STUDIES ON STANDARDIZATION AND STORAGE OF AONLA BASED BEETROOT BLENDED RTS BEVERAGE

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B.Sc. (Hons.) Horticulture

MASTER OF SCIENCE

IN

HORTICULTURE

(FRUIT SCIENCE)

DEPARTMENT OF HORTICULTURE

COLLEGE OF AGRICULTURE, PARBHANI

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PARBHANI – 431402 (M.S.), INDIA

2022

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BY

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B.Sc. (Hons.) Horticulture

A thesis submitted to

Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani

in partial fulfilment of the requirement for the degree of

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2022

DECLARATION BY THE CANDIDATE

I hereby declare that the thesis entitled, **"STUDIES ON STANDARDIZATION AND STORAGE OF AONLA BASED BEETROOT BLENDED RTS BEVERAGE".** submitted by me is based on the actual work carried out by me under the guidance and supervision of **Dr. V.N. SHINDE**. The extent of information derived from the existing literature have been duly cited and referenced. The existing research work or its any part is not submitted anywhere else for the award of any degree or diploma.

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Research guide & Chairman,
Advisory committee

(Dr A M Bhosale)
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(Dr C V Ambadakr)
(Member)

(V.B. Jadav)
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Submitted by	Dr V. N. Shinde
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ACKNOWLEDGMENT

From the core of my heart, I bow my head in extreme regards to the almighty god whose blessings enabled me to reach this destination.

In presenting this text, i feel privileged to ***Dr.V.N. Shinde.****Assistant Professor, Department of Horticulture, College of Agriculture, VNMKV, Parbhani as the chairman of my advisory committee for his valuable guidance, noble inspiration, constructive suggestion and constant support during the course of my investigation and study to accomplish this stupendous task.*

I feel deeply immense pleasure in expressing my wholehearted sense of gratitude to the members of my advisory committee, ***Dr A M Bhosale****, Assistent professor, Department of Horticulture,* ***Dr C V Ambadakr,****Assistent professor, Department of Plant PathologyAnd* ***Prof. V. B. Jadhav****, Department of Agriculture engeneering for constant inspiration, encouragement and constant guidance during the period of present research work.*

I express my sincere and humble thanks to ***Dr. G.M. Waghmare****, Head of the Department and* ***Dr. A. M. Bhosale****, PG in charge, Department of Horticulture, for their useful expert advice, constant supervision and co-operation during the course of study.*

I also extend my deep sense of honour to Honourable, ***Dr.Indra Mani****, Vice-Chancellor,* ***Dr. D. N. Gokhale****, Dean and Director of Instruction and* ***Dr. Syed Ismail****, Associate Dean, Principal, VNMKV, Parbhani,****Dr. S. R. Bharkule,*** *Central Nursery, for providing necessary facilities during the course of this investigation and also for their kind support and generosity during the entire study.*

It is incumbent upon me to express my profound sense of gratitude toDr.R. Bhalerao andDr. SyedSabnam Department of Horticulture, V.N.M.K.V., Parbhani who have been kindly navigated my ship of academic pursuit and I would like to mention my gratitude to them.

I am immensely thankful to my dear friends particularly Abhishek, Akhilesh, Deepak and Tribhuvan who have rendered their immense care and support during my entire research work of MSc. programme.

I am really grateful to my brother ***AmitVerma*** *and* ***ShivamVerma*** *for their endless love, support and motivation during my low times. You have played a main role in strengthening my self-confidence and in developing a clear vision in life.*

I wish to put on record my heartiest thanks to my batchmates and friends,Ravi, Mahanand, Ramprem, Ramniwas, Praveen, Shravani, Raees, Kunalnaik, Dattabise, Nilesh,Sidhant, Vaibhav, Akash, AmbrisPrakurti, Snehal, Nikitha, for their valuable help, lively association and enthusiastic moral support curing the course of this study and motivated me for every good type of step in my life and their joyful company made my life richer.

I am thankful to all workers and labour for their support and help in department during my research programme.

I deeply thank my parents, my beloved father ***Shri.LalsahabVerma****, mother* ***Smt.SarojVerma****, have been a source of encouragement throughout my career, for moral support, personal attention and care without which I would not have reach the present destination. I owe all of my respect to them.*

I wish to thank all the teachers from my schooling days onwards and well-wishers, who have directly or indirectly helped me to reach up to this level in my life.

I am thankful to all the authors in past and present whose literature has been cited.

I think it is a matter of pleasure to glance back and recall the path one travels the day of hard work and preference. It is still great at this juncture to recall the face and spirits in the form of teachers, friends, near and dear one.

Place: Parbhani

Date: 03/02/2023

(Tribhuvan Verma)

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ABBREVIATIONS

%	:	Per cent
@	:	at the rate
AOAC	:	Association of Official Ananlytical Chemistry
CD	:	Critical Difference
CRD	:	Completely Randomized Design
Cv.	:	Cultivar
et al.,	:	and others
Fig.	:	Figure
g	:	Gram
HCL	:	Hydrochloric acid
HPO_3	:	Meta phosphoric acid
Hrs	:	hours
HRS	:	horticultural Research Station
KCL	:	Potassium chloride
Kg	:	kilo gram
mg L^{-1}	:	milligram per liter
ml	:	milliliter
mm	:	milli meter
N	:	Normality
NaoH	:	Sodium hydroxide
NHB	:	National Horticulture Board
nm	:	nano meter
NS	:	Non Significant
^oC	:	Degree Centigrade
pp	:	page number
ppm	:	parts per million
rpm	:	revolution per minute
RTS	:	Ready To Serve
S.E (d)	:	Standard error of deviation
S.E (m)	:	Standard error of mean
SB	:	Sodium benzoate
TSS	:	Total soluble solids
Viz.	:	namely
VNMKV	:	Vasantrao Naik Marathwada Krishi Vidyapeeth
TPC	:	total plate count

THESIS ABSTRACT

THESIS ABSTRACT

1. Title of the Thesis	: "Studies on Standardization and Storage of Aonla based Beetroot Blended RTS beverage"
2. Full name of the candidate	: Tribhuvan Verma
3. Full name of Research Guide	: Dr. Vinod Shinde
4. Department	: Horticulture (Fruit Science).
5. College / University	: College of Agriculture, VNMKV, Parbhani.
6. Degree to be awarded	: M.Sc. (Horticulture).

ABSTRACT

Health benefits of aonla and beetroot motivated us to develop aonla based beetroot blended RTS beverage by blending aonla and beetroot juices in different ratios such as, T_1 - aonla:100, T_2 - aonla:beetroot (95:05), T_3- aonla:beetroot (90:10), T_4 - aonla:beetroot (85:15), T_5 - aonla:beetroot (80:20), T_6 - aonla:beetroot (75:25), T_7 - aonla:beetroot (70:30) were prepared and pasteurized at 85 ℃ for 30 minutes. After preparation the selected samples from each treatment were analyzed for physio-chemical properties, sensory evaluation and microbial analysis at 0 day, 30 days, 45 days, 60 days and 90 days.

In aonla based beetroot blended RTS beverage, the TSS and acidity were fixed to 10° Brix, 0.35% during preparation. Later on the TSS got increased while acidity got decreased in all the treatments due to various biochemical reactions took place during storage. The bio-chemical characters like TSS, total sugars, and reducing sugars were recorded highest in T_4 (aonla:beetroot::85:15) and the traits like pH, non-reducing sugars, color was found to be highest in T_7 (aonla:beetroot::70:30). While, T_1 (aonla:100) had maximum ascorbic acid content and antioxidants. The chemical constituents like TSS, reducing sugars, total sugars, were found increasing trend during storage while, pH, acidity, ascorbic acid, antioxidants and color were found decreased during 90 days of storage.

In sensory evaluation, T_4 (aonla:beetroot::85:15) was found to be the best treatment which was decided on the basis of highest sensory score in terms of colour, taste, flavor and overall acceptability. All these parameters decreased upon storage due to various undesirable changes in RTS beverage during storage. Shelf life of the RTS beverage was decided based on microbial analysis at all stages of staorage where plate count, yeast total, mould and coliform count were recorded. There was no growth of all these organisms in all the treatmrntsupto 60 days of storage. While, at 90 days total plate count, yeast and mould count, and coliform count were 3 cfu/ml, 1 cfu/ml, and nil respectively after 72 hours of incubation.

According to results obtained, it may be suggested that for preparation of aonla based beetroot blended beverage 85% aonla juice and 15% beetroot juice was most acceptable which was at par with 80% aonla juice and 20% beetroot juice. After pasteurization these can be stored till 90 days without considerable quality deterioration.

CHAPTER-I
INTRODUCTION

CHAPTER - I

INTRODUCTION

Fruits and vegetables are the important sources of nutrients especially vitamins, sugars, minerals and fiber due to which these are the important constituents of healthy diet. Regular consumption of fruits and vegetables reduces the risk of cancer, heart diseases, premature aging, stress and fatigue as they have the free radicals scavenging compound like β-carotene, ascorbic acid and other dietary fibers, antioxidants(Gaikwad*et al*., 2013).

The shelf life of fruits and vegetables is very limited because of their perishable nature. In India, over and above 20-25 percent of fruits and vegetables are spoiled before utilization. Despite being the world's second largest producer of fruits and vegetables, in India only 1.5 percent of the total fruits and vegetables produced are processed. Maximum amount of fruit and vegetable juices get bitter after extraction as a result of conversion of chemical compounds. Despite being underutilized, the utilization of highly nutritive fruits and vegetables is very minimal due to high acidity, astringency, bitterness, and some other aspects. To overcome all these, it is necessary to find the new ways for maximum utilization of fruits and vegetables without affecting their nutritive values.

Fruit beverages are delicious as well as nutritious containing the goodness of fresh fruit.Fruit beverages such as ready-to-serve (RTS) are becoming increasingly popular in comparison to synthetic drinks, evidently because of their taste, flavor and nutritive value. They are in great demand in various public eating places like school canteens, cafeterias, snack bars as well as homes. Many of the beverages that are available in the market attract the consumers mainly because of their color and taste without providing nutrients apart from the empty calories in the form of sugar.The advantage of RTS beverage is that there is no need to dilute it whereas squash, syrup, cordial, crush are diluted with water before use. Ready-to-serve beverages are made out of juice, sugar, water and consumed as such.

However, the beverages based on fruit juice or pulp are easily digestible, appetizing, thirst quenching, highly refreshing and are nutritionally superior to many

aerated and synthetic beverages. Since, these beverages are meant for direct consumption without further dilution thus provides the advantage of convenience of consumption. These beverages also serve as healthy alternative for the children and elderly people who have difficulties in handling the whole fruits.

Nowadays, customers demand products which are free from any chemical additives like color or flavors and are natural with assured safety and better shelf life. Thus, beverages based on fruit juices can be used to replace the artificial or synthetic beverages that are available in the market having no nutritional value (Kanchan *et al*., 2020).

In India Aonla(*Emblica officinalis*) has got special place among the indigenous fruits as it has tremendous medicinal value as well as high nutritional value.Aonla fruit is one of the richest known sources of ascorbic acid (300-l000mg per 100g edible portion) depending upon the cultivar and location (Manny and Shadakshara, 1997) and fresh fruit of aonla is also appreciable source of total sugar (7.53mg/100g), calcium (14.91 mg/100g), iron (0.62 mg/100g), and phosphorus (11.81 mg/100g) as reported by Khan (2009). Thereby, only a few fruits can meet the daily requirement of vitamin C (Shankar, 1969). The fruit also contains polyphenols, which have antioxidant property and thus has good free radical scavenging activity. Tannin found in fruits containing gallic acid, ellagic acid and glucose in its molecule, which is naturally present in the fruit, prevents and/or retards the oxidation of the vitamin C. Therefore, even after processing it retains major part of ascorbic acid.

Apart from the above, the fruit also contains epicatechin which is hypoglycaemic, anti-inflammatory and antiviral. This compound also eminently cures menourrhagie in menstruating women by bringing balance of prostaglandin. This fruit also contain appreciable amount of linoleic acid which imparts immunomodulation in human body. Ingestion of linoleic acid by the patients having multiple sclerosis reduces the frequency and intensity of heart attack. And the phyllemblin constituent of fruit regulates blood pressure and respiration in normal tune. The 3 acetic acid and four other similar auxins, of fruit which potentiate externally administered insulin. (Basak, 2005).

Generally,aonla fruits are not consumed fresh because, fruits are highly acidic and astringent. Therefore,aonlafruits are not popular as table fruit.Nayak *et al*. (2011) reported that high medicinal properties of aonla fruits can be processed in to value added Ready to Serve (RTS) beverage.

Beetroot (*Beta vulgaris L.*) having bright crimson colour also known as beet, chard, spinach beet, sea beet, garden beet, white beet and Chukander (in Hindi) belongs to Chenopodiaceae family. It is famous for its high valued juice and medicinal properties. The beetroot can be kept for 4-5 days when refrigerated in the vegetable crisper (Chibber *et al*., 2019). *Beta vulgaris* roots contain significant amounts of vitamin C, while the leaves are an excellent source of vitamin A. It is among the sweetest of vegetables, containing more sugar even than carrots or sweet corn. Incorporation of beetroot in the juice, which contains considerable amount of nitrate helps to dilate and widen the blood vessels, thereby reducing the blood pressure and allowing more blood flow and it can also be effective to boost the athlete's performance (Lansley *et al*. 2011). Muddy flavor in fresh beetroot juice is due to presence of geosmin.

Beetroot being an alkaline food having pH 7.5 to 8.0 contains higher antioxidant compounds (Singh *et al*., 2013). Betaine has several noted effects related to human health and function, including acting as an osmolyte (protecting cells against dehydration), as an antioxidant agent (protecting cells against free radicals), as a methyl group donor (lowering potentially harmful levels of homocysteine), and as a vascular protectant (Bloomer *et al*., 2011). A small increase in the habitual consumption of antioxidant and polyphenol-rich beverages such as beetroot juices blended with aonla juice may have significant positive effect on public health (Mahnoori *et al*. 2020). Juice blending is one of the best methods to improve the nutritional quality of the juice. Thus, blending of Aonlaand beetroot juice offers many opportunities to develop balanced health product high in quality with respect to both sensory and nutritional aspects.Sandhu and Sindhu (1992), Saxsena *et al*., (1996), Attri *et al*., (1998) and Langthasa (1999), have reported that two or more fruit juices/pulp may be blended in various proportions for the preparation of nectar, RTS beverage, etc.

Keeping in view of these facts, an attempt has been made in present investigation titled “Studies onstandardization and storage of aonla based beetroot blended RTS beverage” with the following objectives:

1. To study value addition of aonla based beetroot blended RTS beverage.
2. To study nutritional value of aonla based beetroot blended RTS beverage.

CHAPTER-II
REVIEW OF LITERATURE

CHAPTER - II

REVIEW OF LITERATURE

Fruit and Vegetable juices are valuable source of antioxidants because it contains a significant amount of bioactive compounds. Aonla and beetroot possesses wide range of compounds like flavonoids, phenolic acid, amino acid, ascorbic acid, to copherol and pigments. The antioxidant rich extracts of these two for the preparation of refreshing, thirst quenching & energizing ready to serve beverage that not only improve the health but also fulfils the nutritional requirements.

This chapter deals with comprehensive review of literature relevant to study. It is very necessary to have the thorough knowledge of the literature available related to the present investigation entitled **"Studies on standardization and storage of aonla based beetroot blended RTS beverage"**. It is an essential and important part of scientific investigation with purpose to determine the previous work done and to assist in delineation of objective of hypothesis and research procedure to be followed. The related past studies on this topic are limited. The scattered literature is mentioned under following headings:

2.1 Physicochemical and Nutritional properties of aonla and beetroot
2.2 Medicinal and health benefits of aonla and beetroot
2.3 Processing and value addition of aonla and beetroot
2.4 Formulations and developments of RTS beverages
2.5 Chemical composition of RTS beverage
2.6 Storage stability of RTS beverages
2.7 Microbial analysis of RTS beverages

2.1Physicochemical and Nutritional properties of aonla and beetroot

2.1.1 Aonla

Manny and Swamy, (1997) concluded that Aonla fruit is one of the richest known sources of ascorbic acid (300- 1000mg per 100g edible portion) depending upon the cultivar and location.

Pragathi and Dhawan (2001) evaluated fresh Aonla fruits of cultivar Chakaiya for their physical and chemical characteristics and the fruits were found to be rich source of Ascorbic acid (454.4 mg/100g) and appreciable source of total sugars (7.43 g/l00g), calcium (14.9 m/l00g), iron (0.62mg/100g) and phosphorus (11.81 mg/100g) and reported that this has great potential for processing.

Tewari *et al*., (2001) studied physical characteristics of Aonla fruits, they were nearly spherical or globular, 1.3-1.6 cm in diameter, globose with smooth surface, pale yellow with six obscure vertical furrows enclosing 6 trigamous seeds in 2 seeded three crustaceous cocci.

Basak, (2005) reported aonla (*Emblica officinalis Gaertn*) is a most encouraged medicinal minor fruit crop. Being one of the most cherished fruits in Ayurveda and consumed in various forms, having high amount of ascorbic acid 600 mg/100g of fruit. Fruits also contain fat, Phyllemblin and tannin, besides being rich in mineral matters like Phosphorous, iron, calcium and appreciable amount of Pectin.

Mall and Tandon (2005) carried out investigation on blending of pulp of guava fruit in different ratios with that of aonla to improve the flavour and acceptability of the prepared ready-to-serve (RTS) beverage. The beverages having 10 per cent pulp, 12 or 14 per cent TSS and 0.22 per cent acidity were prepared, filled in pre-sterilized glass bottles; heat processed and stored up to three months under ambient conditions. Chemical analyses and sensory evaluation of RTS beverages were carried out at 0, 45 and 90 days of storage. There were little changes in the quality parameters, viz. TSS, titratable acidity, ascorbic acid, total and reducing sugars and tannins contents during storage. Organoleptic quality score was highest in pure guava beverage having 14 per cent, TSS followed by guava: aonla (80:20) blended beverage having 12 per cent TSS during 90 days of storage. The results indicated that as the concentration of aonla pulp in the beverages increased their acceptability decreased.

Radha and Mathew (2007) opined that, the Aonla is predicted to be the fruit of 21st century, is indigenous to tropical South eastern Asia precisely the central and Southern India. Aonla fruit contains 89% pulp, 0.8-2.0% fibre, 10-14% total soluble solids, 1.4-2.4%acidity, 700-900mg/100g vitamin C, 0.4-3.10% pectin and 2-3% Phenols.

Khan (2009) recorded 7.53mg/100g of total sugar in fresh fruit of aonla and it is also appreciable source calcium (14.91mg/100g), iron (0.62 mg/100g), phosphorus (11.81 mg/100g). he also noticed the antioxidant properties of this fruit as it is the rich source of vitamin C.

Kumar *et al*., (2009) carried out a study on preparation of nectar from aonla-pineapple blended. The nectar was prepared using 20 per cent blended juice, 15 per cent TSS and 0.3 per cent acidity at the time of preparation in all the formulated blends. They concluded that the best combination of the blend for nectar was 50% pineapple + 50% aonla with maximum organoleptic score. They also found TSS, total sugars, reducing sugars, organoleptic rating increased as where non reducing sugar, ascorbic acid and acidity decreased during the storage.

