PREPARATION OF BEETROOT-GINGER READY TO SERVE (RTS) JUICE AND STUDY OF STORAGE STABILITY AT DIFFERENT STORAGE CONDITION

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by

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Preparation of Beetroot-Ginger Ready to Serve (Rts) Juice and Study of Storage Stability at Different Storage Condition

A dissertation submitted to the department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B. Tech. In Food Technology

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Approval Letter

This ***dissertation*** entitled ***preparation of beetroot -ginger ready to serve juice and study of storage stability at different storage condition by*** **Pragya Paudel has been accepted as a partial fulfillment of the requirements for the B. Tech degree in Food Technology.**

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Abstract

The study was aimed for preparation of ready to serve drink from beetroot (*Beta vulgaris*) and ginger (*Zingiber officinale)* juice and study its storage stability. Beetroot and gingers, juice was prepared and chemical analysis was performed. Preliminary trials were performed to find the maximum and minimum quantity of beetroot juice, TSS, acidity and ginger juice. Beetroot- ginger blended RTS was prepared by optimizing beetroot juice at level of 15%, TSS (15°Bx) and acidity 0.3% and ginger juice 1.5%. Pasteurized (88°C 30s), filled, sealed and cooled to room temperature. Juice was stored for 28 days at room temperature (25±3°C), refrigeration temperature (5±1°C) in glass bottles and PET bottles and chemical as well as microbial changes were analyzed (TPC and coliform).

During analysis Total Soluble Solids (TSS), acidity as citric acid, pH and vitamin C of beetroot juice was found to be 9°Bx, 0.014%, 6.5 and 14 mg%, and for ginger juice these values were 4°Bx, 0.12%, 5 and 9 mg % respectively. Among the different proportions, the RTS prepared from 15% beetroot juice and 1.5% ginger juice was selected as superior from sensory analysis. The pH, acidity (as citric acid), TSS (°Bx), vitamin C (as ascorbic acid), reducing sugar (as dextrose), total sugar (as dextrose) of optimized blended RTS was found to be 3.1, 0.3% m/v, 15°Bx, 4.11 mg%, 1.38% m/v, 4.16% m/v. Similarly, Total plate count (TPC) not detected at initial stage but was found to be increased with successive week while there no coliform detected even at end of storage. During storage the chemical analysis at different interval showed there was not significant ($P<0.05$) change in TSS, browning index, acidity, vitamin C, betaxanthin content and betacyanin content. Packaging materials did not show any significant ($P<0.05$) reduction in rate of change in properties of product packed in glass as compared to PET bottle. Changes were observed more in room temperature than refrigeration temperature. RTS stored at the refrigeration temperature retained the desire quality attributes better than stored at room temperature. Glass bottles gave greater protection against degradation of the chemical attributes of the blended RTS.

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List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of variance
BI	Browning index
LSD	Least significance difference
NFC	Not from concentrate
PCA	Plate count agar
PET	Polyethylene terephthalate
POD	Peroxidase
PME	Pectin methyl esterase
PPOs	Polyphenol oxidases
RTS	Ready to serve
TSS	Total soluble solids
TPC	Total plate count
GRAS	Generally recognized as safe
PEF	Pulsed electric field
EDTA	Ethylenediamine tetraacetic acid

Part I

Introduction

1.1 General introduction

Ready to serve juice are type of beverages which are not diluted before serving. Generally, it is prepared from juice or pulp or both by mixing the sweeteners (sugar), acidulants (citric acid) and colorings and flavorings materials are optional. Ready-to- drink beverages are carbonated (Thapaliya, 2004).

Beetroot is found to be 10^{th} most powerful vegetables with antioxidant properties (Kushwaha *et al.*, 2018). Beets contain a substance called geosmin that imparts earthy smell to beetroot. Human beings are sensitive to this so even low dose can give intense scent. Some people adore the sweet and earthy flavor of beets, while others can't stand the thought of them (Anonymous, 2014). The Beetroot is the taproot portion of the beet plant. Other than as a food, it plays another role as a natural colorant in textile industries and as a medicinal plant. Beetroots are eaten boiled either as a cooked vegetable, or cold as a salad after cooking and adding oil and vinegar, or raw and shredded, either alone or combined with any salad vegetable. A large proportion of the commercial production is processed into boiled and sterilized beets or into pickles. In Eastern Europe beet soup, such as cold borscht, is a popular dish (Grubben and Denton, 2004).

Pickled beets are a traditional food of the American South. It is also common in Australia and New Zealand for pickled beetroot to be consumed on a burger. Garden beet juice is a popular health food. Betalains, obtained from the roots, are used industrially as red food colourants to improve the color of tomato paste, sauces, desserts, jams and jellies, ice cream, sweets and cereals. Roots can be round shaped, cylindrical or tapered. Their color can be white, yellow or red according to the color of the flesh. The leafy tops can also be used as a tasty spinach substitute (Kumar, 2015).

