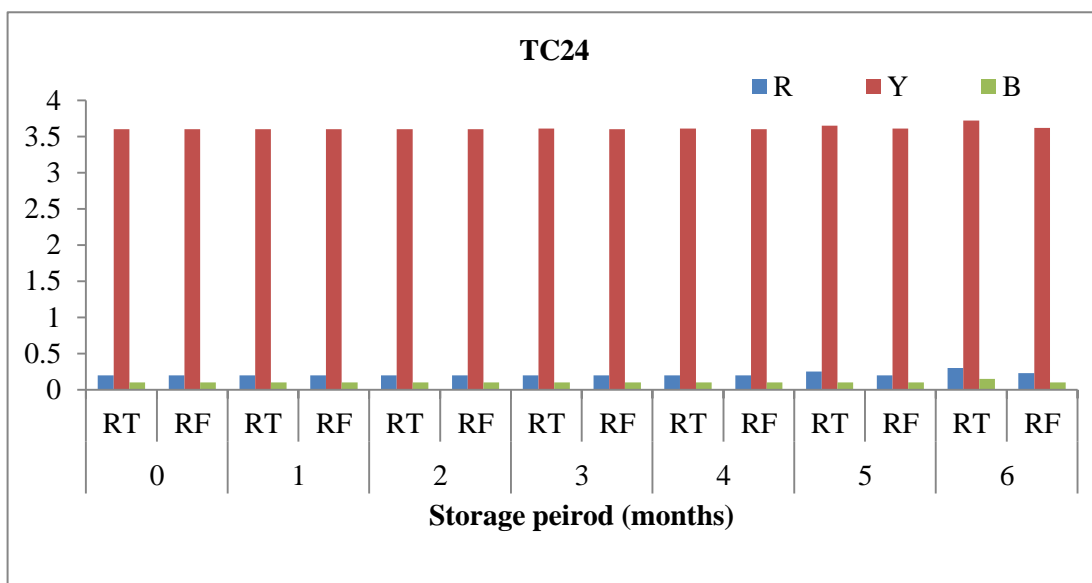
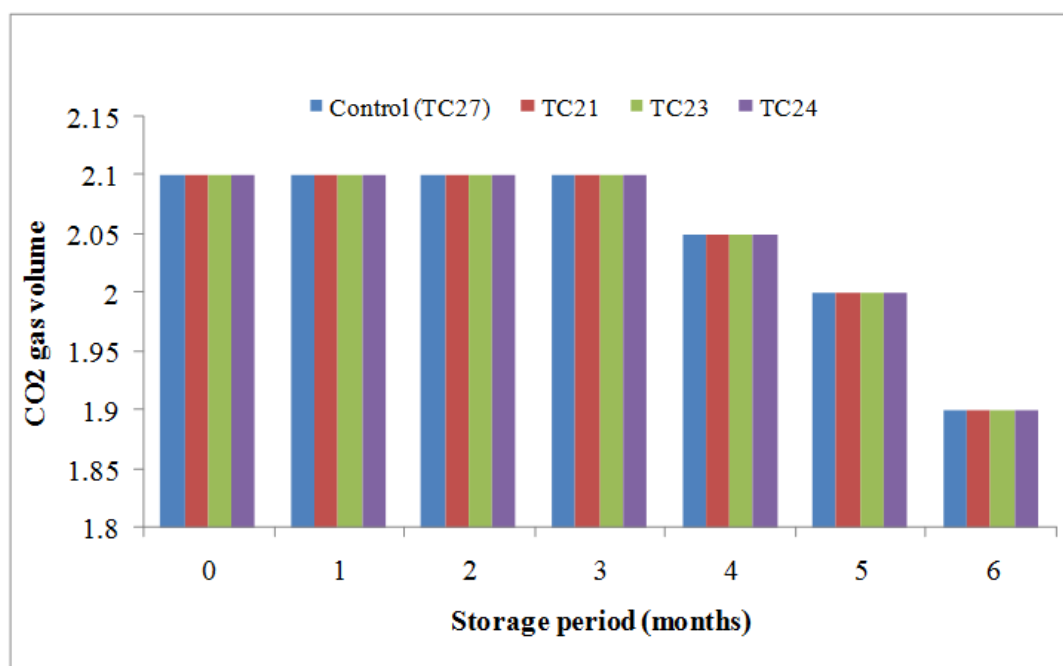


Fig. 10: Effect of storage period and storage temperatures on colours (Tintometer colour units) of carbonated sugarcane juice beverages

4.4.13 Effect of storage period and storage temperature on CO₂ gas volume of sugarcane juice carbonated beverages

The effect of storage period on CO₂ gas volume (CO₂ gas dissolved by one volume of water) the data depicted in Fig. 11, it was observed that, no significant change was observed in CO₂ gas volume during storage of 6 months, all the treatments had the same gas volume, while the refrigerated samples (2.09) showed greater values than stored at room temperature (2.01) but statistically no significant difference.