Kothari and Bhatnagar (2010) investigated the development and quality assessment of aonla-pineapple and aonla-sweet lime juice blends. The juice was blended with each juice in varying proportion with addition of spices (black salt, cardamom and ginger juice). They have observed that the most acceptable blends were aonla- pineapple (15% + 85%) with 0.35% black salt and aonla- sweet lime (20% + 80%) with 0.5% black salt. Overall acceptability of blended juice was found maximum and nutritionally superior in the terms of ascorbic content compared to sole.

Syamal *et al*., (2011) studied blended nectar of bael and aonla fruits. They found that the nectar blended with 50 per cent aonla and 50 per cent bael pulp at 15 per cent TSS was rated best with good storage stability up to 60 days with respect to increased level of reducing sugars and total sugars whereas decreased level of acidity.

Simirtha *et al*., (2013) mentioned that aonla is highly nutritious and is an important dietary source of vitamin-C. So they conducted an experiment to develop vitamin-C enriched fruit drink from aonla which was blended with two different concentrations of aonla, apple, watermelon juice, ginger and sugar i.e. V1 and V2 in the ratio (45:25:15:1:15 and 35:30:20:1:15, respectively). The result revealed that the treatment V2 i.e. 35:30:20:1:15 was more acceptable.

Singh and Singh (2014) carried out investigation with the objective to develop aonla based low calorie blended nectar from aonla, mango, guava, jamun and jackfruit using artificial sugar for low calorie. Blended nectar with ratio of 25 per cent aonla pulp + 75 per cent mango pulp was found to be best and imparted good flavour and colour and also scored highest organoleptic quality, which was followed by the 50:50 ratio of aonla : mango as well as 75:25 ratio of aonla : guava.

Kumari and Khatkar (2016) studied polyphenols, antioxidants and antimicrobial properties of aonla where he evaluated phenolic content, antioxidant activities and antimicrobial activities of methanolic, ethanolic and ethyl acetate extracts of five different varieties of aonla (*Emblica officinalis*) fruits as well their powders. Total polyphenolic content in fresh aonla fruit extracts varied from 70.6 to 159.4 mg GAE/g and their EC 50 (effective concentration) values for antioxidant activity ranged from 46.72 to 359.7 pg/ml.

Kulkarni *et al*., (2017) detailed fruits of aonla have average diameter of 38.80 ±2.18 mm (vertical) and 33.28 ± 1.53 mm (horizontal). Average weight of whole fruit, pulp and seed were 31.80 ± 5.96 g, 28.21 ± 4.83 g and 2.48 ± 0.26 g respectively. Specific volume and specific gravity of the fruits were 29.50 ± 4.97 ml and 1.07 ± 0.06 respectively. Fresh aonla juice contained 120.95 mg/100 ml of ascorbic acid with acidity of 2.34 % and pH of 1.97. TSS, reducing and total sugars of the juice were found to be 12.7 °B, 7.6 % and 7.91 % respectively. Tannin content of the fresh juice was higher (1.45 %) which contributed for astringent taste to the juice.

Singh *et al*., (2018) stated aonla fruit is nutritious and is a rich source of Vitamin-"C(600-800 mg/100g pulp). Ascorbic acid and other constituents are well retained in dried aonla fruits. Apart from ascorbic acid, it is also a rich source of pectin, polyphenols. Potassium and iron content are relatively high in the fruit. The fresh fruit contains as much as 4.45% tannin compounds of which ellagic acid, gallic acid, corilagin etc. are important. The fruit contains an appreciable amount of linoleic acid, kaempferol, quercetin, and rutin.

Aditya *et al*., (2021) reported aonla has the richest source of vitamin C, it has 500 to 1500mg of ascorbic acis per 100g of fresh aonla pulp and it also had other nutrients such as polyphenols, pectin, iron, calcium and phosphorus. However, owing to its highly acidic and astringent taste, low total soluble solids (TSS), and poor flavor and color, it is not

popular as a table fruit.The edible fruit tissues of aonla contain about 3 times more protein and 160 times more vitamin C as compared with apples. Normally, a single aonla fruit contains 20 times more vitamin C than two oranges.

2.1.2 Beetroot

Buyanov and Voronyuk (1985) concluded that the physical properties of table beet were: length of root 5.7-6.2 cm. diameter of roots 5.2-6.2 cm, mass of root 110-151 g and the length of top (leaf cluster) 17-27 cm, diameter of top (leaf cluster) 2.6-2.9 cm and mass of top (leaf cluster) 54-75g.

Zaalouk (1994) analyzed the physical properties of sugar beet were length of root 29.14 cm, diameter of lo.82 cm, mass of root 1910.7 g and diameter of top (leaf cluster) 8.32cm. vegetative growth height of 43.61 cm and leaves mass 1033.2gr. They also concluded that the physical properties of table beet were: length of root 5.7-6.2 cm. diameter of roots 5.2-6.2 cm. mass of root 110-151 g and the length of top (leaf cluster) 17-27 cm. diameter of top (leaf cluster) 2.6-2.9 cm and mass of top (leaf cluster) 54-75g.

Bose (1999) reported that the shape of beetroot is globular or round, the color of beetroot is dark red, the average weight of peel is 20 g, the average width of beetroot is 8 cm and the average weight of beetroot is 241 g. The chemical composition of beet root varies according to environmental condition and variety, the edible portion of beetroot contains 86.90 per cent water, protein 1.66 g, carbohydrate 9.7 g, fats 0.18 g and ash 0.8 g, while calcium 16 mg, iron 0.8 mg, sodium 77 mg, potassium 305 mg, magnesium 23 mg, vitamin C 3.6 mg, thiamine 0.7 mg, riboflavin 0.4 mg

Shyamala and Jamuna (2010) reported the moisture content of samples ranged from 79 - 84 percent. The protein content was high in beetroot (13.23 mg/ 100g) and low in carrot (6.21mg/100g). Total polyphenols were higher in methanol extracts of samples (220-250 mg 100g) compared to ethanol and aqueous extracts. The antioxidant activity determined by the DPPH method exhibited 40 percent and 78 percent activity in methanol extracts of carrot and beetroot pulp waste (20 mg) respectively.

Straus *et al*., (2012) determined the internal quality (the total phenol, sugar, organic acid contents, antioxidant activity, and mineral components). Organic cropping of red beetroot plants significantly reduced the yield by 27 percent of some macro minerals (P at 23.1. K at 13.1. and Mg at 7.7 %) in comparison with conventional cropping but increased the ascorbic acid by 23.3 percent, antioxidant activity by 30.3 percent and some micro minerals (Na at 39.1. Cu at 5.0. Fe at 17.9, Mn at 3.4, and Zn at 2.1%).

Odoh and Okoro (2013) shown proximate composition analysis of beetroot indicates that it contains 1.35. 0.3, 1.9. 2.56, 87.4 and 1.4 percent of protein, fats, and oils, dietary fibre, total fibre, moisture, and ash value respectively, 0-carotene (11.64 mg100g) and energy (42kcal). The elemental analysis also indicates the presence of the following minerals: iron, magnesium, copper, sodium, potassium, manganese, calcium and zinc in these ratios 0.76, 18.60, 0.08, 73.60, 31.20, 0.86, 13.80 and 0.29mg/100g respectively. Vitamins found were vitamin A (2.6pg/100g), vitamin K (3.2pg/100g), vitamin C, vitamin E, vitamin B3, vitamin B6, vitamin B2, vitamin B, panthotenic acid and cholesterol (4.36, 0.18, 0.35, 0.03, 90.053, 0.034, 0.151 and 0.04 mg/100g respectively).

Chawla *et al*., (2015) reported that Beta vulgaris (Chenopodiaceae) is generally known as beet root or garden beet. Beet root is most commonly dark red in colour, however it also come in hues ranging from white to yellow to a "candy cane" red and white variety known as Chioggia.

Dambalkar *et al*., (2015) studied proximate analysis of beetroot that carbohydrates 8.8 percent: protein 1.7 percent; fat 0.1 percent; minerals 0.8 percent; vitamins 5.2 percent; moisture 87.7 percent; fibers 0.9 percent.

Kumar (2015) noticed the beetroot is the taproot portion of the beet plant. It is an excellent food which impart very important role for the development and growth of human body. It also acts as fruits as well as vegetables. Fresh form of beetroot consumed generally as a salad.

Burcu *et al*., (2016) studied the effects of home-processing on the antioxidant properties and in vitro bioaccessibility of red beetroot bioactives were investigated. This study provides comparative data to evaluate the effects of various home-processing techniques on antioxidant potential of red beetroot products.

2.2 Medicinal and health benefits of aonla and beetroot

2.2.1 Aonla

Singh *et al*., (2008) assessed the activation and mutagenecity of 2 Acetamidofluorene (2AAF) was inhibited by *Emblica officinalis*. It also supress the cytochrome P-450, aniline hydroxylase. The antimutagenic potential of different solvent viz; water, acetone and chloroform extracts of *Emblica officinalis* has been evaluated on sodium azide and 4-nitro-o-phenylenediamine induced his revertants in TA100 and TA97 tester strains of Salmonellatyphimurium. From the study it is revealed that chloroform extract was less active compared to water and acetone extracts.

Mitra and Pathak, (2008) aonla fruit is one of the rich sources of vitamin-C (500-700 mg/l00g), pectin and minerals like iron, calcium and phosphorous and known for its nutraceutical, antioxidant and anti-inflammatory properties. It possesses antibacterial, anticarcinogenic, antiemetic, antioxidative, antipyretic, antitumour, antiviral and expectorant activities.

Akhtar *et al*., (2011) evaluated the anti-hyperglycemic and lipid-lowering properties of *Emblica officinalis Gaertn*. fruit in normal and diabetic human volunteers. The results indicated a significant decrease ($P < 0.05$) in fasting and 2-h post-prandial blood glucose levels on the 21st day in both normal and diabetic subjects receiving 1, 2 or 3 g *Emblica officinalis* powder per day as compared with their baseline values. Significant ($P < 0.05$) decreases were also observed in total cholesterol and triglycerides in both normal and diabetic volunteers on day 21 that were given either 2 or 3 g *Emblica officinalis* powder per day. However, diabetic volunteers receiving only 3 g *Emblica officinalis* powder exhibited a significant ($P < 0.05$) decrease in total lipids on day 21. Both normal and diabetic volunteers receiving 2 or 3 g *Emblica officinalis* powder significantly ($P < 0.05$) improved high-density lipoprotein-cholesterol and lowered low-density lipoprotein-cholesterol levels.

Srivasuki (2012) reported aonla is assessed for its unique tannins and flavanoids which exhibit strong powerful antioxidant properties. The inhibition of tumour incidences by fruit extract of this plant has been evaluated on a two-stage process of skin carcinogenesis in Swiss albino mice. The chemo-preventive potential of aonla fruit extract on 7, 12-dimethylbenz (a) anthracene (DMBA) induced skin tumori-genesis in Swiss albino mice have been found. Triphala indicates its potential use as an anticancer drug for clinical treatment.

Kumari and Khatkar (2016) studied antimictobial properties in different aonla varieties where they observed methanolic extracts of various varieties had maximum TPC and antioxidant activity. Variety NA-7 showed high TPC and antioxidant activity. Almost, similar trend was observed among the extracts of aonla powders for TPC and AOA (antioxidant activity). A high positive correlation coefficient existed between TPC and AOA of different aonla extracts. All the extracts analyzed, exhibited a strong antimicrobial potential against E. coli, Salmonella typhi, Staphylococcus aureus and Candida albicans. This study suggests aonla as potential natural source of antioxidants and antimicrobial agents.

Singh *et al*., (2018) reported that aonla used in curing chronic dysentery, bronchitis, and diabetes in the traditional Indian system of medicine. The fruits of aonla possess expectorant, purgative, spasmolytic, antibacterial, hypoglycaemic, hepatoprotective and hypolipidemic activity. The aqueous extract has been reported to have anti-pyretic laxative and tonic properties and also showed antibacterial activity. The fruit has a very high content of ascorbic acid and is analgesic, antiinflammatory, and antipyretic. Vitamin- “C” is also antihepatoxic, antinephrotoxic, antioxidant, and promotes chromosomal stability. The fresh fruit contains as much as 4.45% tannin compounds of which ellagic acid, gallic acid, corilagin, etc. are important. Ellagic acid is antimutagenic and anticarcinogenic. Gallic acid scavenges free radicals generated by various metabolic processes. The fruit contains (-) epicatechin which is hypoglycemic, antiinflammatory and its antiviral action is effective against Moloney murine leukaemia virus.

Aditya *et al*., (2021) noted some of the medicinal properties of the aonla as it is a potent antioxidant, hypolipidemic and antibacterial; it has antiviral and antacid properties. It is used in treating many diseases such as diabetes, cough, asthma, bronchitis, headache, ophthalmic disorders, dyspepsia, colic, flatulence, skin diseases, leprosy, jaundice, scurvy, diarrhoea, greyness of hair and cancer (e.g., liver cancer and skin cancer). The fruit is used as an antiscorbutic, diuretic and laxative, and for treating the common cold, gastric troubles, acidity, scurvy, dysentery, bronchitis, diabetes, jaundice and dyspepsia. Dried fruits are useful in chronic dysentery, diarrhea, diabetes, dyspepsia, cough, anemia and jaundice. Aonla is one of the three constituents of the famous ayurvedic preparation, triphala, which is prescribed in many digestive disorders

2.2.2 Beetroot

Jajja *et al*., (2014) reported that inorganic nitrate and beetroot juice supplementation are associated with decreased systolic blood pressure (BP), these results have primarily been obtained from short-term trials that focused on healthy young adults. Therefore, the oral supplementation of beetroot juice concentrate would decrease systolic BP in overweight older participants but that the decline in BP would not be sustained after a 1-week interruption of the beetroot juice supplementation.

Kuru (2014) studied the health benefits of tamarind. It is preferred to be used for abdominal pain, diarrhea and dysentery, some bacterial infections and parasitic infestations wound healing, constipation and inflammation.it is a rich source of most of the essential amino acids and phytochemicals, and hence the plant is repotted to possess antidiabetic, antimicrobial, antivenomic, antioxidant, antimalarial. cardioprotective, hepatoprotective,antiasthmatic, laxative and anti-hyperlipidemic activity. Tamarind has ameliorative effects on many diseases.

Meher *et al*., (2014) repotted that currently there has been an increased interest globally to identify plants and explore there therapeutic potential. As because drugs which obtained from nature pharmacologically potent and have low or no side effects for use in preventive medicine and the food industry. It discussed about the therapeutic potential and chemical constituents of Tamarindusindica. It is available all over the country. Tamarindusindica is

having some reported activities like antidiabetic, hypolipidemic, hepatoprotective and antimicrobial properties. This plant is consumed by rural people as vegetable.

Pinna *et al*., (2014) reported the beneficial effects of beetroot juice supplementation which have been tested during cycling, walking, and running. The purpose of the present study was to investigate whether beetroot juice supplementation can also improve performance in swimmers. Fourteen moderately trained male master swimmers were recruited and underwent two incremental swimming tests randomly assigned in a pool during which workload, oxygen uptake, carbon dioxide production, pulmonary ventilation, and aerobic energy cost of swimming were measured.

Clifford *et al*., (2015) studied that beetroot is also being considered as a promising therapeutic treatment in a range of clinical pathologies associated with oxidative stress and inflammation. Its constituents, most notably the betalain pigments, display potent antioxidant, anti-inflammatory and chemopreventive activity in vitro and in vivo.

Menezes *et al*., (2016) stated tamarind is a plant that can be used traditionally in wound healing, snake bite, abdominal pain, colds, inflammations, diarrhea, diarrhea, helminth infections, and fever. It may also play a role as antimicrobial, antidiabetic, antiinflammatory and effects on the control of satiety, playing a potential role in the treatment or prevention of obesity and other chronic diseases. These effects are probably due to the presence of polyphenols as n-Hexacosane, eicosanoic acid, b-sitosterol, octacosanylferulate, 21-oxobehenic acid, and pinitol and phenolic antioxidants for proanthocyanidins.

Miraj (2016) noticed that beetroot possess anti-inflammatory effect, antioxidant Properties, anti-stress effect, anti-anxiety and anti-depressive effect, anti-cancer, antihypertensive effect, hydrophobic properties, anti-sterility effects. The result of this study has found various constituents of Beta vulgaris exhibit a variety of therapeutic effects with little or no associated toxicity.

2.3 Processing and value addition of Aonla and beetroot

2.3.1 Aonla

Jain *et al*., (2004) developed an RTS containing the blend of aonla juice and Pusa Navrang grape juice in different ratio. Indian gooseberry juice contained the highest vitamin C (478.56 mg/100 ml). Hence, when gooseberry juice was blended with other fruit juice for the preparation of ready-to-serve (RTS) beverages, it boosted their nutritional quality in terms of vitamin C content. On the basis of overall sensory quality and vitamin C content, RTS beverage prepared by blending gooseberry and Pusa Navrang grape juice in 20:80 ratio was found to be the best.

Chandan *et al*. (2010) prepared an aonla RTS beverage with drained aonla syrup. They obtained the drained syrup from blanched slices of aonla steeped in salt for 2 h, followed by steeping in 70 °Brix syrup for 24 h, and adjusted it to 20 °Brix containing 2% lime juice + 1% ginger juice, which was found to be acceptable, with good organoleptic scores.

Pareek and Kaushik (2012) studied the effect of drying method on quality of aonla powder during storage. Among 4 drying methods, fluidized bed drying found best. At the start of experiment dried powder with fluidized bed drying had highest ascorbic acid content (272.74 mg / 100 g), total sugars (39.41 g / 100g), reducing sugar (33.69 g / 100g), and lowest content of tannin (8.8 g / 100g), browning index (0.02 OD at 440 nm) and acidity (9.94 g / 100g). The quality parameters deteriorated under storage irrespective of the drying method and ascorbic acid steadily lost during 90 days of storage. However, powder making with fluidized bed drying was acceptable even after 90 days of storage and contains 205.5 mg / 100g ascorbic acid. It is recommended that aonla can be dried with fluidized bed drying at a temperature of 65°C with air velocity of 90 m/min.

Vijaykumar *et al*., (2013) stated that there are numerous products that can be prepared from dry aonla fruits such as dried whole fruit, flakes, slices, supari, shreds and powder. Blanching with hot water or with potassium metabisulfite before drying prevents enzymatic browning and improves the color and texture of the shreds. Solar drying is cheaper, whereas hot air oven drying is a more convenient method of removing moisture from the product.

Singh *et al*., (2018) prepared aonla candy where he concluded that the treatment R3D1 (80 % sugar solution and sun drying) was best for the chemical characteristics of aonla and citrus peel candies because the sun drying regulates the process of osmosis and concentration of sugar in the candies. Different drying methods reduce the growth and development of microbes in the product during storage. Hence, it is the best method for the value addition of the fruits like aonla for earning the more income.

Singh and Singh (2019) evaluated various recipes for commercial processing of new aonla products viz. herbal squash, herbal jam, candy and toffee. The results reveal that the composition of 25 percent aonla pulp+5 percent asparagus root extract + 2 percent ginger juice with 50 percent total soluble solids (TSS) and 1.2 percent acidity for herbal squash; 50 percent aonla pulp + 5 Percent asparagus root extract + 2 percent ashwagandha extract with 68 percent TSS and 1.2 % acidity for herbal jam; candy with pectin coating particularly segmented candy; 55 percent aonla pulp + 2.5 percent butter + 0.5 percent custard powder and 42 percent sugar for toffee was found excellent for preparation of value added products from aonla fruits. The recipes developed for commercial processing of new aonla products will be helpful for establishing agro processing industry so that produce could be utilized in an effective manner.