Ginger *(Zingiber officinale*), a subtropical perennial, herbaceous plant grown for its underground branched stem called rhizome belongs to family Zingiberacea. Ginger rhizome, as fresh or dried used as spice, flavoring agent, and traditional herbal remedy as anti-emetic, anti-oxidant, and anti-inflammatory against respiratory tract infections

(Shahrajabian *et al.*, 2019). Nepal is ranked as fourth largest ginger production country after India, China, and Nigeria which contributes about 10.16%, of global production in 2018. About 99% of Nepal's ginger is exported to India, about three fourth of which is fresh ginger while the remaining is in dried form locally known as "sutho" (Acharya *et al.*, 2019).

Ginger is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases. Ginger is a food spice that also has been accepted by the American Diabetic Association as a nutraceutical which are known to be functional food. Ginger has direct anti-microbial activity and thus can be used in treatment of bacterial infections (Aleem *et al.*, 2020). Ginger is relatively inexpensive due to their easy availability, universally acceptable and well tolerated by the most people. It has also "Generally Recognized as Safe" (GRAS) by the US FDA (Aleem *et al.*, 2020).

While improving flavor, palatability, and nutritive and medicinal value of various fruit juices such as aonla, mango, papaya, pineapple, citrus, pear, apple, watermelon, and vegetables including bottle gourd, carrot, beet root, bitter gourd, medicinal plants like aloe vera and spices can also be used for juice blending. All these natural products are valued very highly for their refreshing juice, nutritional value, pleasant flavor, and medicinal properties. Fruits and vegetables are also a rich source of sugars, vitamins, and minerals. However, some fruits and vegetables have an off flavor and bitterness although they are an excellent source of vitamins, enzymes, and minerals. Therefore, blending of two or more fruit and vegetable juices with spices extract for the preparation of nutritive ready-to-serve (RTS), beverages is thought to be a convenient and economics alternative for utilization of these fruits and vegetables (Bhardwaj and Pandey, 2011).

1.2 Statement of the problem

In spite of being nutrition rich and having several health benefits the knowledge about beetroot consumption is very low in the context of Nepal. Consumption is limited to a small group of people. Thus, main problem is lack of knowledge among the people ranging from farmer to consumer. It has great potential to become a profitable product in small scale farming using organic cultivation.

Intense red color of beetroot is due to pigment betalains, a group of phenolic secondary plant metabolites. These are used as natural colorants in different industrial purpose also utilization increased due to its health benefits in human, especially their antioxidant and anti-inflammatory, inhibition of lipid peroxidation, etc, (Wruss *et al.*, 2015). Beetroot is being utilized for making different products and for different research purpose. Beetroot is utilized for its red color in making jam, ketchup, sweets, sauce, dessert, juices, burgundy wine so on (Kumar, 2015). Beetroot was used for preparation and standardization of beetroot-based products i.e., halwa and lassi (Dwivedi *et al.*, 2017).

Beetroot is an underutilized nutrient rich crop with its unique combination of vitamins, minerals and antioxidants. In Nepal, beetroot is being commercially produced in various parts of the country but still they aren't being efficiently utilized in the market. Nowadays, consumers are much more concerned about their health and demand the food products conferring health benefits with reduced calories, low sugar content and rich in dietary fiber, etc. Due to the change in life-style, evidences of diseases such as cardiac arrest, infertility, eye problems, hypertension and similar other diseases is on the rise. Hence, presence of vitamin C and betalain as well as antioxidant property of beetroot helps to reduce above all health problems. Beetroot has earthy flavor which is found to be not desirable by peoples which can be mask by blending it with other fruits or spices. Addition of ginger to beetroot RTS increase appealing as well as nutritional value of juice.

1.3 Objectives

1.3.1 General objective

General objective of this work is preparation of beetroot- ginger Ready to Serve (RTS) juice and study its storage stability at different storage condition.

1.3.2 Specific objectives

- Extraction and analysis of beetroot and ginger juice (TSS, pH, acidity, moisture and juice yield).
- Recipe optimization for preparation of beetroot- ginger blended Ready-To-Serve juice (RTS) (% of beetroot juice, %ginger juice, %TSS, %acidity) by sensory analysis.

- Chemical analysis of the optimized RTS. (pH, acidity, reducing sugar, TSS, betalain, vitamin C, Browning index, etc.)
- Study storage stability of blended beetroot-ginger RTS at room temperature and refrigeration temperature in PET bottles and glass bottles in terms of chemical and microbial changes.