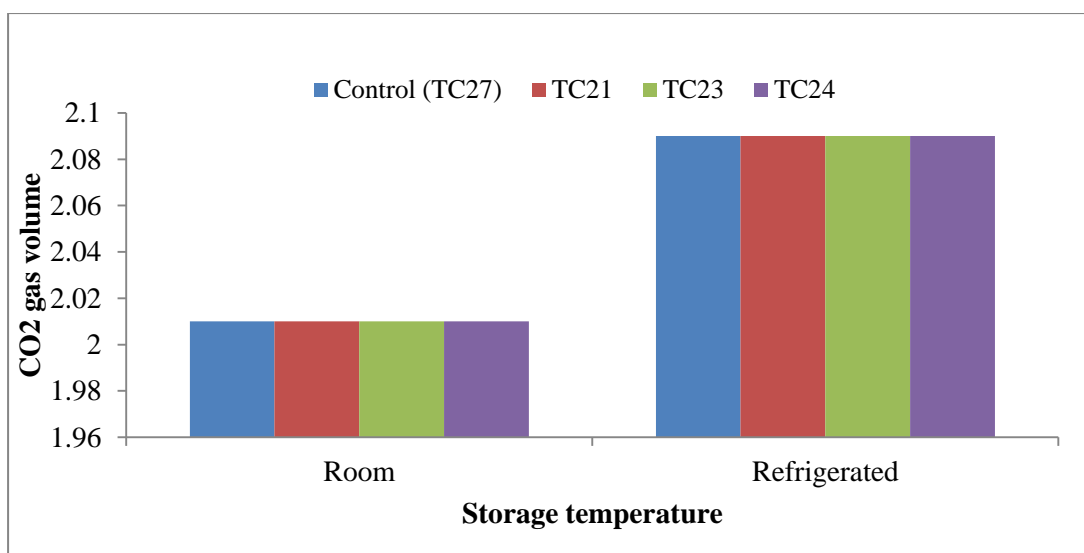


Fig. 11 :Effect of storage period and storage temperature on CO₂ Gas: Volume (CO₂ Gas dissolved by I volume of water) of carbonated sugarcane juice beverages

4.4.14 Effect of storage period and storage temperature on CO₂ gas absorption of carbonated sugarcane juice beverages

The data shown in Table 4.20 for the effect of storage period on CO₂ content in carbonated sugarcane juice beverages, it was observed that the significant reduction in CO₂ content was observed in all treatments TC27, TC21, TC23, TC24 and reduction found was 4.56%, 4.55%, 4.55%, 4%, respectively during storage of 6 month. The more CO₂ retention was observed in refrigerated samples than room temperature but statistically no significant difference.

Table 4.20: Effect of storage period and storage temperature on CO₂ content (mg/100ml) of carbonated sugarcane juice beverages

Treatment	Carbonated sugarcane juice beverages			
	TC27 (Control)	TC21	TC23	TC24
Storage period (months)				
0	570	571	570	574
1	570	571	570	574
2	568	569	569	572
3	563	563	563	570
4	557	557	558	563
5	550	551	551	556
6	544	545	544	551
CD at 5%	0.32			
Storage temperature				
Room	555	556	555	560
Refrigerated	565	566	566	571
CD at 5%	NS			

4.4.15 Effect of storage period and storage temperature on microbiological quality of carbonated sugarcane juice beverages