Garg *et al*., (2021) developedanaonla based bael fortified fermented beverage where the gooseberry juice was extracted by first crushing the fruits using fruit mill and then pressed with hydraulic press to get the juice. Two separate batches of ameliorated bael pulp containing 0.25 per cent bael leaf extract and 10 per cent gooseberry juice were prepared for fermentation under ambient conditions.

2.3.2 Beetroot

Azeredo (2009) noticed consumers are increasingly avoiding foods containing synthetic colourants, which lead food industries to replace them by natural pigments, such as carotenoids, betalains, anthocyanins and carminic acid. Betalains are water-soluble nitrogen-containing pigments, composed of two structural groups: the red-violet betacyanins and the yellow-orange betaxanthins.

Amnah and Alsuhaibani (2013) used the red beet roots powder and extract in the preparation of biscuits and the effect of consumption of these biscuits on injured liver in experimental rats were studied. The results revealed that beet powder or beet extract incorporation to biscuits increased protein and ash contents as well as fiber, moisture contents and caloric value.

Nisa *et al*., (2015) extracted natural red dye from red beetroots for coloring the food products instead of the synthetic colors to avoid the harmfulness to humans. The unique crimson red color of red beet acts as best natural dye. The prepared red dye paste exhibits the highest DPPH radical scavenging activity (33.32%) with water extract (lOmg/ml). The stability of red dye depends on temperature and high temperature decreases its stability.

Amirasgari and Mirsaeedghazi (2016) evaluated red beet extract was concentrated using osmotic distillation method, and the best pretreatment was evaluated to increase the efficiency of the concentration process. According to the results, osmotic distillation can produce red beet concentrate with more nutritional value than the concentrate produced with thermal method.

Guine *et al*., (2016) developed different jams. It produced from pear jam, pear with beetroot, apple, apple with beetroot and finally apple with beetroot and cinnamon. The results obtained revealed that the apple jam with beetroot and cinnamon had a low sugar content and a high antioxidant activity, enhancing the potential health benefits.

Singh and Hathan (2016) studied the beetroot cubes were osmotically pretreated in 60°Bx of sucrose solution at 55°C osmotic solution temperature for 180 min with fruit to solution ratio 1:4 (w/w). The osmotically dehydrated Beetroot cubes were further dehydrated convectively at different drying air temperatures of 55, 65 and 75°C up to final moisture content of 9 per cent (w.b). Finally, osmo-convectively dried beetroot cubes were coated with sucrose for candy preparation.

Ingle *et al*., (2017) analyzed the nutritional qualities of cookies with incorporation of different levels of beetroot powder i.e., 0, 5, 7, 10, 15 and 20 %, and examined for its physical and chemical composition. The proximate composition of cookies enriched with beetroot powder from 5 to 20 per cent indicated that protein was increased from 7.39 to

9.12 per cent, crude fibre 0.95 to 1.90 per cent and ash content 0.93 to 1.89 per cent. The incorporation of beetroot powder in cookies lowered the lightness (L*) and yellowness (b*) but increased redness (a*) of cookies.

Hailu and Mekonnen (2017) was focused on the effects of yeast and oxygen on quality parameters of wine produced from Ethiopian beetroot such as Ethanol. Volatile Acidity, Titratable acidity. Specific Gravity and pH. The basic ingredients used for fermentation were water, sugar, yeast, beetroot juice and citric acid. Finally, it can be concluded that beetroot juice treated with pure water, yeast, acid and sugar under anaerobic fermentation is produced wonderful wine.

2.4 Formulations and developments of RTS beverages

Dhamsaniya and Varshney (2013) investigated the developed delicious and nutritious RTS beverage from the ripe banana juice and milk whey. The M. anensis extract was used as a natural flavoring agent. The proportion of banana juice, M. arvensis extract and milk whey was varied from 5-15 ml. 1-5 ml and 72-86 ml per 100 ml of the prepared beverage, respectively. As a result of various studies conducted for optimizing the proportions; an acceptable whey banana RTS beverage was prepared having 15 ml banana juice, 3 ml M. arvensis extract, 8 g sugar powder and 77 ml milk whey per 100 ml of the prepared beverage, respectively.

Sindumathi and Premalatha (2013) noticed feasibility of blending papaya and pineapple juice in combination with different naturally flavored extracts in different ratio for preparation of flavored and blended Ready-to-Serve beverage. Extracts of ginger and cardamom in the ratio of 1:1 was found to be more suitable as flavoring agents in papaya and pineapple blended RTS beverages.

Singh *et al*., (2014) developed aonla based low calorie blended RTS and squash from aonla, mango, guava, jamun and jackfruit using stevia for low calorie. A blend containing 25 per cent aonla pulp and 75 per cent mango pulp was found better for the preparation of RTS and squash both. In the preparation of low calorie RTS and squash half amount of the sugar can be successfully into juice and treated with 0. 5, 10, 15 and 20 percent sugar syrup. Twenty percent (20%) sugar syrup was further used with lemon

(30%), ginger (0, 2, 4 and 6%), ehuru (Mondoramyristica) (0, 2, 4 and 6%) and a blend of ginger and ehuni (1:1) in the treatment of beetroot juice. Sensory result of beetroot juice samples treated with indigenous spices showed that 4 percent ginger and 2 percent "ehuru" successfully improved all the sensory attributes more than the control sample.

Panjiar *et al*., (2015) developed a ready-to-serve (RTS) beverage using tamarind dried fruit. The results of physico-chemical analysis revealed that TSS, acidity and reducing sugar content increased while the ascorbic acid content decreased with the advancement of storage period. From the results of quality assessments, the formulated beverage with 12 per cent blended juice of tamarind and ginger (3:1), 21 per cent TSS and 0.3 per cent acidity was found superior in quality and could be stored at ambient conditions for a period of five months without any significant changes in quality.

Zaman *et al*., (2016) studied color properties of pineapple and mango juice blends at ratio of 70P:30M; 50P:50M; 30P:70M and evaluated in term of L*, a*, b* hue, Chroma, color difference (AE). Physicochemical properties of juice blends ratio of 70P:30M also give more perishable results of pH (4.32) titratable acidity (0.66% malic acid), total soluble solid (13.67), vitamin C (54.25 mg ascorbic acid/100 ml), and turbidity (438 NTU).

Hossain *et al*., (2017) developed functional ready-to-serve (RTS) beverage was prepared from jackfruit and aloe vera juice. The blended juice was made by using the different combination of jackfruit and aloe vera juice as TO (100:0), T1 (90:10), T2 (80:20). T3 (70:30) and T4 (60:40) to improve the flavour and therapeutic properties. The blends were homogenized and filled into 200 ml colourless sterilized PET bottle and pasteurized at 85°C for 10 minutes, cooled and stored at refrigerated temperature. The blended RTS beverages were prepared to optimize the mixed ratio of jackfruit and aloe vera juice.

Sarkar and Bulo (2017) standardized process for blending of guava pulp with pineapple juice for preparation of ready-to-serve (RTS). The added 50 per cent guava pulp and 50 per cent pineapple juice improved the drink quality with special reference to TSS (15.54 °Bx), total sugar (8.65%) and TSS acid ratio (49.85). But the ratio of 60 per cent guava pulp and 40 per cent pineapple juice reached the highest sensory characters for

overall acceptability (7.74 score out of 9 point hedonic scale) with 15.29 °Bx TSS and 0.317 per cent acidity.

Balaji and Purnima (2018) formulated RTS by blending different concentrations of kinnow, aonla and aloe vera gel with calculated amount of sugar, water, citric acid and preservative KMS (350 ppm) and he concluded that the treatment combination 5% Kinnow + 70% Aonla juice + 25% Aloe vera gel was found to be the most suitable in terms of quality and sensory scores was remained acceptable up to 4 months (120 days) under ambient storage conditions.

2.5 Chemical composition of RTS beverage

Chandan *et al*., (2010) prepared an aonla RTS beverage with drained aonla syrup. They obtained the drained syrup from blanched slices of aonla steeped in salt for 2 h, followed by steeping in 70 °Brix syrup for 24 h, and adjusted it to 20 °Brix containing 2% lime juice + 1% ginger juice, which was found to be acceptable, with good organoleptic scores.

Jan and Masiha (2012) developed a blend of pineapple, carrot and orange Juice. Physicochemical characteristics were evaluated. Marginal changes in pH, total soluble solids, acidity, vitamin C and beta-carotene were observed. The beta carotene content of juice was found (1583pg) to be increased with increasing the proportion of carrot juice. Estimation of vitamin C content of sample (19.50mg) showed high improvement in nutritional value of pineapple juice incorporated with carrot and orange juice. The acidity increased (0.97-1.83) and pH of the juice decreased progressively during the storage period. This may be due to the excessive fermentation and presence of lactic acid reducing micro- organism.

Balaswamy *et al*., (2013) revealed that fruit pulps from mango, papaya, sapota, banana; juices from green grapes and pineapple were blended at various proportions (10-60%) by considering their individual properties such as pulpy, juicy, sweetness, sourness and colour to yield palatable smoothies without addition of external sugar and acidulant. Smoothies containing sapota, pineapple and pomegranate showed the highest polyphenol

content of 158 mg/100 g on the day of preparation, which increased to 164 mg TOO g after six months storage period.

Amaravathi (2014) estimated the nutrientritional content of spiced pineapple ready-to-serve beverages during fresh period. The pineapple RTS beverages were processed with extracts of ginger, green chillies, pepper, cardamom and nutmeg. The combined spices extracts such as ginger and pepper, ginger and cardamom and ginger and nutmeg were blended with pineapple juice and prepared the RTS beverages. The spiced pineapple RTS was standardized based on organoleptic evaluation.

Archana and Laxman (2014) standardized the protocol for the preparation of tamarind squash. The chemical composition of tamarind squash and changes in chemical constituents during storage at ambient temperature were studied. Results showed that higher amount of mean TSS (54.13%), reducing sugar (9.51%) and total sugar (30.25%) were noticed in recipe having 30 per cent juice, 50 per cent TSS and 1 per cent acidity, whereas higher retention of ascorbic acid (3.68 mg 100 g-1) was observed in recipe having 30 per cent juice, 40 per cent TSS and 1 per cent acidity. However, minimum TSS (40.48%). reducing sugar (8.82%) and total sugar (22.77%) were noticed in recipe having 25 per cent juice. 40 per cent TSS and 1 per cent acidity and titrable acidity in the recipe having 30 per cent juice, 50 per cent TSS and 1 per cent acidity. Among the recipes the tamarind squash prepared from 30 per cent juice, 50 per cent TSS and 1 per cent acidity was found superior to other recipes. The beverage retained its characteristic colour, aroma and taste up to 3 months of storage at room temperature.

Afreen *et al*., (2016) formulated four formulations of the blends of carrot juice with Sour-orange juices at the ratios of 100:0. 60:40. 50:50 and 40:60 were prepared. The results of the nutritional properties showed that titrable acidity, ascorbic acid, total sugar and TSS increased and pH decreased when increase the sour orange percentage. Carrot juice with Sour-Orange juices at the ratios of 50:50 showed 0.32 per cent titrable acidity. 7.3 mg 100g ascorbic acid, 16.9 per cent total sugar, 16.1° Brix TSS and 3.03 pH.

Balaji and Purnima (2018) prepared squash with the combination of different concentrations of kinnow juice, Aonla juice and aloe vera gel. The TSS, acidity, non-reducing sugars found to increase significantly irrespective of the different concentrations

till the end of four months of storage. Ascorbic acid, reducing sugars decreases gradually with the storage period.

2.6 Storage stability of RTS beverages

Jain and Khurdiya (2009) reported that the increase in non-enzymatic browning (NEB) values in juice pasteurized at higher temperatures was faster during storage than in juice pasteurized at lower temperatures. Aonla juice treated with SO2 alone was least brown as compared to pasteurization at 90 °C + SO2 or pasteurization alone after 6 months of storage. They concluded that Maillard reaction (reaction between sugars and amino acids) might contribute to browning.

Bhattacherjee *et al*., (2011) pasteurized aonla juice at different temperatures among them at 75 and 80 °C were of best quality in terms of least browning and maximum retention of ascorbic acid during 9 months of storage, but juice pasteurized at 75 °C was found contaminated with microbes after 9 months of storage. Hence, it can be concluded from the study that pasteurization temperature of 80 °C was optimum for preservation of aonla juice under ambient condition.

Kumar *et al*., (2012) developed products, RTS beverage and nectar which were stored for 120 days at ambient temperature and studied its storage stability and overall acceptability in terms of variation in sugar, pH, acidity, ascorbic acid, calcium and iron contents at an interval of 30 days. A panel of 10 judges evaluated the products at the end of 120 days storage for their quality attributes like appearance, flavor, taste and overall acceptability. The RTS beverage prepared with 15 per cent juice, l00Bx TSS and 0.30 per cent acidity and nectar prepared with 20 per cent juice, 200Bx TSS and 0.25 per cent acidity were rated as best recipes with highest scores for organoleptic quality.

Profir and Vizireanu (2013) noticed the effect of pasteurization, ohmic heating and lactic fermentation on the storage stability of juice prepared by carrot, celery and beetroot. The juice acidity, pH and vitamin C have been measured during two weeks to quantify the changes occurred on storage after this preservation process. Also, it has been tested the viability of probiotic bacteria in vegetables juice. The lactic fermented juice has been shown a better stability during storage, with a good viability of probiotic bacteria.

Sasi *et al*., (2013) the storage studies revealed that blended therapeutic RTS made from aloe vera gel aonla fruit juice and ginger juice extracts could be successfully stored for the period of four months without significant change in chemical and sensory qualities.

Ramachandran and Nagarajan (2014) studied the quality characteristics and storage stability of the spiced beverage blend and compared with spiced papaya RTS beverage. Periodic analysis was carried out up to five months for various physicochemical parameters, sugar profile, bioactive compounds, microbial quality, instrumental color, and sensory acceptability. The spiced beverage blend exhibited superior quality characteristics compared to spiced papaya RTS beverage both in fresh and in stored samples. The spiced papaya RTS beverage was acceptable up to four months and spiced beverage blend for five months. The results indicate that nutraceutical rich aloe gel (AG) could be successfully utilized to develop functional fruit beverages with improved quality and shelf life.

Kathivaran *et al*., (2015) optimized the beetroot-passion fruit blended juice prepared in bulk, thermally pasteurized and also studied the effects on pigments, antioxidant activity, CIE color, browning index, native microflora and other physicochemical quality parameters were also evaluated during the storage at 27-30 °C. The processing and storage had a significant effect on degradation in the pigments, antioxidant activity during storage. The decrease in antioxidant activity was correlated ($r = 0.9895$) with a decrease in betalain pigment. The product was safe from microflora after thermal pasteurization and during 180 days of storage with good sensory acceptability.

Sharma *et al*., (2016) developed products and analyzed for their bio-chemical, antioxidant potential and sensory quality characteristics at different storage intervals (0, 3 and 6 months). The bitter gourd- kiwi blended squash contained 44.83°B TSS, 1.25 per cent acidity and 37.54 per cent total sugars. Blending has increased ascorbic acid content (33%) and total phenolics (24%) of the blended beverage over bitter gourd squash. The products exhibited only slight changes in their physicochemical, nutritional and sensory characteristics during storage, yet were shelf-stable and remained acceptable up to a period of six months. The high antioxidant potential (68.29%) of the developed product compared to the control sample revealed its health benefits.

Hamid *et al*., (2017) developed RTS beverage from nutritious underutilized mulberry (*Morisalba L.*) fruits. Further standardized product was packed in PET (polyethylene terephthalate) and glass bottles under ambient and refrigerated storage. Minimum decrease was recorded in various chemical characteristics like titratable acidity (0.30 to 0.25 %). anthocyanins (2.90 to 1.89 mg 100 ml), total phenols (21.16 to 16.88 mg/100 ml) and increase in total sugars (10.27 to 11.19 %) was observed after 6 months of storage. Drink could be stored safely for a period of 6 months; however, quality of the product was retained better in glass than PET bottles stored under refrigerated conditions as compared to ambient storage conditions.

Garg *et al*., (2021) conducted sensory evaluation of aonla fortified bael fermented beverage on the basis of parameters like colour, clarity, aroma and taste, revealed that addition of gooseberry juice enhanced the organoleptic qualities of the product. It obtained 8.2 score out of 10 at zero day, followed by 7.9 of leaf extract sample and 7.0 of control. The scores reached peaks at 4 months which might be due to increased clarity of cider as a result of settling of suspended viscous bael particles during aging. After that slight decrease in score was observed this might be due to reduction in vitamin C and phenolic content.

2.8 Microbial analysis of RTS beverage

Bhardwaj and Mukherjee (2011) stated the juice blends were preserved by pasteurization (75°C for 15 min) and by addition potassium meta-bi-sulphite (750 ppm). These blends were stored in 200 ml. colourless glass bottles at room temperature (28 = 4°C) for six months and tested at two months interval for physico-chemical, sensory evaluation and microbial population.

Bhardwaj and Mukherjee (2012) analyzed the prepared juice blend. The ratio of Kinnow juice: Aonla juice: Ginger juice (92:5:3) was best in view of non-enzymatic browning (0.081 to 0.104) and minimum population of bacteria (4.0 x 103), mould (1.5 * 103) and yeast (2.1 x 103) at end of storage (six months). It contained fair amount of vitamin ‘C’ (38.95 mg/100 ml juice) at six months of storage. It was also observed that the addition of ginger juice in blends improves the quality and reduces microbial growth. Further, the juice was found acceptable after six months of storage at room temperature.

Kumar *et al*., (2011) prepared RTS beverages using fermented carrot juice and sweet lime. Carrots were fermented with Streptococcus lactis and Lactobacillus plantarum at ambient temperatures with 2 percent bacteria and 2.5 percent salt for 24-48 hours. There was no microbial growth in RTS beverages prepared with fermented carrots up to 30 days of storage. The increase in microbial load after 45 days of storage was negligible and safe for consumption; however, the increase was substantial in the standard.

Raj *et al*., (2011) revealed that the brix to acid ratio of the beverage was optimum when sand pear juice and apple juice were blended in the ratio of 50:50 to 60:40. Storage of blended beverage containing 50-60 per cent sand pear juice was found more shelf stable during 6 months storage.

Jan and Masih (2012) studied that the heat pasteurization (90 °C for 25 sec) treatment for pineapple juice blended with orange and carrot juice was more effective for inactivating the microbial flora. However, the shelf life of juice was established within 21 days. The product is recommended for children, youth and elderly persons to be used upto 21 days.

Divyashree *et al*., (2013) analyzed juices for the microbial quality with respect to type of organisms and number of colonies by serial dilution technique, pour plate method, gram's staining method and staining for fungi; and physicochemical properties. Results showed that the pineapple juice from two sources was highly contaminated with bacterial pathogens (20x104 cfu/ml). It is contended that contamination was mainly due to poor quality of water used for dilution as well as prevailing unhygienic conditions related to washing of utensils and maintenance of the premises.