1.4 Significance of the study

The study can be beneficial in possible utilization of beetroot for the purpose of juice production so that loss occurs due to under-utilization can be minimized and addition of ginger can impart a pronounce flavor and somehow mask the earthy smell of beetroot. In the current context of world people are being more conscious about nutrition and diet in every food so production of this juice at commercial level may result a great advantage in economy of nation. Beetroot and ginger both are rich source of antioxidants and different minerals which result it being a highly nutritious. Despite of its high health benefits majority of people are totally unknown about its importance. Hence, this work might also provide enthusiastic market for beetroot which would also help the economy of people involved in its cultivation, production and marketing, ultimately uplifting their living standards.

1.5 Limitations of the study

- Storage stability analysis was carried out for only for 28 days, long period analysis wasn’t carried out.
- All the component (Anti-nutritional components, bioactive components and so on) of beetroot was not determined.

Part II

Literature review

2.1 Background

The global juice market is expanding and it is likely driven by the fitness conscious consumer and the demand for healthy food products. Nowadays juice manufacturers are customer centered and focus on introducing different juices varieties, flavors, and mix juices along with innovative packaging and detailed nutrition and health claims. The global juice market is predicted to witness strong growth at a compound annual growth rate of 3% during the period 2016-20 (Ceclu and Nistor, 2020) .

2.2 Beetroot

Beetroot (*Beta vulgaris*) is a vegetable consumed worldwide due to its high content of biologically active substances, such as betalain, inorganic nitrates, polyphenols, folates, as well as its minerals and vitamins. Its juice serve as traditional medicine, food colorant and additive to cosmetics and has high antioxidant and anti-inflammatory properties, and could be an important aid in the treatment of many diseases (Ceclu and Nistor, 2020).

The beetroot being an alkaline food with pH from has been acclaimed for its health benefits, in particular for its disease fighting antioxidant potential, significant amount of vitamin C and vitamins B1, B2, niacin, B6, B12 and excellent source of vitamin A (Kharode *et al.*, 2019). Beetroot is known to be a powerful antioxidant (Winkler *et al.*, 2005). The juice of beetroot is also consumed as a natural remedy for sexual weakness and to expel kidney and bladder stones (Sharma *et al.*, 2011). The claimed therapeutic use of beetroot includes its antitumor, carminative(reliving of discomfort of gas in digestive track), emmenagogue (drug to improve mensuration) and hemostatic and renal protective properties and is a potential herb used in cardiovascular conditions (Vali *et al.*, 2007). Its taxonomic classification is shown in Table 2.1

Table 2.1 Taxonomic classification of beetroot

Kingdom	Plantae
Sub kingdom	Tracheobionta
Division	Magnoliophya
Class	Magnoliopsida
Family	Amaranthaceae
Species	B. Vulgaris

Source: Ceclu and Nistor (2020)

2.2.1 Historical background and current situation of beetroot

Beets are native to the Mediterranean. Although the leaves have been eaten since before written history, the beetroot was generally used medicinally and did not become a popular food until French recognized their potential in the 1800's. Beet powder is used as a coloring agent for many foods. Some frozen pizzas use beet powder to color the tomato sauce. The most common garden beet is a deep ruby red in color, but yellow, white, and even candy striped are available in specialty markets. Outside the United States, beets are generally referred to as beetroot. It is estimated that about two-thirds of commercial beet crops end up canned (Yashwant, 2015).

2.2.2 Varieties of beetroot

Beet seeds are somewhat bigger than the seeds of other root crops, and they look like bits of cork. Each one is actually a cluster (or corm) with three or four seeds. Beets come in many shapes and sizes, and in colors ranging from red to white to golden or striped. Some varieties are grown for their greens rather than their roots. Here are descriptions of several popular varieties; (Anonymous, 2021a).

- Detroit dark red
- Crimson globe
- Crosby Egyptian
- Early wonder

2.2.3 Climate and soil

Beetroot is essentially a cool-season crop but it can be grown in moderately warm climate as it attains best color, texture and quality in cool weather. The plant can withstand moderate frosts, but growth will be affected. Bolting to seed in spring can be induced if the crop is exposed to prolonged periods of low temperatures during the winter months. Beetroot can be grown on a wide range of soils, but best results are obtained on well-drained sandy to loamy soils, with optimum pH 6 to 8. Hard, compacted soils should be avoided, as they impede seedling emergence and symmetrical root development. Fairly susceptible to boron deficiencies (Anonymous).

2.2.4 Cultivation

Cultivated beets (Beta vulgaris) are biennials, although they are usually grown as annuals. Beetroot produces green tops and a swollen taproot during its first growing season. The nutrients stored in the taproot are used to produce flowers and seeds in the second season. The cultivated forms of Beta vulgaris must be propagated from seed. The seed occurs in the form of a seed cluster, glomerular or seed ball. Germination usually takes between 10 to 24 days, depending on temperature and other factors, although under ideal conditions it can occur in less than 10 days. Beetroot germinates relatively well at high temperatures, but germination becomes slow and erratic at temperatures below 7°C. Beetroot seed has a relatively low germination rate compared to other crop seed (Nottingham, 2004).