It was observed from Table 4.21 that the total plate count was found absent in all the treatments for storage of 5 months storage both at room and refrigerated temperatures. The total plate count was found present only in TC27, TC21 and TC23 samples after 6 months of storage at room temperature and the values found were 10, 3 and 2cfu/ml those were far below the prescribed limit of FSSAI. The total plate count was found absent in all the treatments stored at refrigerated temperature. The total plate count in the samples of all treatments was found far below the prescribed limit of FSSAI law. The yeast and mould count was found absent in all the treatments at both the temperatures of storage except TC27 as 1cfu/ml at room temperature was detected after 6 months. It is observed that all the treatments were found storage stable during the storage of 6 months as the beverage was carbonated along with the ascorbic acid content and acidity due to lemon and citrus fruit juices, also the presence of ginger juice in the beverages. The beverage also possessed stability due to the presence of prescribed limit of preservative (sodium benzoate 60 ppm). The samples stored at low temperature had better stability than stored at room temperature.

Table 4.21: Effect of storage period and storage temperature on microbiological quality of carbonated sugarcane juice beverages

Treatment	Storage period (months)														
	0		1		2		3		4		5		6		
	RT	RF	RT	RF	RT	RF	RT	RF	RT	RF	RT	RF	RT	RF	
	Total plate count (CFU/ml)														
TC27	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-
TC21	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-
TC23	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-
TC24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Yeast and Mould count (CFU/ml)														
TC27	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
TC21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TC23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TC24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CHAPTER V

SUMMARY

The investigation was carried out to develop carbonated and non carbonated sugarcane juice beverages with the objectives to optimize the formulation and processing conditions for development of carbonated and non carbonated sugarcane juice beverages from sugarcane juice blended with fruit juices, fresh ginger and mint, to assess the physicochemical and sensory qualities of developed beverages, to study the effect of blends and carbonation levels on the bioactive compounds of developed carbonated and non carbonated sugarcane juice beverages and evaluation of storage stability of prepared carbonated and non carbonated beverages. The fresh sugarcane (Var. CoPb 91) was procured from Sugarcane Research Station, Kapurtala, Punjab Agricultural University, Ludhiana and fresh fruits of kinnow, aonla and lemon of commonly grown varieties were purchased from the Department of Fruit Science, PAU, Ludhiana and also from local market. Fresh ginger and mint were procured from the local market of Ludhiana. The juices of sugarcane, kinnow, aonla, lemon and ginger were extracted following the standard methods. The juices of sugarcane, kinnow, aonla, lemon and ginger were analyzed for the physicochemical and phytochemical constituents and antioxidant activity. The juices were analyzed for the juice yield and proximate composition viz. moisture content, total solids, ash, content, titratable acidity, pH, ascorbic acid, antioxidant activity, total polyphenols, total flavonoids, total sugars, reducing sugars and viscosity. The values for per cent juice yields of sugarcane, kinnow, aonla (with equal quantity of water), lemon, ginger (with double quantity of water) were found 54.61, 47.15, 129.46, 40.7 and 182 respectively. The values for total solids were found maximum in sugarcane (20.73 %) and minimum in ginger juice (2.70 %). The total soluble solids (⁰B) in juices of sugarcane, kinnow, aonla, lemon and ginger were found 20.54, 10.30, 8.6, 9.16 and 2.1 while the ash content (per cent) values were 0.29, 0.35, 0.23, 0.33 and 0.27, respectively. The maximum titratable acidity (per cent) was found in lemon juice (4.95 %) while less was reported in sugarcane juice (0.21%) followed by aonla (1.38%), kinnow (1.16%) and ginger (0.27%). The total sugar content values (%) reported in sugarcane juice, kinnow, aonla, lemon and ginger were 18.57, 9.59, 1.48, 4.39 and 1.75, respectively. The ascorbic acid (mg/100ml), antioxidant activity (% inhibition), total phenolic content (mg GAE/100ml) and total flavonoids (mgQE/100ml) values for juices of sugarcane, kinnow, aonla, lemon and ginger were reported as 3.65, 23.6, 253.42, 28.86, 5.6; 43.23, 69.3, 88.32, 67.41, 78.2; 487.3, 1266.61, 1536.6, 1021.3, 505; 2.56, 16.42, 16.73, 17.63, 150, respectively.

The mineral content of the juices of sugarcane , kinnow, aonla, lemon and ginger are the good source of calcium, iron, potassium, sodium, magnesium, phosphorus, sulphur, copper, zinc and the respective values (mg/100ml) for the sugarcane juice were 13.87, 1.78,

49.52, 13.89, 13.42, 25.27, 62.123, 0.12, 0.39.