Dambalkar *et al*., (2015) evaluated the effect of storage period on microbial quality of RTS made from beetroot, orange and ginger juice. The population of bacteria was 1.7 x 103 cfu/ml at the end of storage of 90 days. So, the product was microbiologically safe during 90 days of storage with good acceptability.

Sangma *et al*., (2016) scrutinized the developed formulations of aloe vera blend by mixing with ginger, sweet lime and amla and also studied its storage stability. The physico-chemical and the sensory quality of the RTS blends were acceptable up to 60 days of

storage. Microbial analysis of the RTS during storage period up to 60 days revealed that it was free from any spoilage.

Garg *et al*., (2021) conducted microbial analysis in aonla beverage which indicated that there was no microbial growth in any of the samples at any stage of storage. Compared to apple cider where the starting TSS is kept up to 14^{o}B.

CHAPTER-III
MATERIALS AND METHODS

CHAPTER - III

MATERIALS AND METHODS

The present investigation entitled **"Studies on standardization and storage of aonla based beetroot blended RTS beverage"** was carried out in Department of horticulture. The analytical study was carried out in Department of horticulture, VNMKV, Parbhani during year 2021-22. Materials used and methods adopted for the present investigation are presented under the following subheadings.

3.1Experimental details

The experiment was carried out in Department of Horticulture VNMKV, Parbhani during year 2021-22.

3.2 Materials required

3.2.1 Raw materials

Fully developed, mature fruits of aonla, tender roots of beetroot were procured from the local market of Parbhani and brought to Department of Horticulture, College of Agriculture, VNMKV, Parbhani during November-2021. Grading was done before washing to select the good quality, green colour, uniform size and fresh aonla for processing and dark red in color, uniform in size and fresh beetroots were selected for processing.

3.2.2 Packaging material

Packaging material (PET bottles) used for packing of juice.

3.2.3 Chemicals used for chemical analysis of RTS

All the chemicals used in this investigation were of analytical grade, which were available at Department of Horticulture, VNMKV, Parbhani

3.3 Processing and analytical equipments used

The processing and analytical equipments included juice grinder, digital refractometer, pH meter and electronic weighing balance facilities available at Department of horticulture, VNMKV, Parbhani.

3.4 Methodology

The present study was conducted for preparation of RTS beverage by blending of aonla, beetroot comprising of different number of treatments. Each treatment was repeated thrice times. Principal steps adopted for the preparation of above-mentioned product are detailed under captions 3.2.1 to 3.2.3.

3.4.1 Raw materials preparation

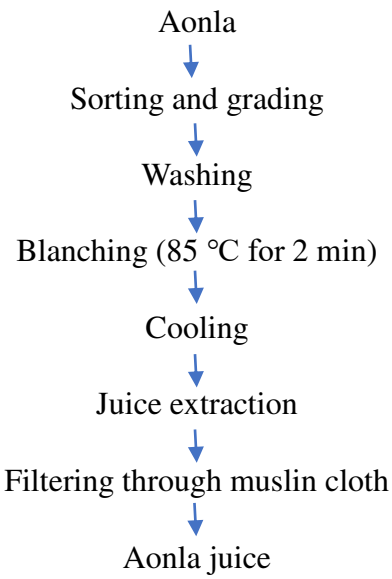
Aonla and beetroot after bringing from market were sorted and graded, then washed to remove dirt particles from their surface and then processed further for extraction of juice to be utilized for preparation of beverage.

3.4.1.1 Methodology for preparation of aonla juice

The fully matured fruits of aonla were used for extraction of juice. The fruits were washed in running water to remove adhering dirt and dust particles. Then washed fruits were steam blanched for 2 minutes. After blanching the fruits were cooled by keeping in running tap water. Then they were sliced into small segments and seeds were separated manually. Then juice was extracted by using screw type juice extractor and filtered through muslin cloth. The juice was further used for preparation of beverage. Principal steps used for aonla juice extraction are illustrated in flow chart below. (Kulkarni 2017).

Plate 4.1 preparation of RTS

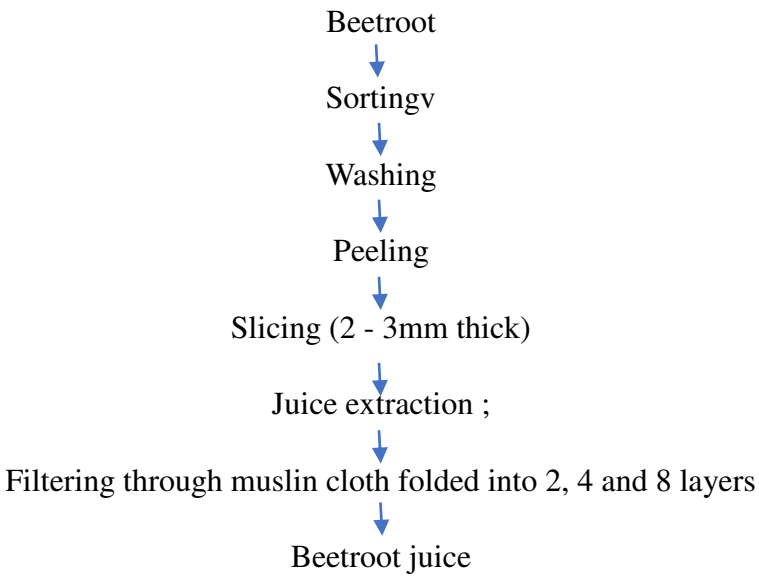
Plate 4.2 packaging of RTS bottels



Flow Sheet 1: Preparation of Aonla juice

3.4.1.2 Methodology for preparation of beetroot juice

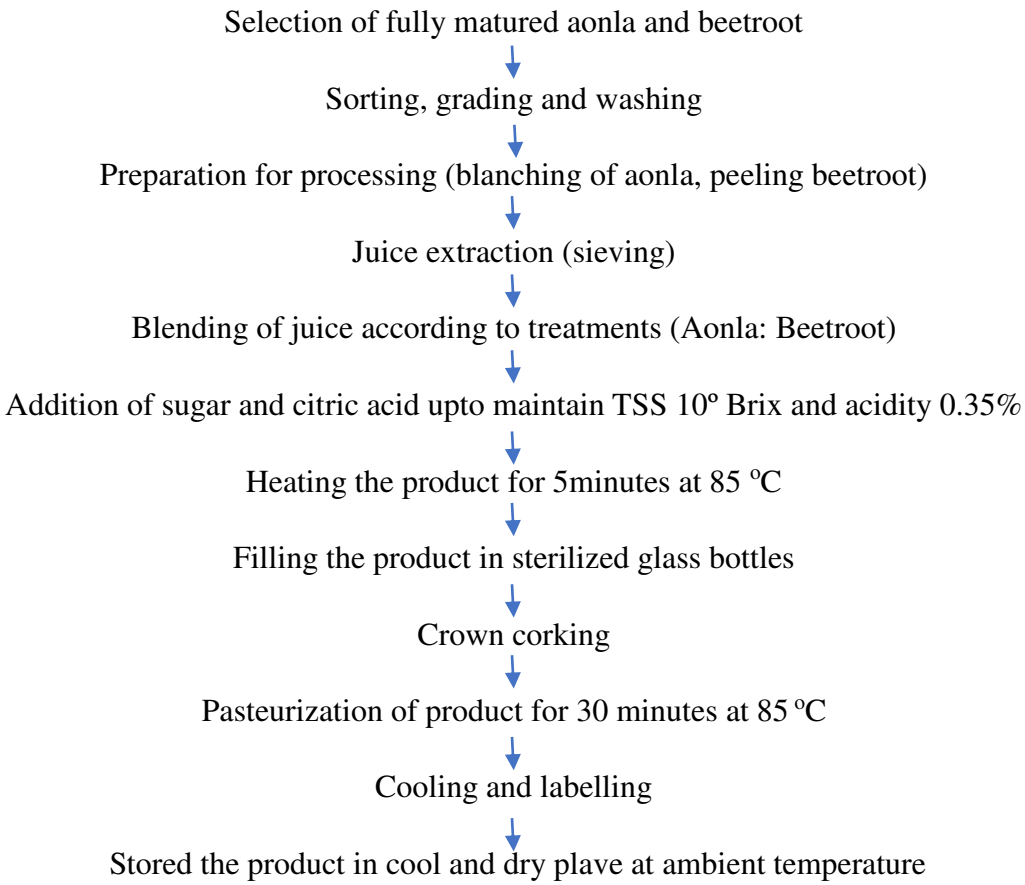
Fresh beetroots were procured from local market of Parbhani. After grading the beetroots washed to remove unwanted impurities like mud, dust and dirt particles. Peel were removed. The peeled beetroots were cut into small slices and passed through mixer with addition of little water. Obtained pulp was passed through muslin cloth to get clear beetroot juice. Beetroot juice was prepared as per the method given by Emelike (2016).



Flow Sheet 2: Preparation of Beetroot juice

3.5 Recipie details

Extraction of both aonla and beetroot juice both the juices were blended according to the treatments ratio. Sugar and citric acid was added to maintain the TSS (10º Brix) and acidity (0.35%) then boiled separately till it reached 85 ℃. After reaching 85 ℃ it was boiled for five minutes then it was filled hot into the glass bottles. Immediately corking of the bottle was done. After filling these bottles were pasteurised for 30 minutes at 85 ºC then allowed to cool and labelled. These bottles were stored at cool and dry temperature.



Process flow chart for preparation of beetroot blended aonla RTS

3.6 Treatment details

Treatments	Fruit Combination	Ratio/treatment
T1	Aonla	100
T2	Aonla and beetroot	95:05
T3	Aonla and beetroot	90:10
T4	Aonla and beetroot	85:15
T5	Aonla and beetroot	80:20
T6	Aonla and beetroot	75:25
T7	Aonla and beetroot	70:30

3.7 Bio-chemical parameters of processed product (RTS)

3.7.1 Total Soluble Solids (°Brix):

The total soluble solids (TSS) were determined with the help of digital brix meter and expressed as °Brix. The TSS of juice or beverage was determined directly by putting a drop of filtrate on the prism of brix meter.

3.7.2 Determination of pH

The pH value of the RTS beverage was determined potentiometrically by means of a digital pH meter. The pH meter was operated according the manufacturer's instructions. First the apparatus was calibrated using buffer of 4, 7 and 9 pH. The digital pH meter probe was immersed in the beverage directly and the pH was recorded.

3.7.3 Determination of titratable acidity

The acidity of sample was calculated as described by Ranganna (1997).

Reagents

1. Sodium hydroxide (0.1N)

2. Phenolphthalein indicator

5 ml of fresh sample was taken in 100 ml volumetric flask and volume made up to 100 ml with distilled water. A measured aliquot (10ml) was then titrated against 0.1 N NaOH using phenolphthalein as indicator to a light pink colour. Here, the blend of fruit juices involve aonlaso the predominant acid is citric acid and hence, acidity was expressed as per cent citric acid. Milliequivalent Factor for citric acid is 0.064.

$$\text{Acidity (\%)} = \frac{\text{Titre x Normality of alkali x Volume made up x Equivalent weight of citric acid}}{\text{Volume of sample taken x Weight of sample taken x 1000}} \times 100$$

3.7.4 Sugars (%):

Sugars were determined by Lane and Eynon methods detailed by Ranganna (1997). Invert sugar reduces the copper in Fehling's solution to red insoluble cuprous oxide. The sugar contents in samples were estimated by determining the volume of the unknown sugar solution required to completely reduce a measured volume of Fehling's solution. Before using, the mixture (1:1) of Fehling's solution A and B (5 ml of each) was standardized against standard glucose for obtaining glucose equivalent and to arrive at using a conversion factor.

3.7.4.1 Reducing sugar (%):

The reducing sugar content was determined by the method given by Ranganna (1986) using Fehling's A and Fehling's B solution.

Standardization of Fehling's Solution

Equal quantities (20 mL each) of Fehling's solution A and B were mixed in a 250 mL conical flask with 100 mL water. The mixed Fehling's solution was then titrated with

standard glucose solution (1%) till blue colour just disappears. Content in the flask was then heated on a hot plate with wire gauge. When liquid begin to boil, 3 drops of methylene blue indicator were added without removing flask from hot plate. Then further titration is continued with glucose solution till the brick red colour is observed and dye colour is decolorized. The volume of glucose solution required to reduce the Fehling's solution was noted as titer value.

$$\text{Fehling's factor} = \frac{\text{Titer value of std. glucose solution x 2.5}}{1000}$$

Preparation of sample

25 g of macerated sample was taken and homogenized with few quantities of distilled water and then transferred to 250 mL volumetric flask. The sample was neutralized with 0.1 N NaOH and decolorized by adding 2 mL lead acetate. After shaking, the sample was allowed to stand for 10 min. The excess lead was removed by adding potassium oxalate and final volume was made upto 250 mL with distilled water.

Assay

The neutralized and decolorized sample was filled in burette and titrated against mixture of Fehling's solution as did for standardization of Fehling's solution. The per cent reducing sugar present in sample was determined by using following formula.

$$\text{Reducing sugar (\%)} = \frac{\text{Fehling's factor x Dilution of sample made x 100}}{\text{Titre value of sample x Wt. of sample x 1000}}$$

3.7.4.2 Total sugar (%):

For the estimate of total sugars, the titrate obtained in the estimation of reducing sugars was used. An aliquot from the filtrate was taken. 10 mL of dilute HC1 was added

and the inversion was carried out at room temperature for 24 h. Subsequently, contents were cooled and neutralized with 40% sodium hydroxide solution using phenolphthalein as indicator and the final volume was made. The solution was filtered and titration was carried out using filtrate as detailed for reducing sugars. The total sugars content was expressed as percentage in terms of invert sugars according to the formula (Ranganna. 1986).

$$\text{Total sugars (\%)} = \frac{\text{Glucose equivalent x Total vol. made up x Volume made up after inversion x 100}}{\text{Titre x weight of sample x aliquot taken for inversion}}$$

3.7.4.3 Non reducing sugars (%)

The amount of non reducing sugars in the sample were calculated using the formula

Non reducing sugars (%) = total sugars (%) – reducing sugars (%)

3.7.5 Ascorbic acid (mg/ 100 ml):

The vitamin-C i.e. ascorbic acid content was determined by dye solution method as detailed by Ranganna (1997). The samples were prepared in 3 per cent metaphosphoric acid solution and then titrated against 2, 6-dichlorophenol indophenol dye solution till pink colour persist for few seconds. Ascorbic acid content was expressed as mg of ascorbic acid per 100 g sample.

$$\text{Ascorbic acid (mg/100 ml)} = \frac{\text{Titre x Dye factor x Volume made up x 100}}{\text{Volume of filtrate taken x Weight of sample taken}}$$

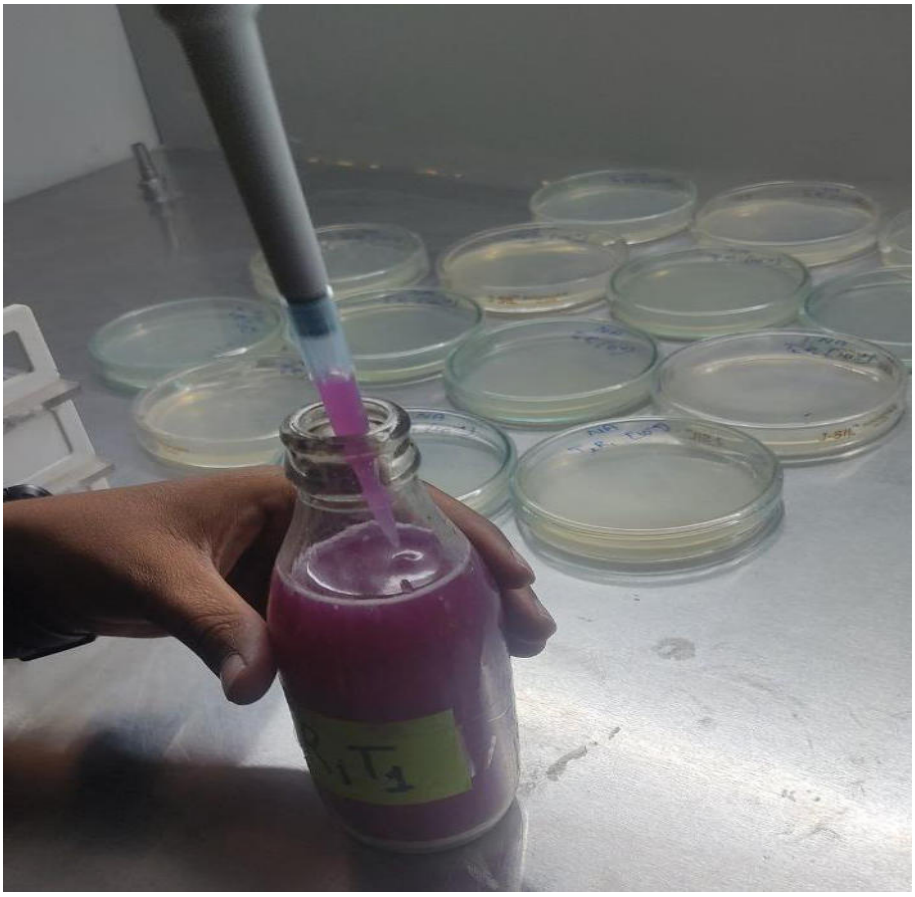


Plate 4.3 Microbial count

Plate 4.4 Estimation of acidity by titration

3.7.6 Colour

Colour of the RTS was measured after the preparation and at the intervals of 30, 45, 60 and 90 days using colorimeter. Colour was measured in terms of L, a, b values.

3.7.7 Antioxidants (mg/ml AAE)

The determination of antioxidants was done by FRAP method (Ranganna 1997) at initial days.

3.8 Organoleptic evaluation of the blended beverage:

Sensory evaluation of RTS was conducted during storage to assess the consumer's acceptance for the products. The prepared samples of beverage were evaluated for sensory qualities on the basis of colour, taste, flavour and overall acceptability on a 9-point Hedonic scale (Appendix-I) according to the method of Amerine et al. (1965).

Sensory panelist's near about 9 to 11 members at time comprised of faculty members and PG students of Department of Horticulture, VNMKV, Parbhani (Maharashtra) were used for sensory analysis throughout the entire period of storage. Coded samples of products were served. Plain drinking water was provided to the panelist's for mouth rinsing in between the sensory evaluation. The organoleptic evaluation were carried out of the beverage was done according to 9 points hedonic scale, for assessing colour, flavour, taste and overall acceptability. Higher product scoring was treated as more acceptable from the quality point of view.

3.9 Microbial analysis of aonla- beetroot RTS beverage

Microbial analysis is the perfect quality assessment protocol performed in food products. These products pose the highest food safety risk and have the shortest shelf life because they are the most susceptible to microbiological deterioration and the possibility of the growth of pathogenic organisms. The results obtained for each count was recorded as colony forming unit per ml of sample i.e. cfu/ml.

3.9.1 Total plate count

Microbial analysis was done to determine total plate count (TPC) of the samples on the nutrient agar media for bacterial count by the method recommended by Harrigan and McCance (1966). Nutrient agar media was prepared and the samples were serially diluted up to IO'5 dilution factor. 0.25 ml of the samples, suspended in saline solution, was transferred to the respective petri dishes of nutrient agar media. Three replicates were taken for each dilution. The inoculated petri dishes were incubated for 48 hours at 37+l°C and total colonies were calculated by the following formula.

$$\text{TPC (cfu/ml)} = \frac{\text{No. of colonies x dilution factor}}{0.25}$$

3.9.2 Yeast and mould count

Microbial analysis was done to determine total yeast and mould count of the samples on the potato dextrose agar media for yeast and mould count by the method recommended by Harrigan and McCance (1966). Potato dextrose agar media was prepared and the samples were serially diluted up to 10^{-5} dilution factor. 0.25 ml of the samples, suspended in saline solution, was transferred to the respective petri dishes of potato dextrose agar media. Three replicates were taken for each dilution. The inoculated petri dishes were incubated in a incubator for 48 hours at 37+l°C for counting of yeast and mould.

3.9.3 Coliform count

The Coliform and basically E. coli are the indicator microbes of water contamination by faeces and therefore it is mandatory to examine the contamination. The Coliform gives red pink colonies on Violet Red Bile (VRB) agar during analysis.

Using the pour-plate technique, appropriately 0.1 ml aliquots was taken in duplicate plates and tempered VRB agar was added. The agar was allowed to solidify and then overlay of about 5 ml of VRB agar was added. Allow agar to solidify. Plates were

inverted and incubated at 35°C for 24 hours. Red colonies surrounded by a zone of precipitate and report as “presumptive coli forms cfu/ml.

3.10 Storage study of aonla- beetroot RTS beverage

The prepared RTS was subjected to storage at ambient temperature. Samples were drowned at specific time interval 30 days, 45 days, 60 days and 90 days. The stated RTS beverage were periodically analyzed for their chemical properties and microbial analysis.

3.11 Statistical analysis:

The experimental data were analyzed by completely randomized design (CRD) according to procedure described by Panse and Sukhatme (1967). The treatment differences were tested by ‘F’ test of significance on the basis of null hypothesis. The appropriate standard errors (S.Em.±) were calculated in each case and the critical differences (C.D.) at 5 per cent level of probability were worked out to compare two treatment means wherever the treatment effects were significant.

CHAPTER-IV
RESULTS AND DISCUSSION

CHAPTER -IV

RESULT AND DISCUSSION

Aonla is well known for its medicinal properties and its consumption has huge health benefits. Beetroot is a rich source of iron and antioxidants and unique compounds like betalins also have their own health benefits. Therefore the present investigation was undertaken to develop the process of beetroot blended aonla RTS with the title "Studies on standardization and storage of aonla based beetroot blended RTS beverage" was conducted in the Department of Horticulture, Vasantrao Naik Marathwada Agricultural University, Parbhani, Maharashtra during 2021-22 are presented in this chapter. The data collected during experimentation on chemical and sensory changes of various treatments during storage period of three months were subjected to statistical analysis using Completely Randomized Design. The results along with statistical inference are presented and described in this chapter under the following headings:

4.1. Effect of blending and storage of beverage on physico-chemical characteristics.

4.2. Effect of blending and storage on sensory characteristics.

4.3. Effect of blending and storage on microbial analysis.

4.1. Effect of blending and storage of beverage on physico-chemical characteristics.

4.1.1 Total Soluble Solids (°Brix)

Data regarding the changes in TSS content of aonla based beetroot blended RTS beverage was influenced by various treatments during the storage have been presented in table 4.1 and graphically depicted in Fig. 4.1. Furthermore, it was found in increasing trend up to 90 days of storage period. The data clearly indicated that the TSS content of beverage was found non-significant at 0 days while it was found significant at rest of storage period.

At 0 days, the TSS was found non-significant between the treatments as the TSS of the RTS beverage was fixed to 10.00° Brix.

At 30 days, change in TSS was found significant. The significantly highest TSS was found in T_7 (10.47° Brix) which was followed by T1, T_4, T_5 (10.45° Brix). While, the lowest TSS was found in T_3 (10.00° Brix).

Table 4.1 Changes in TSS (^{o} Brix) of aonla based beetroot blended RTS beverage duringstorage

	Treatments (Ratio/treatment)	Storage period			
		0 days	30 days	60 days	90 days
T_1	**Aonla 100**	10.00	10.45	11.20	11.90
T_2	**Aonla and beetroot (95 :05)**	10.00	10.32	11.02	11.46
T_3	**Aonla and beetroot (90:10)**	10.00	10.20	10.80	11.42
T_4	**Aonla and beetroot (85:15)**	10.00	10.45	11.16	12.00
T_5	**Aonla and beetroot (80:20)**	10.00	10.45	11.00	11.60
T_6	**Aonla and beetroot (75:25)**	10.00	10.29	11.00	11.80
T_7	**Aonla and beetroot (70:30)**	10.00	10.47	11.17	11.70
S.Em±		**0.13**	**0.16**	**0.16**	**0.14**
CD at 5%		**NS**	**NS**	**NS**	**NS**

At 60 days, significant results of the TSS was recorded. The significantly highest TSS was observed in T_1 (11.20° Brix) which was followed by T1 (11.17° Brix). The lowest TSS was found in T_3 (10.80° Brix).

At 90 days, the results of TSS was significant. The significantly highest TSS was observed in T_4 (12.00° Brix) which was followed by T1 (11.90° Brix). The lowest TSS was found in T_3 (11.42° Brix).

The overall TSS data showed increasing trend of TSS with storage time. At 90 days all the treatments had highest TSS in comparison to their 0 day TSS. From these results it is inferred that, beetroot juice had more sweetness compared to aonla. Thus, the high concentration of beetroot juice must be the reason for high TSS in T_7.

The increase in TSS (°Brix) during storage may be caused by the hydrolysis of polysaccharides like starch, cellulose, and pectin substances into simple sugars, the reduction of the product's moisture content during storage, as well as the higher atmospheric temperature during storage conditions, which causes more of the product's polysaccharides to break down into simple sugars and increases the level of TSS. Similar kind of observation also found by Lal *et al.* (1999) in apple-ginger squash, Deka *et al*. (2001) in mix fruit juice, Kumar *et al*. (2009) in aonla-pineapple

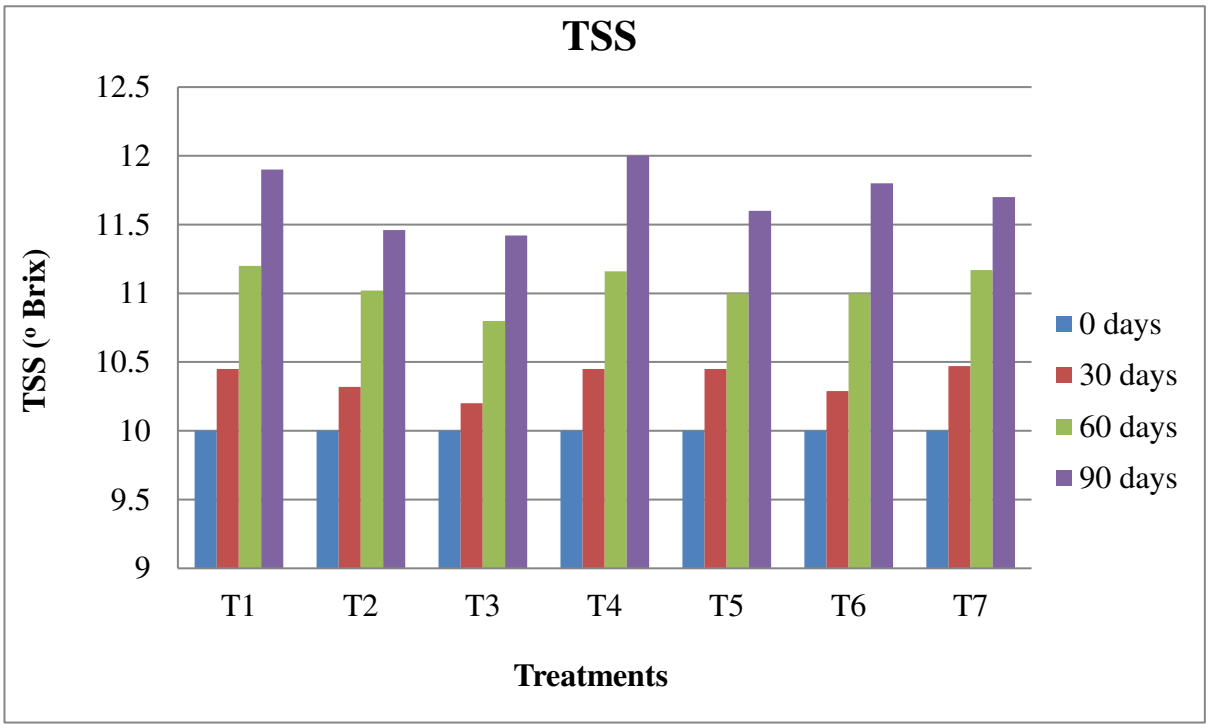


Fig. 4.1 Changes in TSS (° Brix) of aonla based beetroot blended RTS beverage during storage

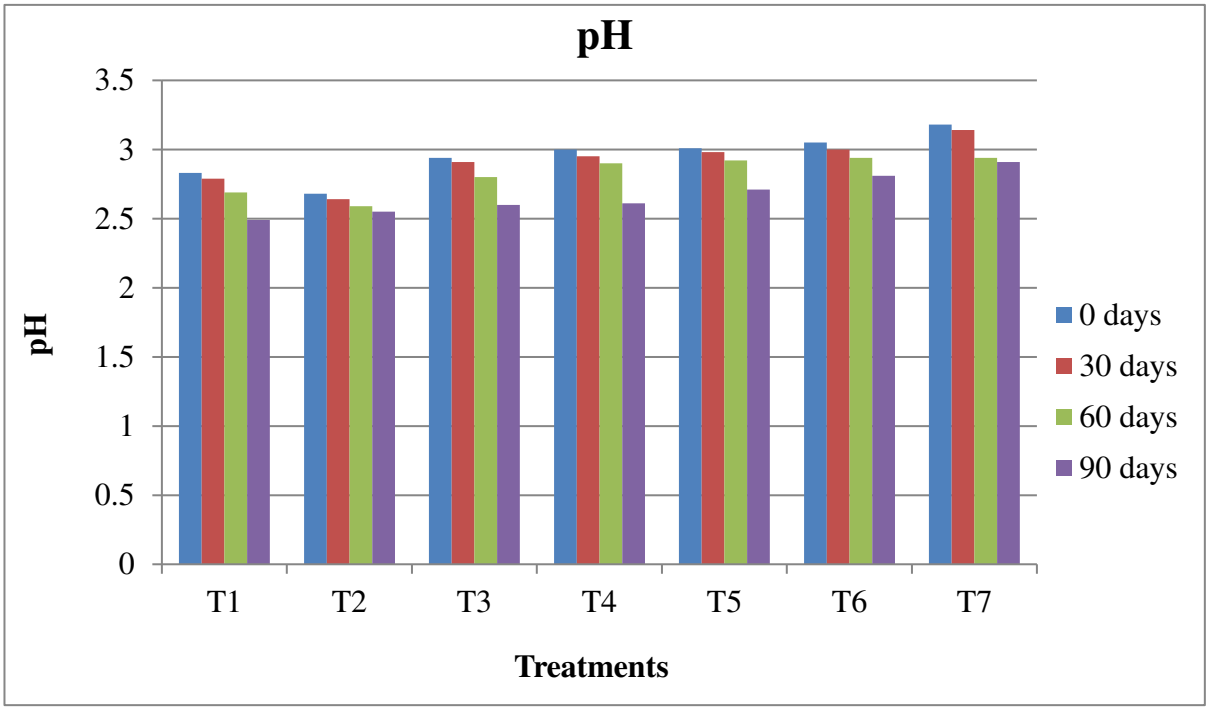


Fig 4.2 Changes in pH of aonla based beetroot blended RTS beverage during storage

nectar, Jan and Masih (2012) in pineapple, carrot and orange blended juice, Jain and Meena (2013) in aonla-kinnow ready to serve, Rustagi and Kumar (2013) in aonla-mango RTS, Balaji and Prasad (2014) in aonla-kinnow RTS, Ullah *et al*. (2015) in carrot, kinnow and ginger blended RTS.

4.1.2 pH

Data regarding the changes in pH of aonla based beetroot blended RTS beverage was influenced by various treatments during the storage have been presented in Table 4.2 and graphically depicted in Fig. 4.2. It was found to have decreasing trend up to 90 days storage period. The data clearly indicated that the pH of beverage was found non-significant result at 0 days while it was found significant at rest of storage period.

At 0 days, pH in RTS beverage of different treatments were found to be different. The significantly highest pH was found in T_7 (3.18) which was followed by T_6 (3.05). While, the lowest TSS was found in T2 (2.68).

Table 4.2 Changes in pH of aonla based beetroot blended RTS beverage during storage

	Treatments (Ratio/treatment)	**Storage period**			
		0 days	**30 days**	**60 days**	**90 days**
T_1	**Aonla 100**	2.83	2.79	2.69	2.49
T_2	**Aonla and beetroot (95 :05)**	2.68	2.64	2.59	2.55
T_3	**Aonla and beetroot (90:10)**	2.94	2.91	2.80	2.60
T_4	**Aonla and beetroot (85:15)**	3.00	2.95	2.90	2.61
T_5	**Aonla and beetroot (80:20)**	3.01	2.98	2.92	2.71
T_6	**Aonla and beetroot (75:25)**	3.05	3.00	2.94	2.81
T_7	**Aonla and beetroot (70:30)**	3.18	3.14	2.94	2.91
S.Em±		**0.05**	**0.04**	**0.04**	**0.03**
CD at 5%		**0.15**	**0.14**	**0.14**	**0.09**

At 30 days, change in pH was found significant. The significantly highest pH was found in T_7 (3.14) which was followed by T_6 (3.00). While, the lowest TSS was found in T2 (2.64).

At 60 days, significant results of the pH was recorded. The significantly highest pHwas observed in T_7 (2.94) which was followed by T_6 (2.94). The lowest pH was found in T2 (2.59).

At 90 days, the results of pH was significant. The significantly highest pH was observed in T_7 (2.91) which was followed by T_6 (2.81). The lowest pH was found in T1 (2.49).

The overall pH data showed decreasing trend of pH with storage time. At 90 days all the treatments had lowest pH in comparison to their 0 day pH. The blending effect had highest influence on pH. High pH in T_7 was due to alkaline nature of beetroot juice which was in high concentration in this treatment. The pH is altered as a result of a chemical reaction between organic acids and pigments, which would result in a commensurate decrease in acidity (Kannan and Thirumaran, 2004). Similar findings with jackfruit RTS drinks stored at room temperature were reported by Krishnaveni*et al*. in (2001), Murtaza et al. (2004) in strawberry drinks. These results were in agreement with the results of Desai *et al.* (2016) in anola-carrot blended beverage, Devra *et al.*in anola RTS (2017), and Sita and Komal *et al. in* anola blended beverage(2022).

4.1.3 Reducing sugars (%)

Data regarding the change in reducing sugars in aonla based beetroot RTS beverage was influenced by different treatments and also storage period which was presented in the table 4.3 and also depicted in figure 4.3. The results of reducing sugars found in increasing trend with storage period. These results were found to be significantly different between the treatments.

At 0 day, treatment T_4 (8.06%) had maximum reducing sugars followed by T_7(7.78%) and the minimum reducing sugars was recorded in the treatment T_6 (7.20%).

At 30 days, the change in reducing sugars was significant for all the treatments. The treatment T_4, T_5 (8.15%) had maximum reducing sugars followed by T_7 (8.09%) and the minimum reducing sugars was recorded in the treatment T_6 (7.35%).

Table 4.3 Changes in reducing sugars (%) of aonla based beetroot blended RTS beverage during storage

	Treatments (Ratio/treatment)	Storage period			
		0 days	30 days	60 days	90 days
T_1	Aonla 100	7.47	7.69	10.16	10.73
T_2	Aonla and beetroot (95 :05)	7.50	7.70	10.07	10.46
T_3	Aonla and beetroot (90:10)	7.31	7.56	9.97	10.36
T_4	Aonla and beetroot (85:15)	8.06	8.15	9.97	11.22
T_5	Aonla and beetroot (80:20)	7.78	8.15	10.01	10.46
T_6	Aonla and beetroot (75:25)	7.20	7.35	9.98	10.52
T_7	Aonla and beetroot (70:30)	7.78	8.09	10.01	10.19
S.Em±		0.11	0.09	0.20	0.17
CD at 5%		0.33	0.29	NS	0.51

At 60 days, the change in reducing sugars was seen non-significant for all the treatments.

At 90 days, the change in reducing sugars was significant for all the treatments. The treatment T_4 (11.22%) had maximum reducing sugars followed by T_1(10.73%) and the minimum reducing sugars was recorded in the treatment T_7(10.19%).

The overall reducing sugars data showed increasing trend of reducing sugars with storage time. At 90 days all the treatments had highest reducing sugars in comparison to their respective 0 day values.

These results revealed that the high reducing sugars in T_4 with blending ratio of aonla: beetroot:(85:15) was due to higher breakdown of polysaccharides leading to more conversion of non-reducing sugars into invert sugar. Increased polysaccharide breakdown causes a greater amount of non-reducing sugars to be converted into invert sugar. This can be explained by the hydrolysis of juice's starch and disaccharides into invert sugars as well as the inversion of a portion of non-reducing sugars into glucose and fructose by the partial acids. High temperatures at ambient settings sped up these

chemical processes, increasing the amount of reducing sugars in the final product. Analogous trends in reducing sugars were also recorded by Byanna and Gowda (2011) in orange- pomegranate RTS, Rustagi and Kumar (2013) in aonla-mango blended RTS, Ullah *et al*. (2015) in carrot, kinnow and ginger blended RTS.

4.1.4 Non-reducing sugars (%)

Data regarding the change in reducing sugars in aonla based beetroot RTS beverage was influenced by different treatments and also storage period which was presented in the table 4.4 and also depicted in figure 4.4. The results of non-reducing sugars found in decreasing trend with storage period. These results were found to be significantly different between the treatments.

At 0 day, treatment T_7 (0.69%) had maximum non-reducing sugars followed by T_6 (0.65%) and the minimum reducing sugars was recorded in the treatment T_1 (0.46%).

At 30 days, the change in non-reducing sugars was significant for all the treatments. The treatment T_7 (0.68%) had maximum non-reducing sugars followed by T_6 (0.65%) and the minimum non-reducing sugars was recorded in the treatment T1 (0.45%).

At 60 days, the change in non-reducing sugars was significant for all the treatments. The treatment T_7 (0.60%) had maximum non-reducing sugars followed by T_6 (0.57%) and the minimum non-reducing sugars was recorded in the treatment T_1 (0.38%).

At 90 days, the change in non-reducing sugars was significant for all the treatments. The treatment T_7 (0.46%) had maximum non-reducing sugars followed by T_6 (0.45%) and the minimum non-reducing sugars was recorded in the treatment T_1 (0.24%).

At 90 days all the treatments had lowest non-reducing sugars in comparison to their respective 0 day values.