The values for pasteurization temperature, time and the preservative concentration (sodium benzoate) were optimized for the extracted juices of sugarcane, kinnow, aonla, lemon and ginger also for the carbonated and non carbonated sugarcane juice beverages of sugarcane juices. The pasteurization temperature of 82⁰C for 5 min and 120 ppm of sodium benzoate were found optimum for the preservation of juices of kinnow, aonla, lemon, ginger and both the non carbonated sugarcane juice beverages and the juice base of carbonated beverages. The final concentration of sodium benzoate in the carbonated sugarcane juice beverages was 60 ppm.

The sugarcane juice beverage prepared by using treatment T23 (sugarcane juice 95%, lemon juice 3%, ginger juice 2 %) was found superior on sensory evaluation and scored 8 for overall acceptability and was further used as a control sample, while the non carbonated sugarcane juice beverage samples blended with juices of kinnow, aonla, lemon and ginger by using treatments of T11 (80% sugarcane juice, 10 % kinnow juice, 5 % aonla juice, 2 % lemon juice and 3 % ginger juice), T12 (80% sugarcane juice, 10 % kinnow juice, 5 % aonla juice, 2.5 % lemon juice and 2.5 % ginger juice) and T14 (75% sugarcane juice, 15 % kinnow juice, 5 % aonla juice, 2 % lemon juice and 3 % ginger juice) were scored high over the other treatments for overall acceptability and other parameters on sensory evaluation by judges of semi trained panel using 9 point hedonic scale. The samples of non carbonated sugarcane juice beverages of treatments T23 (control), T11, T12 and T14 were chosen for further study, prepared in bulk and further studied for its physicochemical characteristics, storage stability and microbial quality.

The non carbonated sugarcane juice beverages T23, T11, T12 and T14 on physicochemical analysis showed that the per cent values for ash content, acidity, total sugars and reducing sugars were 0.16, 0.52, 17.73, 0.44; 0.12, 0.47, 16.25, 0.54; 0.16, 0.65, 16.35, 0.56 and 0.17, 0.61, 15.78, 0.63, respectively. Also the values for the ascorbic acid (mg/100ml), antioxidant activity (% inhibition), total polyphenols (mg GAE/100ml) and total flavonoids (mgQE/100ml) for respective beverages were observed as 4.3, 16.53, 16.62, 17.3; 53.2, 80.21, 80.7, 81.9; 496.6, 621.3, 624.2, 660.3; 5.22, 15.42, 15.3, 16.2, respectively. The no significant difference were observed in viscosities of samples. The mineral content values (mg/100ml) for the non carbonated sugarcane juice beverage samples viz. T23, T11, T12 and T14 were found at par and showed good amount of minerals in the beverages.

The effect of storage period on sensory qualities of non carbonated sugarcane juice beverages (T23, T11, T12 and T14) were studied for the 6 months at room (30±4⁰C) and refrigeration (4⁰C) temperature and the periodic observations were noted after each month. The results showed that all the samples of non carbonated sugarcane juice beverages were found acceptable with slight decreased sensory score by semi trained panel during the storage

of 6 months for all parameters viz. appearance, mouth feel, aroma, taste, consistency and overall acceptability though the beverages were found stable during the storage period. The effect of storage temperature both at room ($30\pm 4^{\circ}\text{C}$) and refrigeration (4°C) on sensory qualities of non carbonated sugarcane juice beverages showed that the beverages stored at refrigerated temperature scored slightly high for all the sensory parameters than the stored at room temperature. The non carbonated sugarcane juice beverages were found slightly superior in quality at refrigerated storage for 6 months over the storage at room temperature but both beverages were scored well on sensory evaluation.