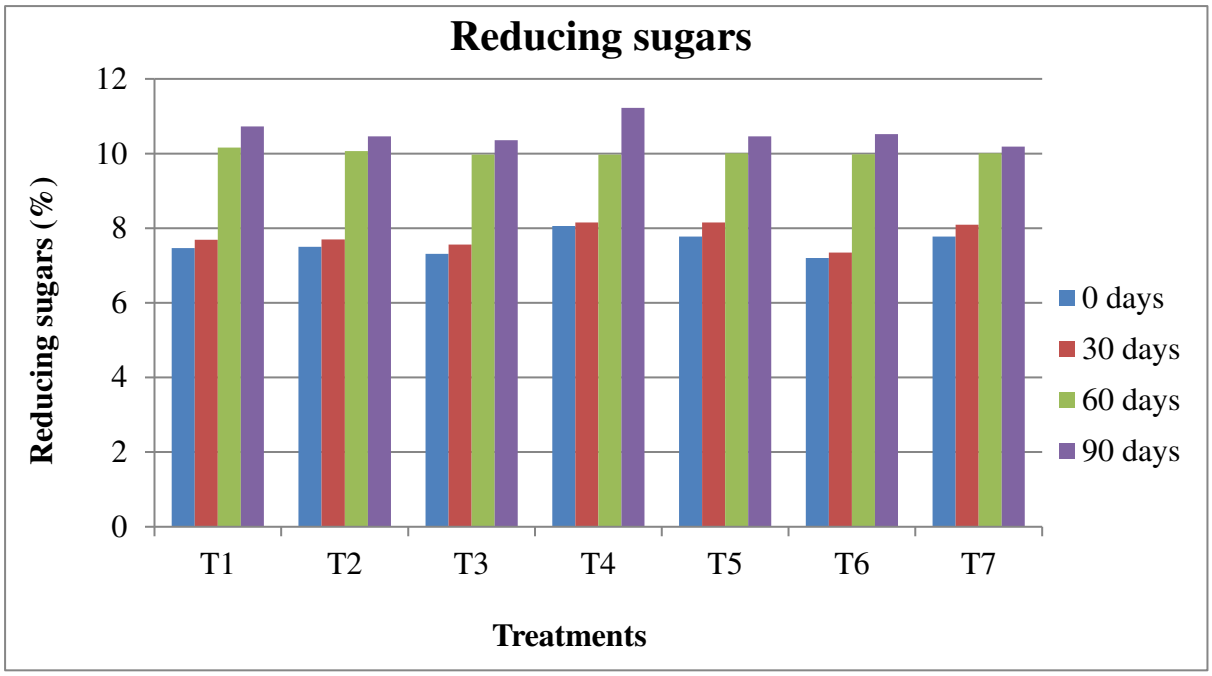


Fig 4.3 Changes in reducing sugars (%) of aonla based beetroot blended RTS beverage during storage

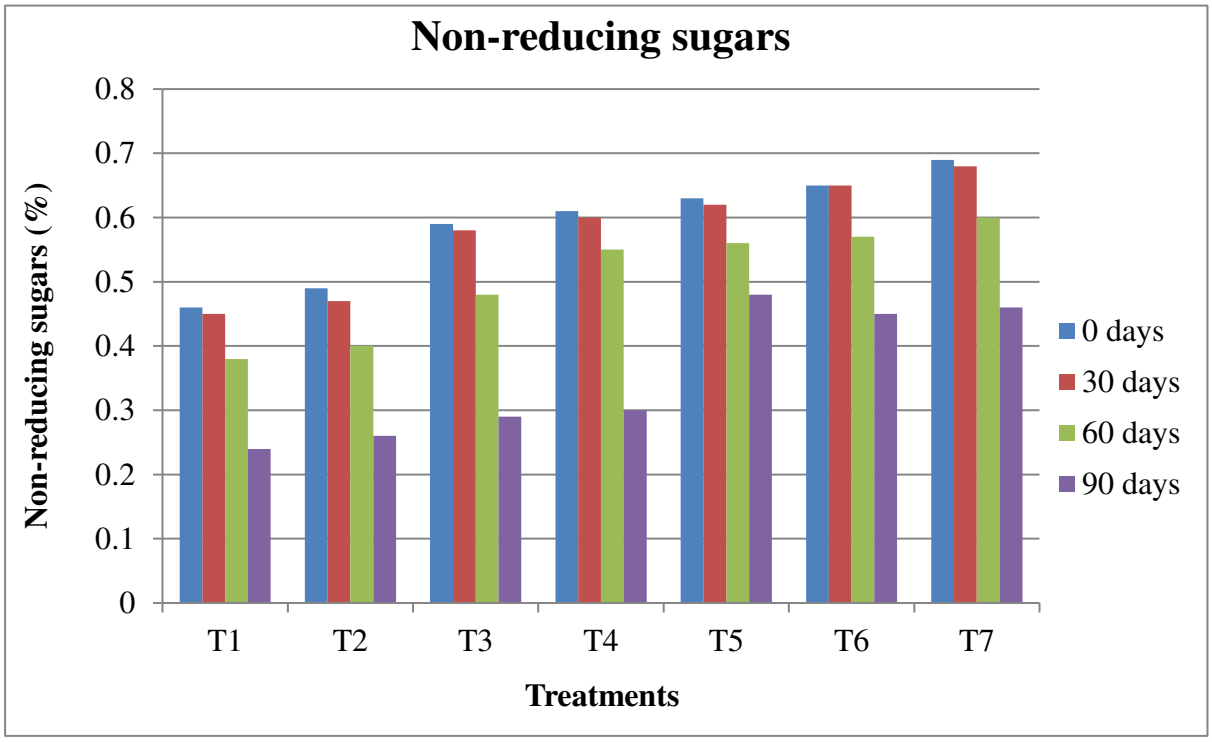


Fig 4.4 Changes in non-reducing sugars (%) of aonla based beetroot blended RTS beverage during storage

Table 4.4 Changes in non-reducing sugars (%) of aonla based beetroot blended RTS beverage during storage

	Treatments (Ratio/treatment)	Storage period			
		0 days	**30 days**	**60 days**	**90 days**
T_1	**Aonla 100**	0.46	0.45	0.38	0.24
T_2	**Aonla and beetroot (95 :05)**	0.49	0.47	0.40	0.26
T_3	**Aonla and beetroot (90:10)**	0.59	0.58	0.48	0.29
T_4	**Aonla and beetroot (85:15)**	0.61	0.60	0.55	0.30
T_5	**Aonla and beetroot (80:20)**	0.63	0.62	0.56	0.48
T_6	**Aonla and beetroot (75:25)**	0.65	0.65	0.57	0.45
T_7	**Aonla and beetroot (70:30)**	0.69	0.68	0.60	0.46
S.Em±		**0.01**	**0.01**	**0.01**	**0.01**
CD at 5%		**0.03**	**0.04**	**0.03**	**0.04**

These results revealed that, high concentration of beetroot juice contain maximum non-reducing sugars, because of low concentration of polysaccharides and starch in this treatment. During storage period these non-reducing sugars concentration has decreased due to non-availability of polysaccharides and high content of starch. These results were in agreement with the results of Bhardwaj and Mukherjee (2011) in kinnow blended juice with aonla, pomegranate and ginger, Kumar *et al*. (2018).

4.1.5 Total sugars (%)

Data regarding the change in total sugars in aonla based beetroot RTS beverage was influenced by different treatments and also storage period which was presented in the table 4.5 and also depicted in figure 4.5. The results of total sugars found in increasing trend with storage period. These results were found to be significantly different between the treatments.

Table 4.5 Changes in total sugars (%) of aonla based beetroot blended RTS beverage during storage

	Treatments (Ratio/treatment)	Storage period			
		0 days	30 days	60 days	90 days
T_1	Aonla 100	7.93	8.14	10.54	10.98
T_2	Aonla and beetroot (95 :05)	7.99	8.18	10.48	10.72
T_3	Aonla and beetroot (90:10)	7.91	8.15	10.45	10.65
T_4	Aonla and beetroot (85:15)	8.67	8.75	10.51	10.52
T_5	Aonla and beetroot (80:20)	8.41	8.77	10.57	10.95
T_6	Aonla and beetroot (75:25)	7.86	8.00	10.56	10.97
T_7	Aonla and beetroot (70:30)	8.57	8.77	10.61	10.65
S.Em±		0.11	0.10	0.16	0.13
CD at 5%		0.34	0.32	0.35	NS

At 0 day, treatment T_4 (8.67%) had maximum total sugars followed by T_7 (8.57%) and the minimum total sugars was recorded in the treatment T_6 (7.86%).

At 30 days, the change in total sugars was significant for all the treatments. The treatment T_5 (8.77%) had maximum total sugars followed by T_4 (8.75%) and the minimum total sugars was recorded in the treatment T_6 (8.00%).

At 60 days, the change in total sugars was non-significant for all the treatments. The treatment T_7 (10.61%) had maximum total sugars followed by T_5 (10.57%) and the minimum total sugars was recorded in the treatment T_3 (10.45%).

At 90 days, the change in total sugars was seen non significant for all the treatments

The overall total sugars data showed increasing trend of total sugars with storage time. At 90 days all the treatments had highest total sugars in comparison to their respective 0 day values. As expected, the total soluble solids of beverages determined their overall sugar content. Both reducing and total sugars sow significant increases. This may be because the acids in the blended RTS beverages made with aonla may trigger an inversion of non-reducing sugars to reducing sugars. Similar results were also reported by Verma and Gehlot (2007) in bael RTS, Reddy and Chikkasubbanna (2008) in lime-Aonla blended squash.

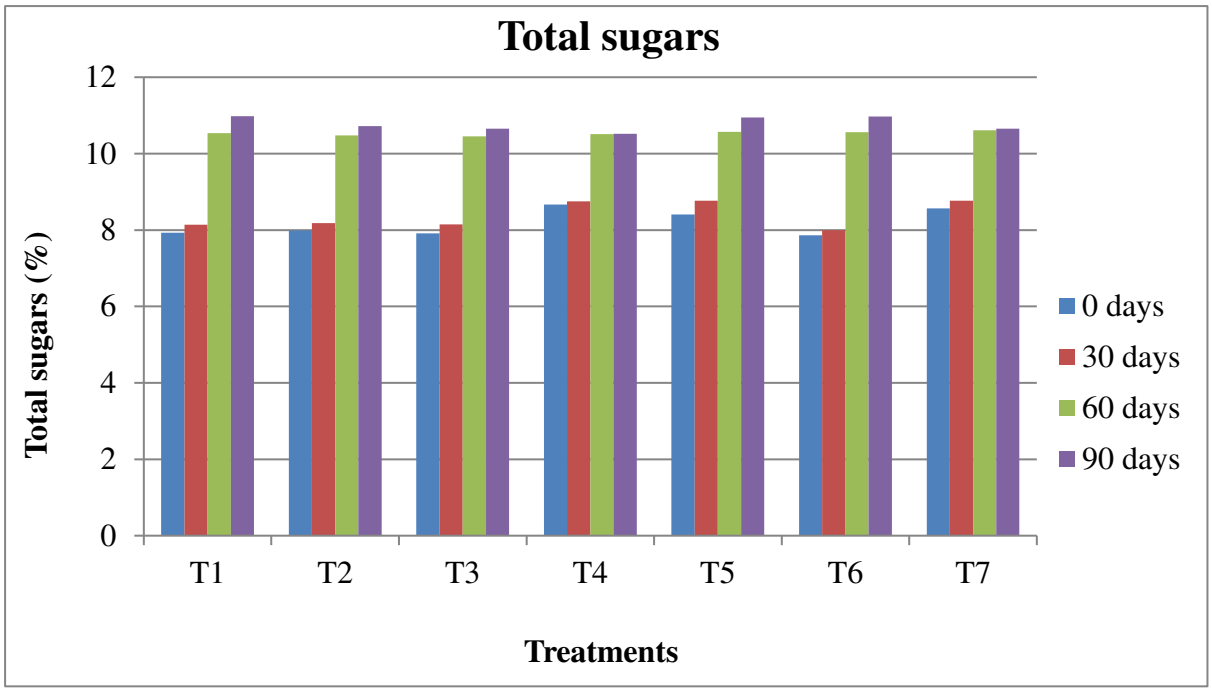


Fig 4.5 Changes in total sugars (%) of aonla based beetroot blended RTS beverage during storage

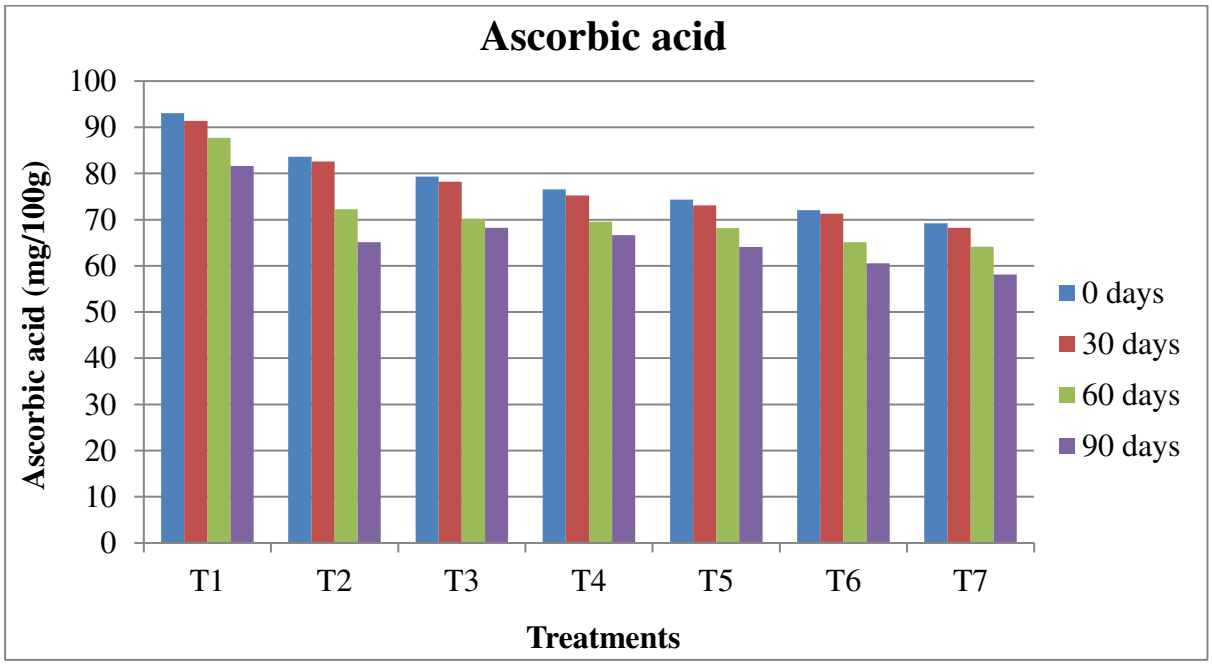


Fig 4.6 Changes in ascorbic acid (mg/100g) content of aonla based beetroot blended RTS beverage during storage

4.1.6 Ascorbic acid (mg/100ml)

Data regarding the change in ascorbic acid content in aonla based beetroot RTS beverage was influenced by different treatments and also the storage period which was presented in the table 4.6 and also depicted in figure 4.6. The results of non-reducing sugars found in decreasing trend with storage period. These results were found to be significantly different between the treatments.

At 0 day, treatment T_1 (93.05 mg/100ml) had maximum ascorbic acid content followed by T_2(83.63 mg/100ml) and the minimum ascorbic acid content was recorded in the treatment T_7 (69.19 mg/100ml).

At 30 days, the change in ascorbic acid content was significant for all the treatments. The treatment T_1 (91.39 mg/100ml) had maximum ascorbic acid content followed by T_2 (82.56 mg/100ml) and the minimum ascorbic acid content was recorded in the treatment T_7 (68.22 mg/100ml).

At 60 days, the change in ascorbic acid content was significant for all the treatments. The treatment T_1 (87.70 mg/100ml) had maximum ascorbic acid content followed by T_2 (72.23 mg/100ml) and the minimum ascorbic acid content was recorded in the treatment T_7 (64.18 mg/100ml).

At 90 days, the change in ascorbic acid content was significant for all the treatments. The treatment T_1 (81.60 mg/100ml) had maximum ascorbic acid content followed by T_3 (68.23 mg/100ml) and the minimum ascorbic acid content was recorded in the treatment T_7 (58.14 mg/100ml).

A gradual and significant decrease in ascorbic acid content with the increasing period of storage was observed in all the treatments. At 90 days all the treatments had highest ascorbic acid content in comparison to their respective 0 day values.

Antioxidants were found highly sensitive to oxidation, being sensitive to oxygen, light and heat, it could be easily oxidized in presence of oxygen by both enzymatic and non-enzymatic catalyst and subsequently suffer loss or decline during storage. This gradual reduction may be due to heat processing and the presence of air in the head space of glass bottles, L-ascorbic acid may have been oxidised or irreversibly converted into ascorbinase, which may have caused the ascorbic acid concentration to significantly decrease during storage.

Table 4.6 Changes in ascorbic acid (mg/100g) content of aonla based beetroot blended RTS beverage during storage

	Treatments (Ratio/treatment)	Storage period			
		0 days	30 days	60 days	90 days
T_1	**Aonla 100**	93.05	91.39	87.70	81.60
T_2	**Aonla and beetroot (95 :05)**	83.63	82.56	72.23	65.12
T_3	**Aonla and beetroot (90:10)**	79.30	78.21	70.18	68.23
T_4	**Aonla and beetroot (85:15)**	76.55	75.21	69.54	66.67
T_5	**Aonla and beetroot (80:20)**	74.36	73.11	68.19	64.11
T_6	**Aonla and beetroot (75:25)**	72.03	71.26	65.12	60.54
T_7	**Aonla and beetroot (70:30)**	69.19	68.22	64.18	58.14
	S.Em±	**0.79**	**1.12**	**0.69**	**1.01**
	CD at 5%	**2.40**	**3.40**	**2.11**	**3.08**

These results were supported by the earlier findings of Oliveira *et al*. (2012) in mango juice storage, Thakur *et al*. (2018) in wild anola RTS, Wruss *et al*. (2015) in beetroot juice.

4.1.7 Acidity

Data regarding the change in acidity in aonla based beetroot RTS beverage was influenced by different treatments and also storage period which was presented in the table 4.8 and also depicted in figure 4.8. The results of non-reducing sugars found in decreasing trend with storage period. These results were found to be significantly different between the treatments.

At 0 day, the acidity was found to be non-significant. Treatments T_1, T_2, T_3, T_4, T_5 (0.35) had maximum acidity and the minimum acidity was recorded in the treatment T_7 (0.34).

At 30 days, the change in acidity was significant for all the treatments. The treatment T_5 (0.34) had maximum acidity followed by T_2 (0.33) and the minimum acidity was recorded in the treatment T_7 (0.26).

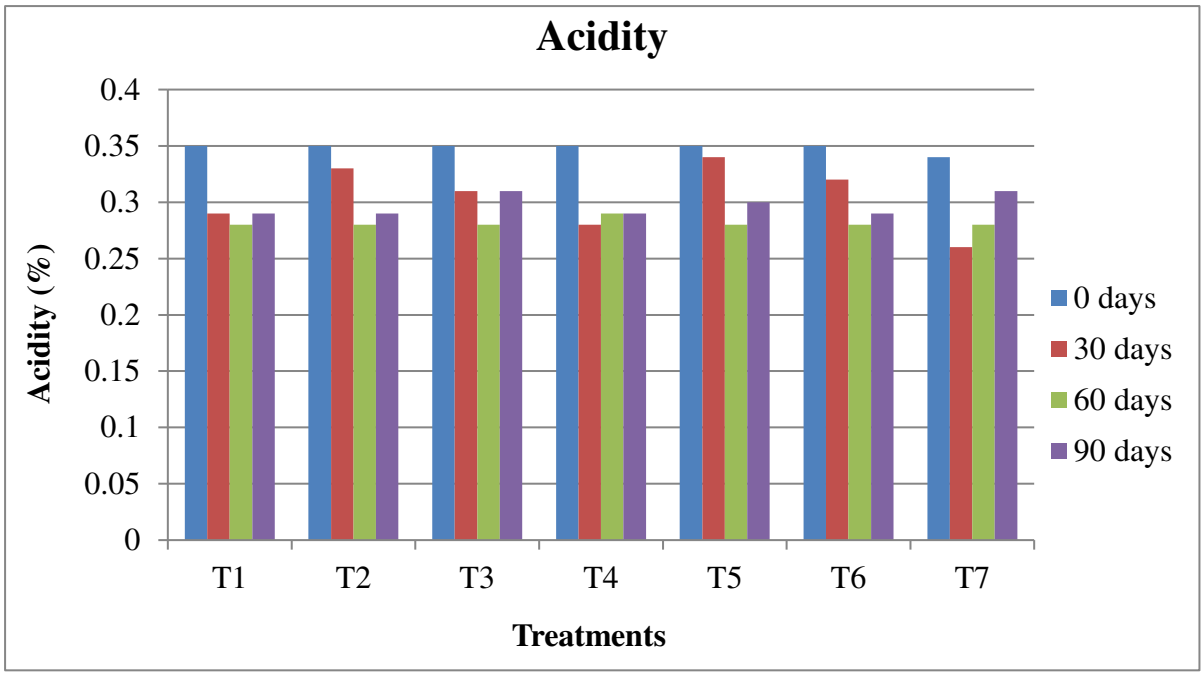


Fig 4.7 Changes in acidity (%) of aonla based beetroot blended RTS beverage during storage

Table 4.7 Changes in acidity (%) of aonla based beetroot blended RTS beverage during storage

	Treatments (Ratio/treatment)	Storage period			
		0 days	30 days	60 days	90 days
T_1	Aonla 100	0.35	0.29	0.28	0.29
T_2	Aonla and beetroot (95 :05)	0.35	0.33	0.28	0.29
T_3	Aonla and beetroot (90:10)	0.35	0.31	0.28	0.31
T_4	Aonla and beetroot (85:15)	0.35	0.28	0.29	0.29
T_5	Aonla and beetroot (80:20)	0.35	0.34	0.28	0.30
T_6	Aonla and beetroot (75:25)	0.35	0.32	0.28	0.29
T_7	Aonla and beetroot (70:30)	0.34	0.26	0.28	0.31
S.Em±		**0.006**	**0.006**	**0.003**	**0.004**
CD at 5%		**0.020**	**0.020**	**NS**	**0.012**

At 60 days, the change in acidity was seen non-significant for all the treatments.

At 90 days, the change in acidity was significant for all the treatments. The treatment T_3 and T_7 (0.31) had maximum acidity, rest of the treatments recorded acidity of (0.29).

The overall acidity data showed decreasing trend of acidity with storage time. At 90 days all the treatments had lowest acidity in comparison to their respective 0 day values. The highest acidity was recorded in T4, T5 (0.353) at 0 day and lowest acidity was recorded in most of the treatments (0.29) at 90 days. It is reported that gradual decrease in acidity of mixed fruit juice beverage prepared by blending pineapple, pomegranate and pear (Kumar and Manimegalai, 2001), RTS carbonated beverage from jamun fruit juice showed similar results (Pandurnikar, 2004). (Deka, 2000) found that the acidity of the RTS beverage prepared from lime-aonla, mango pineapple, guava-mango blends decreased with addition of spices with advancement of storage period up to six months under different storage conditions. Similar results were found by Tiwari (2000) in guava and papaya blended RTS and Dhaliwal and Hira (2001) in carrot juice blends.

4.1.8 Colour

Data regarding the change in colour in aonla based beetroot RTS beverage was influenced by different treatments and also storage period which was presented in the table 4.9 and also depicted in figure 4.9. The colour of the beverage was recorded using colourimeter and represented through L, a, b values. Where (L*) indicates lightness and redness by (a*), yellowness by (b*) of the samples. These results were found to be significantly different between the treatments during storage period.

At0 days, the change in colour was significant for all the treatments. The treatment T_7 had maximum L (48.43), a (2.09), b (32.31) values followed by T_6, L (43.83), a (2.37), b (31.68) values and the minimum colour was recorded in the treatment T_1 L (32.79), a (3.16), b (24.33) values

At 30 days, the change in colour was significant for all the treatments. The treatment T_7 had maximum L (45.21), a (1.98), b (30.45) values followed by T_6, L (41.23), a (2.25), b (30.11) values and the minimum colour was recorded in the treatment T_1 L (31.05), a (2.99), b (22.93) values.

At 60 days, the change in colour was significant for all the treatments. The treatment T_7 had maximum L (35.77), a (1.59), b (27.02) values followed by T_6, L (34.17), a (1.83), b (24.40) values and the minimum colour was recorded in the treatment T_1 L (28.06), a (1.66), b (20.87) values.

At 90 days, the change in colour was significant for all the treatments. The treatment T_7 had maximum L (31.01), a (1.35), b (25.40) values followed by T_6, L (30.23), a (1.56), b (21.95) values and the minimum colour was recorded in the treatment T_1L (26.18), a (1.45), b (18.57) values.

The overall colour data showed colour deterioration with storage time. At 90 days all the treatments had lowest colour in comparison to their respective 0 day values. The colour of aonla juice has been covered by beetroot juice except in aonla juice alone. The blending ratio led to the smallest change in total colour difference, considered as not perceptible by human eyes between T_3, T_4, T_5 and T_6, T_7. Similar results were observed by Tobolkova*et al.* (2020) in apple-beetroot long term juice storage, Flavia*et al.* (2021) in storage quality of beetroot and apple mixed juice.

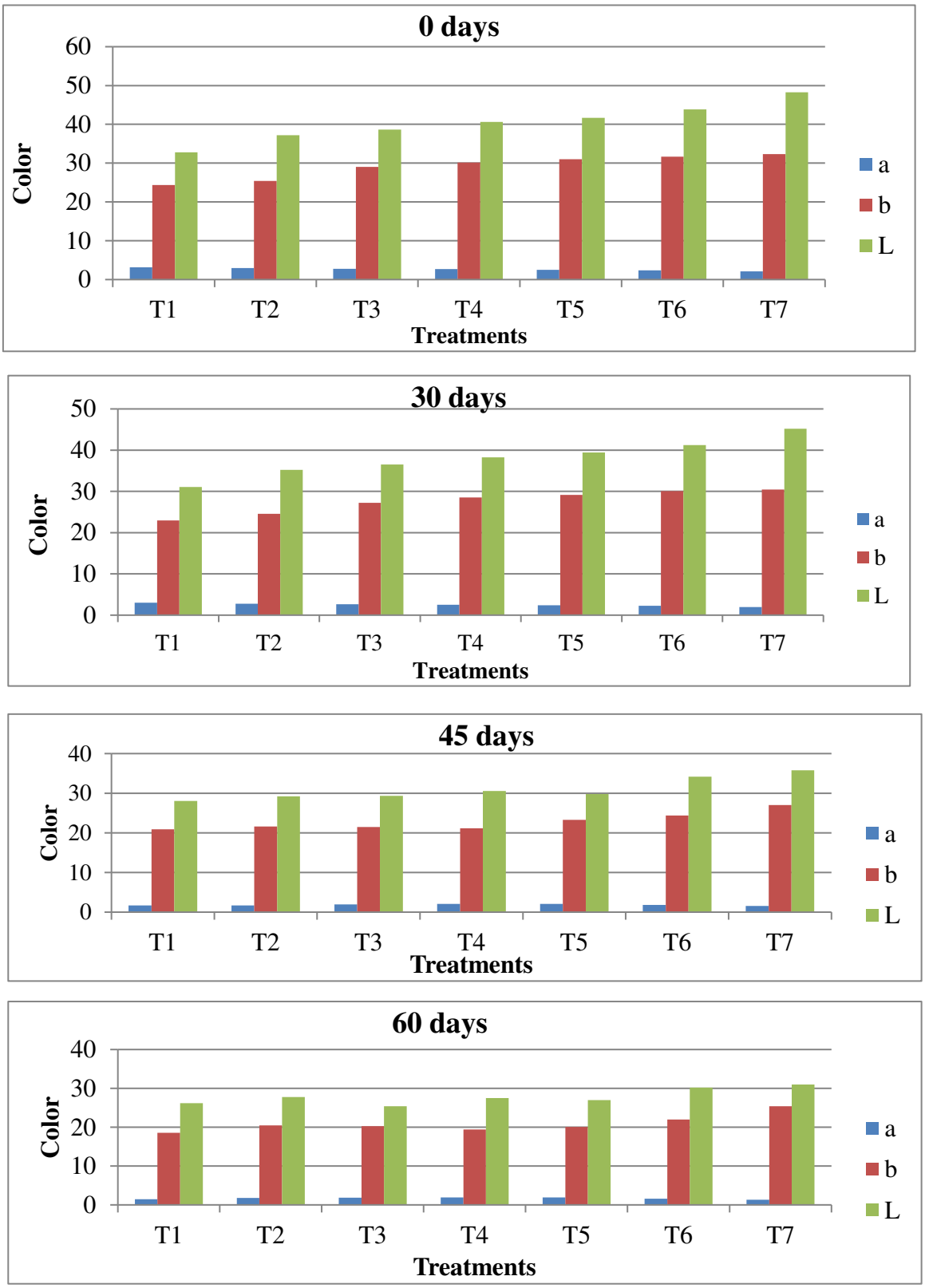


Fig 4.8 Changes in color of aonla based beetroot blended RTS beverage during storage

Table 4.8 Changes in color of aonla based beetroot blended RTS beverage during storage

	Treatments (Ratio/treatment)	Storage period											
		0 days			30 days			60 days			90 days		
		a	b	L	a	b	L	a	b	L	a	b	L
T_1	Aonla 100	3.16	24.33	32.79	2.99	22.93	31.05	1.66	20.87	28.06	1.45	18.57	26.18
T_2	Aonla and beetroot (95 :05)	2.98	25.39	37.17	2.76	24.56	35.23	1.71	21.58	29.23	1.78	20.48	27.74
T_3	Aonla and beetroot (90:10)	2.80	29.05	38.66	2.62	27.23	36.51	1.97	21.49	29.37	1.84	20.26	25.43
T_4	Aonla and beetroot (85:15)	2.68	30.16	40.65	2.54	28.55	38.28	2.07	21.17	30.59	1.95	19.43	27.50
T_5	Aonla and beetroot (80:20)	2.53	31.00	41.69	2.41	29.16	39.44	2.05	23.28	29.78	1.94	20.05	26.95
T_6	Aonla and beetroot (75:25)	2.37	31.68	43.83	2.25	30.11	41.23	1.83	24.40	34.16	1.56	21.95	30.23
T_7	Aonla and beetroot (70:30)	2.09	32.31	48.23	1.98	30.45	45.21	1.59	27.02	35.77	1.35	25.40	31.01
S.Em±		**0.04**	**0.33**	**0.52**	**0.03**	**0.25**	**0.56**	**0.03**	**0.43**	**0.35**	**0.03**	**0.22**	**0.54**
CD at 5%		**0.12**	**1.02**	**1.57**	**0.11**	**0.78**	**1.70**	**0.1**	**1.3**	**1.09**	**0.09**	**0.68**	**1.65**

4.1.9 Antioxidants

Data regarding the change in antioxidants in aonla based beetroot RTS beverage was influenced by different treatments and also storage period which was presented in the table 4.10 and also depicted in figure 4.10. The antioxidants of the beverage was estimated using FRAP and DPPH method. The results obtained were found to be significantly different between the treatments during storage period.

Table 4.9 Changes in antioxidants (mg/100gAAE) of aonla based beetroot blended RTS beverage using FRAP method during storage

	Treatments (Ratio/treatment)	Storage period			
		0 days	30 days	60 days	90 days
T_1	Aonla 100	96.86	95.24	89.70	86.12
T_2	Aonla and beetroot (95 :05)	91.94	90.23	84.98	81.75
T_3	Aonla and beetroot (90:10)	87.50	86.12	80.94	79.24
T_4	Aonla and beetroot (85:15)	81.64	80.12	76.24	74.49
T_5	Aonla and beetroot (80:20)	76.88	76.12	71.69	69.54
T_6	Aonla and beetroot (75:25)	70.13	69.23	65.74	63.11
T_7	Aonla and beetroot (70:30)	60.72	60.12	56.51	54.36
S.Em±		0.91	1.23	1.32	1.16
CD at 5%		2.78	3.75	4.02	3.54

At 0 day, the antioxidants was found to be significant for all the treatments. Treatments T_1 had maximum antioxidants content in both FRAP (96.86) and DPPH (96.19) method followed by T_2 with FRAP (91.9) and DPPH (92.03) values and the minimum antioxidants was recorded in the treatment T_7 with FRAP (60.72) and DPPH (60.84) values.

At 30 days, the change in antioxidants was significant for all the treatments. The treatment T_1 had maximum antioxidants content in both FRAP (95.24) and DPPH (95.24) method followed by T_2 with FRAP (90.23) and DPPH (90.23) values and the minimum antioxidants was recorded in the treatment T_7 with FRAP (60.12) and DPPH (60.12) values.

Table 4.10 Changes in antioxidants (mg/100gAAE) of aonla based beetroot blended RTS beverage using DPPH method during storage

	Treatments (Ratio/treatment)	Storage period			
		0 days	30 days	60 days	90 days
T1	**Aonla 100**	96.19	95.24	89.06	86.03
T2	**Aonla and beetroot (95 :05)**	92.03	90.23	84.11	82.34
T_3	**Aonla and beetroot (90:10)**	86.98	86.12	81.61	79.74
T_4	**Aonla and beetroot (85:15)**	81.40	80.12	76.79	74.72
T_5	**Aonla and beetroot (80:20)**	76.96	76.12	71.54	69.33
T_6	**Aonla and beetroot (75:25)**	70.61	69.23	64.73	62.34
T_7	**Aonla and beetroot (70:30)**	60.84	60.12	56.92	55.04
S.Em±		**0.91**	**1.23**	**1.32**	**1.16**
CD at 5%		**2.78**	**3.75**	**4.02**	**3.54**

At 60 days, the change in antioxidants was significant for all the treatments. The treatment T_1 had maximum antioxidants content in both FRAP (89.70) and DPPH (89.06) method followed by T_2 with FRAP (84.98) and DPPH (84.11) values and the minimum antioxidants was recorded in the treatment T_7 with FRAP (56.51) and DPPH (56.92) values.

At 90 days, the change in antioxidants was significant for all the treatments. The treatment T_1 had maximum antioxidants content in both FRAP (86.12) and DPPH (86.03) method followed by T_2 with FRAP (81.75) and DPPH (82.34) values and the minimum antioxidants was recorded in the treatment T_7 with FRAP (54.36) and DPPH (55.04) values.

The overall antioxidants data showed antioxidants deterioration with storage time. At 90 days all the treatments had lowest antioxidants in comparison to their respective 0 day values. This might be due to the effect of storage temperature, light and heat and ascorbic acid. The decrease in antioxidant activities during storage was also observed in beverages developed from beetroot-passion (Kathiravan *et al*., 2015), carrot-mango (Saci *et al*., 2015), carrot-pineapple (Owoladc *et al*., 2017), beetroot-orange (Porto *et al.,* 2017) and banana-carrot-beetroot RTS drinks (Arora *et al.,* 2019).

4.2. Effect of blending and storage on sensory characteristics.

4.2.1 Overall acceptability (Hedonic 9 point scale)

Data regarding the changes in overall acceptability of aonla based beetroot RTS beverage was influenced by different treatments and also storage period which was presented in the table 4.11 and also depicted in figure 4.11. The overall acceptability of the beverage was calculated based on sensory score given by the people who tasted it. The results obtained were found to be significantly different between the treatments during storage period.

At 0 day, the overall acceptability was found to be significant for all the treatments. Treatment T_4 (8.21) had maximum overall acceptability in comparison to other treatments followed by T_5 (7.86) and the minimum overall acceptability was recorded in the treatment T_2 (7.08).

At 30 days, the change in overall acceptability was significant for all the treatments. Treatment T_4 (8.13) had maximum overall acceptability in comparison to other treatments followed by T_5 (7.73) and the minimum overall acceptability was recorded in the treatment T_2 (6.97).

At 60 days, the change in antioxidants was significant for all the treatments. Treatment T_4 (7.87) had maximum overall acceptability in comparison to other treatments followed by T_5 (7.40) and the minimum overall acceptability was recorded in the treatment T_2 (6.60).

At 90 days, the change in antioxidants was significant for all the treatments. Treatment T_4 (7.60) had maximum overall acceptability in comparison to other treatments followed by T_5 (7.27) and the minimum overall acceptability was recorded in the treatment T_2 (6.43).