The effect of storage period on physicochemical characteristics of non carbonated sugarcane juice beverages (T23, T11, T12 and T14) during 6 months storage at room ($30\pm 4^{\circ}\text{C}$) and refrigeration (4°C) temperature showed that there was slight decrease in values of total soluble solids (1.6%), pH (3.63%), ascorbic acid (1.35%), total sugars (2.38%) and viscosity (1.71%) for all the beverages after 3 months during storage of 6 months. The values for titratable acidity and reducing sugars were increased 4.91% and 7.9%, respectively during the storage of 6 months; however the beverages showed stability till 3 months while further storage slightly decreased the values. The products were found acceptable after six months storage. It was observed that, the all treatments (T23, T11, T12 and T14) beverages stored at refrigerated temperature depicted slightly high values for all physicochemical parameters than the samples stored at room temperature. The non carbonated sugarcane juice beverages were found slightly superior in quality those stored at refrigerated storage for 6 months over the storage at room temperature but both beverages showed statistically non significant changes in the values of all parameters. The effect of storage period on colours (Tintometer colour units) of non carbonated sugarcane juice beverages (T23, T11, T12 and T14) showed that there was a slight increase in values of tintometer colour units of R, Y and B values during storage of 6 months for all treatments and the Y values were found major followed by R for all treatments. The storage period had significant effect on colour values during storage of six months though all the samples were found stable for 3 months. But these changes are acceptable on sensory evaluation of products. The samples stored at refrigerated temperature showed no significant effect on colour values.

The samples of carbonated sugarcane juice beverages were made using different combination treatments viz. TC00 to TC27 for the standardization of recipe. The fresh sugarcane juice of double extraction was filtered through the four fold muslin cloths and the different combination treatments for juice blends for base of the carbonated sugarcane juice beverages were made using the juices of kinnow, aonla, lemon and ginger. The juice mixes of different treatments were pasteurized at 82°C for 5 min and the preservative was adjusted to 120ppm level in each juice blend used as base for carbonated beverage samples. The juice blends were cooled to room temperature and then to 15°C temperature. The blended juice

volume of 100 ml was filled in each sterile bottle of 200 ml capacity, and then the bottles were filled with carbonated water of desired CO₂ level at 15⁰C temperature. The different carbonation levels were used viz. 70, 80, 90 and 100 psi CO₂ pressure for the standardization of carbonation level. The prepared carbonated sugarcane juice beverages were chilled and evaluated for overall acceptability using sensory evaluation by panel of semi trained judges. The treatment TC27, TC21, TC23 and TC24 scored high for overall acceptability score on sensory evaluation at 80 psi carbonation level. Then samples of all treatments were made at 80 psi carbonation level, chilled and further tested for all parameters of sensory qualities viz. appearance, mouth feel, aroma, taste, consistency and overall acceptability. The selected treatments were found best at 80 psi carbonation levels over the other treatments. The treatment TC27 was used as a control.

The samples of carbonated sugarcane juice beverages of treatments TC27 (control), TC21, TC23 and TC24 were chosen for further study, prepared in bulk and further studied for its physicochemical characteristics, storage stability and microbial quality. The bottled samples of carbonated sugarcane juice beverages were stored at room (30±4⁰C) and refrigeration (4⁰C) temperature. The carbonated sugarcane juice beverages TC27 (control), TC21, TC23 and TC24 were analyzed for physicochemical and phytochemical characteristics data showed that total soluble solids were found higher (12.74 ⁰B) in control sample than TC21, TC23 and TC24. The per cent values for ash content, acidity, total sugars and reducing sugars in carbonated sugarcane juice beverages viz. TC27 (control), TC21, TC23 and TC24 were recorded as 0.13, 0.33, 11.73, 0.3; 0.13, 0.34, 11.7, 0.3; 0.11, 0.34, 11.72, 0.28 and 0.13, 0.38, 11.6, 0.31, respectively. Also the values for the ascorbic acid (mg/100ml), antioxidant activity (% inhibition) and total polyphenols (mg GAE/100ml) for respective beverages were observed as 2.93, 49.14, 69.10; 6.22, 73.13, 86; 5.44, 70.96, 78.66; 6.52, 78.53, 91.66, respectively. The viscosities (cp) of the treatments were ranged between 3.16 to 4.03 showed significant differences. The mineral content values (mg/100ml) for the carbonated sugarcane juice beverage samples viz. TC27, TC21, TC23 and TC24 were found at par and showed good amount of minerals in the beverages. The mineral content values (mg/100ml) were found in the range as calcium (10.35-12.45), iron (1.39-1.43), potassium (36.7 - 42.55), sodium (2.16 - 2.44), phosphorus (14.90 - 16.26), sulphur (20.87-23.3), zinc (0.31- 0.37), magnesium (4.47- 5.62), copper (0.13 - 0.18) and nickel (0.01).