The overall acceptability score had deteriorated with storage time. At 90 days all the treatments had lowest overall acceptability score in comparison to their respective 0 day scores. The lowest acceptance in the overall acceptability of beverage was attributed to the aonla juice's high astringency, caustic, and bitter flavour. The trend of the total acceptability score consistently dropping throughout storage may be caused by the deterioration of all sensory criteria, such as colour, taste, and flavour, with longer periods of storage. Such identical findings were also revealed by Mall and

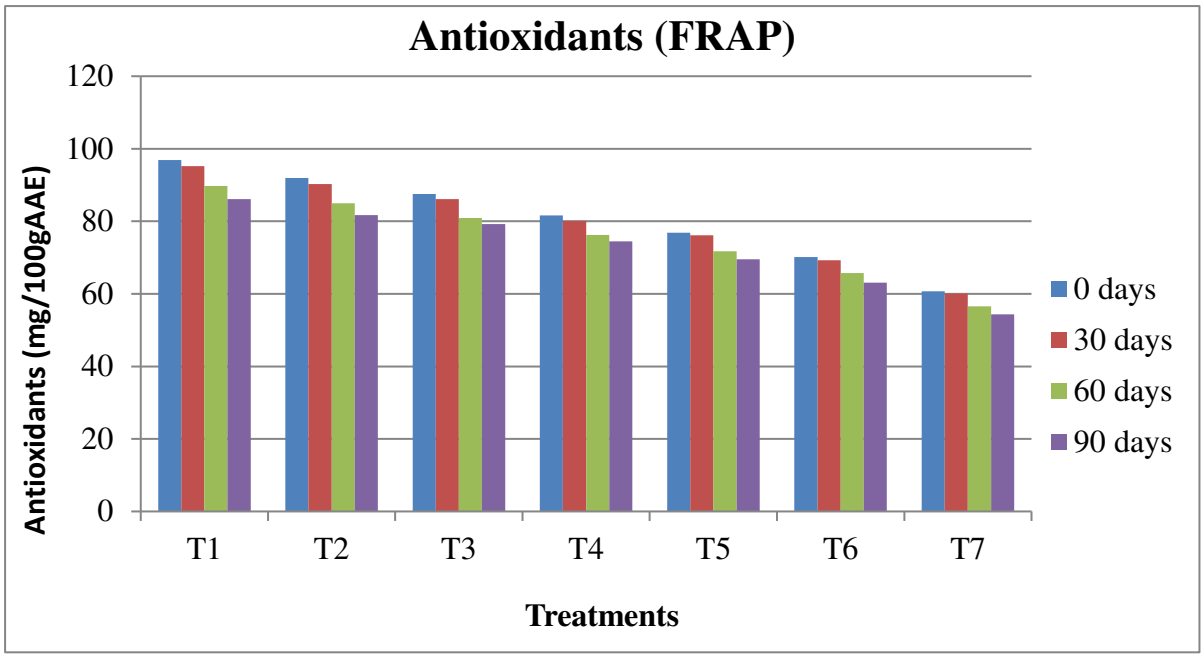


Fig 4.9 Changes in antioxidants (mg/100gAAE) of aonla based beetroot blended RTS beverage using FRAP method during storage

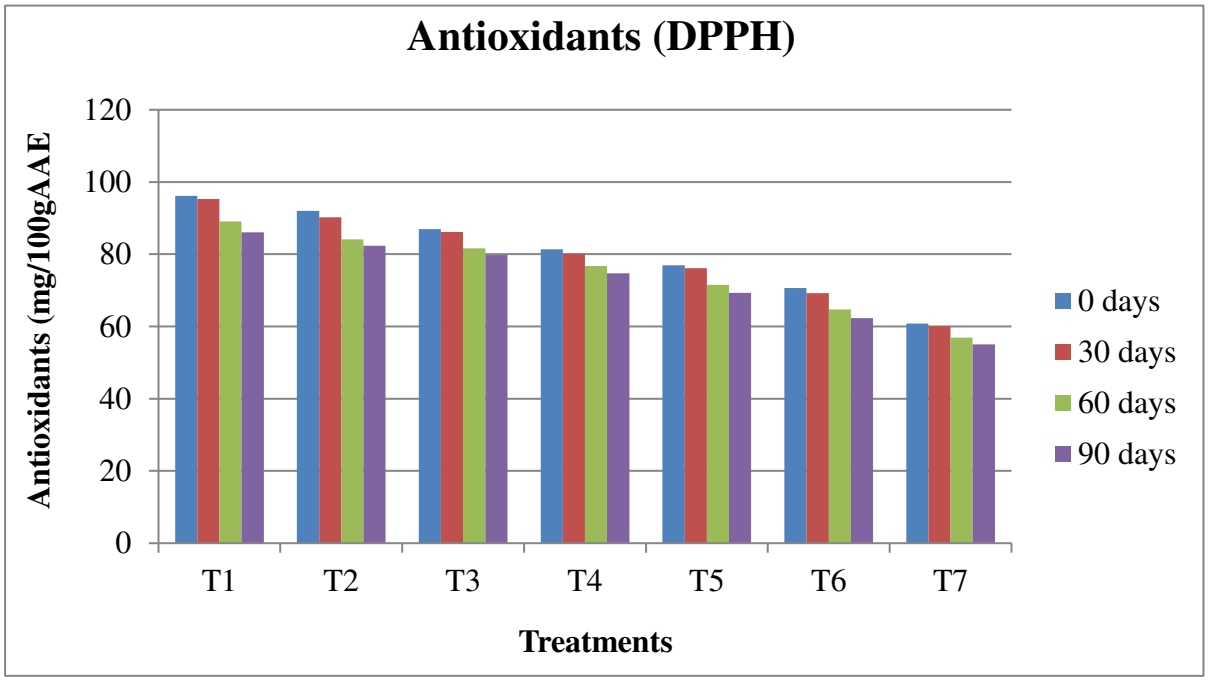


Fig 4.10 Changes in antioxidants (mg/100gAAE) of aonla based beetroot blended RTS beverage using DPPH method during storage

Tandon (2005) in aonla-pineapple nectar, Selvi *et al*. (2013) in guava-lime-ginger RTS, Balaji and Prasad (2014) in kinnow-aonla RTS, Rathod *et al.* (2014) in aonla-bael blended RTS, Ullah *et al.* (2015) carrot-kinnow-ginger blended RTS.

Table 4.11 Changes in overall acceptability of aonla based beetroot blended RTS beverage during storage

Treatments	Treatments (Ratio/treatment)	Storage period			
		0 Day	30 Days	60 Days	90 Days
T_1	**Aonla 100**	7.21	7.10	6.77	6.50
T_2	**Aonla and beetroot (95 :05)**	7.08	6.97	6.60	6.43
T_3	**Aonla and beetroot (90:10)**	7.32	7.23	7.00	6.83
T_4	**Aonla and beetroot (85:15)**	8.21	8.13	7.87	7.60
T_5	**Aonla and beetroot (80:20)**	7.86	7.73	7.40	7.27
T_6	**Aonla and beetroot (75:25)**	7.51	7.40	7.13	6.80
T_7	**Aonla and beetroot (70:30)**	7.56	7.47	7.33	6.93
S.Em±		**0.09**	**0.12**	**0.09**	**0.11**
C.D. 5%		**0.27**	**0.36**	**0.28**	**0.32**

4.3 Effect of blending and storage on microbial analysis.

Table 4.12 Total plate count ((cfu/ml x103) of aonla based beetroot blended RTS beverage during storage

Treatments	Treatments (Ratio/treatment)	Storage period	
		60 days	90 days
T_1	**Aonla 100**	Nil	1
T_2	**Aonla and beetroot (95 :05)**	Nil	2
T_3	**Aonla and beetroot (90:10)**	Nil	3
T_4	**Aonla and beetroot (85:15)**	Nil	2
T_5	**Aonla and beetroot (80:20)**	Nil	1
T_6	**Aonla and beetroot (75:25)**	Nil	2
T_7	**Aonla and beetroot (70:30)**	Nil	2

The microbial analysis of selected sample of prepared RTS beverage which was stored at room temperature was studied in terms of standard plating in nutrient agar was carried out to determine the quality and safety of prepared beverage. The total microbial load (TPC) of RTS beverage was determined in nutrient agar media of stored RTS beverage. The yeast and mould count of prepared RTS beverage concluded in potato dextrose agar (PDA) whereas coliform count determined using violet red bile agar.

The microbiological quality in terms of the total plate count, coliform count, yeast and mould count of aonlabased beetroot juice samples during storage period were analysed and are presented in table 4.12, 4.13, 4.14. Total plate count, yeast and mould count and coliform count were Nil at in all the treatments studied during entire storage period. At 90 days of storage total plate count, yeast and mould count and coliform count were 3 cfu/ml, 1 cfu/ml and nil respectively found highest at 103 dilution after 72 hours in T3 followed by 2 cfu/ml, 1 cfu/ml and nil respectively at 10-3 dilution after 72 hours in T4.While rest of the treatments had 1 to 2 cfu/ml of plate count and there were no yeast,mold and coliform growth.

Table 4.13 Yeast and mold growth (cfu/ml x10^3) in aonla based beetroot blended RTS beverage during storage

Treatments	Treatments (Ratio/treatment)	Storage period	
		60 days	90 days
T_1	Aonla 100	Nil	Nil
T_2	Aonla and beetroot (95 :05)	Nil	Nil
T_3	Aonla and beetroot (90:10)	Nil	1
T_4	Aonla and beetroot (85:15)	Nil	1
T_5	Aonla and beetroot (80:20)	Nil	Nil
T_6	Aonla and beetroot (75:25)	Nil	Nil
T_7	Aonla and beetroot (70:30)	Nil	Nil

Microbial standard for carbonated beverages and ready to serve beverages described as total plate count - not more than 50 cfu/ml. yeast and mold count - not more than 2 cfu/ml and coliform should be absent in 100 ml of sample (FSSAI, 2017).

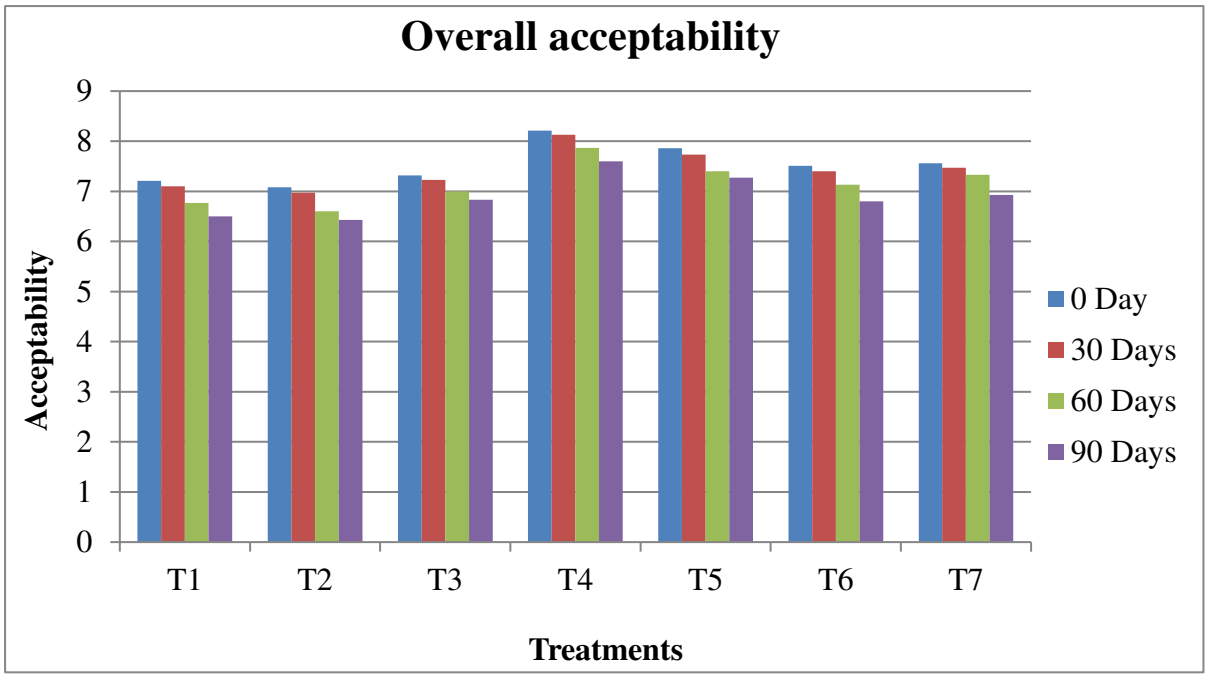


Fig 4.11 Changes in overall acceptability of aonla based beetroot blended RTS beverage during storage

Eventhough, we observed few microbial growths during storage, the count was below safe limits of FSSAI, 2017. The resistance to microbial activity was assumed because of the beetroot and aonla content as they are potent antimicrobials.

Table 4.14 Coliforms growth (cfu/ml x10^3) in aonla based beetroot blended RTS beverage during storage

Treatments	Treatments (Ratio/treatment)	Storage period	
		60 days	90 days
T_1	Aonla 100	Nil	Nil
T_2	Aonla and beetroot (95 :05)	Nil	Nil
T_3	Aonla and beetroot (90:10)	Nil	Nil
T_4	Aonla and beetroot (85:15)	Nil	Nil
T_5	Aonla and beetroot (80:20)	Nil	Nil
T_6	Aonla and beetroot (75:25)	Nil	Nil
T_7	Aonla and beetroot (70:30)	Nil	Nil

In microbiological study, it could be concluded that immediately after preparation of beverage, the total number of viable count was increased significantly. Similar results were also reported by Deka *et al.* (2004), The standard plate count and yeast and mould count of fresh aonla RTS has been reported as 5275 cfu/ml and 232 cfu/ml respectively (Kulkarni, 2011). Presence of yeast in aonla juice has also been reported by Jain *et al*. (2004) at 6 months, Hirdyani (2015) and Afreen *et al*., (2016) reported no microbial growth in Aonla RTS beverage.

CHAPTER-V
SUMMARY AND CONCLUSIONS

CHAPTER - V

SUMMARY AND CONCLUSION

The results of the present investigation titled "Studies on standardization and storage of aonla based beetroot blended RTS beverage" was conducted in the Department horticulture, Vasantrao Naik Marathwada Agricultural University, Parbhani, Maharashtra during 2021-22 are summarized and concluded in this chapter.

As the fruits and vegetables are of primary importance, in the present research development of aonla based beetroot blended RTS beverage was carried out by extracting aonla and beetroot juices and blending them in different ratios. The seven treatments with different blending ratios such as, T_1 = aonla (100), T_2 = aonla : beetroot (95:05), T_3 = aonla : beetroot (90:10), T_4 = aonla : beetroot (85:15), T_5 = aonla : beetroot (80:20), T_6 = aonla : beetroot (75:25), T_7= aonla : beetroot (70:30) were prepared and pasteurized at 85 ^{O}C for 30 minutes. After preparation the selected samples from each treatment were analysed for physio-chemical properties, sensory evaluation and microbial analysis at 0 day, 30 days, 60 days and 90 days.

5.1. Effect of blending and storage of beverage on physico-chemical characteristics.
5.2. Effect of blending and storage on sensory characteristics.
5.3. Effect of blending and storage on microbial analysis.

5.1. Effect of blending and storage of beverage on physico-chemical characteristics.

5.1.1 Total Soluble Solids (°Brix)

The treatment effect and storage period on physio-chemical properties of RTS beverage was found to be significant for all the traits studied except TSS and acidity. The TSS was found to be maximum in T_4 (aonla:100) followed by T_7 (aonla: beetroot::70:30) and the minimum TSS was observed in T_3 (aonla:beetroot::90:10) 0 day, 30 days, 60 days and 90 days of storage. TSS of all the treatments was on par as the intial TSS of the RTS was fixed to 10 ° Brix. It increased rapidly up to 90 days during storage in all the treatments.

5.1.2 pH

The level of pH in aonla based beetroot blended RTS beverage was influenced significantly by various treatments. The overall pH of beverage was found maximum in T_7 i.e. (aonla: beetroot ::70:30) which was at par with T_6 i.e. (aonla: beetroot::75:25) and the minimum in T_2 i.e., (aonlan:beetroot::90:05). It decreased rapidly up to 90 days during storage.

5.1.3 Reducing sugars (%)

Reducing sugars were found to be significantly different in all the treatments during entire storage period except 60 days. The maximum reducing sugars was observed in T_4 (aonla: beetroot::85:15) followed by T_7 i.e. (aonla: beetroot::70:30) and it was found to be minimum in T_6 (aonla: beetroot::75:25). It increased gradually up to 90 days during storage in all the treatments.

5.1.4 Non-reducing sugars (%)

Non-reducing sugars were found to be significantly different in all the treatments during entire storage period. The maximum non-reducing sugars was observed in T_7 i.e. (aonla: beetroot::70:30) followed by T_6 (aonla: beetroot::75:25) and it was found to be minimum in T_1 (aonla:100). It decreased gradually up to 90 days during storage in all the treatments.

5.1.5 Total sugars (%)

Total sugars were found to be significantly different in all the treatments during entire storage period except 60days. The maximum total sugars was observed in T_4 (aonla:beetroot::85:15) followed by T_7 i.e. (aonla: beetroot::70:30) and it was found to be minimum in T_6 (aonla: beetroot::75:25). It increased gradually up to 90 days during storage in all the treatments.

5.1.6 Ascorbic acid (mg/100ml)

Ascorbic acid content was found to be significantly different in all the treatments during entire storage period. The level of ascorbic acid content was found to be maximum in T_1 (aonla:100) followed by T_2 (aonla: beetroot::90:05)and the minimum ascorbic acid

content was recorded in the treatment T_7 (aonla: beetroot::70:30).It decreased gradually up to 90 days during storage in all the treatments.

5.1.7 Acidity

Acidity was found to be significantly different in all the treatments during entire storage period except at 0 day and 60 days. The level of ascorbic acid content was found to be maximum in T_5 (aonla: beetroot::80:20) which was on par with rest of the treatments as the acidity was fixed to 0.350% during RTS beverage preparation. It decreased gradually up to 90 days during storage in all the treatments.

5.1.8 Colour

The overall colour data showed colour deterioration with storage time. At 90 days all the treatments had lowest colour in comparison to their respective 0 day values. The brightest colour was recorded in T_7 at 0 day and lightest colour was recorded in T_1 at 90 days. The colour of aonla juice has been covered by beetroot juice except in aonla juice alone. The blending ratio led to the smallest change in total colour difference, considered as not perceptible by human eyes between T_3, T_4, T_5 and T_6, T_7.

5.1.9 Antioxidants

Overall antioxidant data indicated that antioxidants degraded over the course of storage. All of the treatments showed the lowest antioxidant levels at 90 days compared to their corresponding 0 day values. T_1 (aonla:100) had the highest antioxidant levels at 0 days, T_7 (aonla: beetroot::70:30) had the lowest antioxidant levels at 0 days. Ascorbic acid, light, heat, and storage temperature all may have an impact on this.

5.2. Effect of blending and storage on sensory characteristics.

5.2.1 Overall acceptability (Hedonic 9 point scale)

The overall acceptability score declined with the time during storage. In comparison to their individual 0-day scores, all treatments had the lowest overall acceptability scores at 90 days. T_4 (aonla: beetroot::85:15) had the highest overall acceptability score at 0 days, while T_2 (aonla: beetroot::90:05) had the lowest antioxidants after 90 days. The extreme astringency, caustic, and bitter taste of the aonla juice was

responsible for the beverage's overall low acceptability. The tendency of the overall acceptability score steadily declining throughout the course of storage may be brought on by the deterioration of all sensory criteria over extended periods of storage, including colour, taste, and flavour.

5.3 Effect of blending and storage on microbial analysis.

Throughout the whole storage time, the total plate count, yeast and mould count, and coliform count were all zero. The total plate count, yeast and mould count, and coliform count were 3 cfu/ml, 1 cfu/ml, and nil respectively during 90 days of storage. The highest values were reported at 10^{-3} dilution after 72 hours in T_3, followed by 2 cfu/ml, 1 cfu/ml, and nil at 10^{-3} dilution after 72 hours in T_4. While the other treatments had a plate count of 1 to 2 cfu/ml and no yeast, mould and coliform development. Because beetroot and aonlahave strong antimicrobial activity, it was anticipated that they would be resistant to microbial activity and the microbial growth starts from 90days of storage.

CONCLUSION

Aonla and beetroot are considered as significant crops because of their therapeutic and dietary benefits. High astringency, acrid and bitter taste of aonla had reduced the consumer acceptance. Therefore, it is necessary to blend the aonla juice with other juice. The nutrient richness of beetroot juice paved its importance to with aonla juice as it gives the additional boost up to the nutrient content and taste also. This innovative experimental trail was conducted with seven different concentrations of aonla and beetroot was evaluated for physio-chemical changes, consumer acceptance and shelf life for the period of ninety days.

Ready to serve beverage prepared had high health benefits and its physio-chemical changes during entire study period had increasing effect on the traits like TSS, reducing sugars, total sugars. While the traits like pH, non-reducing sugars, ascorbic acid content, acidity, antioxidants, colour and overall acceptability of the beverage was fond in treatment T_4 (aonla: beetroot::75:25). This indicated that oxidation and other enzyme activity in the stored juice may lead to its quality deterioration with the time. In addition to these the microbial activity present at 90 days of storage had confirmed the reduction in RTS beverage quality and thus it can be consumed till 90 days of storage. Among all the

treatments studied T_4 (aonla: beetroot::75:25) had highest consumer acceptance which was at par with T_5 (aonla: beetroot::80:20). Hence these concentrations are suitable for the preparation of aonla based beetroot blended RTS beverage.

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Academic qualification

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