The effect of storage period on sensory qualities of carbonated sugarcane juice beverages (TC27 (control), TC21, TC23 and TC24) were studied for the 6 months at room (30±4⁰C) and refrigeration (4⁰C) temperature and the periodic observations were noted after each month. The results showed that all the samples of carbonated sugarcane juice beverages were found acceptable with slight decreased sensory score by semi trained panel during the storage of 6 months for all parameters viz. appearance, mouth feel, aroma, taste, consistency

and overall acceptability though the beverages were found stable during the storage period. The effect of storage temperature both at room ($30\pm 4^{\circ}\text{C}$) and refrigeration (4°C) on sensory qualities of carbonated sugarcane juice beverages showed that the beverages stored at refrigerated temperature scored slightly high for all the sensory parameters than the stored at room temperature. The carbonated sugarcane juice beverages were found slightly superior in quality at refrigerated storage for 6 months over the storage at room temperature but both beverages were scored well on sensory evaluation.

The effect of storage period on physicochemical and phytochemical characteristics of carbonated sugarcane juice beverages ((TC27, TC21, TC23 and TC24)) during 6 months storage at room ($30\pm 4^{\circ}\text{C}$) and refrigeration (4°C) temperature showed that there was slight decrease in values of total soluble solids (1.27%), pH (2.73%), ascorbic acid (5.11%), total sugars (2.11%) and viscosity (1.85%) for all the beverages after 3 months during storage of 6 months. The values for titratable acidity and reducing sugars were increased 18% and 14.9%, respectively during the storage of 6 months; however the beverages showed stability till 3 months while further storage slightly decreased the values. The products were found acceptable after six months storage.

The effect of storage temperature on physicochemical characteristics of carbonated sugarcane juice beverages observed that, the all treatments (TC27, TC21, TC23 and TC24) beverages stored at refrigerated temperature depicted slightly high values for all physicochemical parameters than the samples stored at room temperature. The carbonated sugarcane juice beverages were found slightly superior in quality those stored at refrigerated storage for 6 months over the storage at room temperature but both beverages showed non significant changes in the values of all parameters.

The effect of storage period on colours (Tintometer colour units) of carbonated sugarcane juice beverages (TC27, TC21, TC23 and TC24) data showed that there was a slight increase in values of (Tintometer colour units) of R, Y and B values during storage of 6 months for all treatments, the Y values were found major followed by R for all treatments. The storage period had significant effect on colour values during storage of six months though all the samples were found stable for 3 months. But these changes are acceptable on sensory evaluation of products. The samples stored at refrigerated temperature showed no significant effect on colour values.

The effect of storage period on CO_2 gas volume (CO_2 gas dissolved by one volume of water) data showed that, no significant change was observed in CO_2 gas volume during storage of 6 months, all the treatments had the same gas volume, while the refrigerated samples (2.09) showed greater values than stored at room temperature (2.01) but statistically no significant difference. The data for the effect of storage period on CO_2 content in carbonated sugarcane juice beverages showed that the significant reduction in CO_2 content

was observed in all treatments TC27, TC21, TC23, TC24 and reduction found was 4.56%, 4.55%, 4.55%, 4%, respectively during storage of 6 month. The more CO₂ retention was observed in refrigerated samples than room temperature but statistically no significant difference.

Conclusion

The carbonated and non carbonated sugarcane juice beverages were developed by blending sugarcane juice with nutritious juices of kinnow, aonla, lemon and ginger. These beverages were found enriched in nutritional and phytochemical quality parameters. These beverages were prepared without addition of external sugar, citric acid, by use of low heat treatment and only limited preservative. The presence of ascorbic acid in good amount along with other acids and phytochemicals in the fruit juices, became the cause to achieve the required acidity, pH, antimicrobial functionality also to impart taste and flavour for the development of quality beverages from natural juices. These bottled beverages were found self stable during storage of more than six months at room and refrigerated temperatures without significant loss in nutritional and sensory qualities. The non carbonated beverages contained 75 to 80 per cent sugarcane juice and 20 to 25 per cent other juices while, carbonated beverages contained 41 to 43 per cent sugarcane juice and 7 to 9 per cent other juices. Thus these carbonated and non carbonated sugarcane juice beverages could be the best and healthy substitute for the existing non nutritious, artificial and health hazardous beverages in the market as Govt. of India approved the law for the incorporation of at least 5% fruit juice in the existing non nutritious soft drinks.

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