2.2.5 Morphology

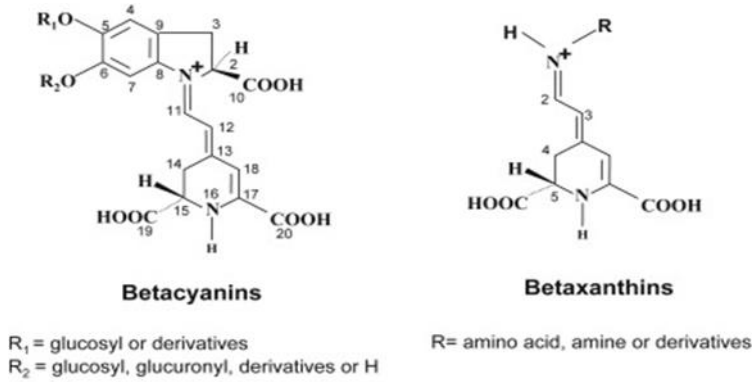
The beetroot is true biennial, producing thickened root and rosette of leaves during the first year and flowers and seeds the second year. Beetroots are mainly grown for their swollen root. The stem is short and plate, producing simple leaves that are arranged in a closed spiral. Leaves are heart shaped. The leaves can also be eaten as spinach. Flowers are very small with diameter of 3 to 5 mm and are produced in dense spikes. They are green or tinged reddish, with five petals. Fruit is a cluster of hard nut and dark color. (Neha P, 2018).

2.2.6 Functional component of beetroot

Beetroot juice is found in red in color due to the pigment betalains and somehow earthly due to geosmin. The betalains are subdivided into two structural groups: the red-violet

betacyanins and the yellow betaxanthins.as shown in Fig 2.1. The characteristic purple red violet color of beetroot is mainly derived from a betacyanin pigment called betanin (200mg of betanin is found in one beetroot). Cultivars with deep purple-red roots have a high ratio of betanin to betaxanthin pigments, while yellow and gold cultivars such as Burpee's Golden have relatively high levels of betaxanthins and very little or no red betanin pigment (Nottingham, 2004).

Betalains are important natural colorings within the food industry. During commercial extraction, beet roots are first crushed, and the colored juice is collected and concentrated. Betalain pigments are sold to the food industry either as juice concentrates or powders. Juice is concentrated under vacuum until it comprises around 60-65% of total solids. Freeze drying techniques are used to produce a powder, typically containing 0.3-1.0% pigment. (Nottingham, 2004).



(a) (b)

Source: Pereira *et al.* (2022)

Fig. 2.1 Structure of betacyanins (a) and betaxanthins (b)

2.2.7 Chemical composition of beetroot

Beetroot juice is rich source of carbohydrates, vitamins, minerals and poor in fats. Chemical composition of beetroot juice is given in Table 2.2.

Table 2.2 Chemical composition of beetroot juice.

Chemical composition	Average value
TSS(◦Bx)	9.0
pH	6.3
Titrable acidity (%)	0.014%
Reducing sugar (%)	4.2%
Total sugar (%)	7.93
Ascorbic acid(mg/100g)	10.01
Betalain (mg/100g)	14.20

Source: Kale *et al.* (2018)

2.2.8 Adverse effect of beetroot

The ability of beetroot juice to lower blood pressure depends on the nitrate concentration, which can vary widely among different beetroot juices. It is found that concentration of 4 millimoles per liter (mmol) of nitrate to lower blood pressure in healthy adults (Jamie, 2019). In most cases, people can safely eat beets or drink beetroot juice without experiencing any negative side effects.

1. Drinking beetroot juice regularly can affect the color of urine and feces due to the natural pigments in beets. People may notice pink or purple urine, which is called beeturia, and pink or purple feces.
2. Though rare, beetroot may cause anaphylaxis, which is an acute allergic reaction to an allergen to which the body has become hypersensitive.
3. The nitrates in beetroot juice affect blood pressure. Anyone who has low blood pressure or is currently taking blood pressure medication should speak with a healthcare professional before adding beets or beetroot juice to their diets.
4. Beets contain high levels of oxalates, which can cause kidney stones in people with a high risk of this condition (Jamie, 2019).

2.2.9 Products of beetroot

There are several products that are being making at home traditionally and at industrial level. At the point when beetroot is eaten crude, it is generally ground and served in a plate of mixed greens. It has an appealing crunchy texture. Marinating crude ground beetroot in a little vinegar can upgrade its flavor. Pickling is the good way of preserving beetroot. Boiled or roasted beetroot are skinned and placed on wide mouthed sterilized jar. Several recipes are found for preparation of beetroot pickle. Beetroot roasted chips as snacks having the nutritional value of different nutrients such as protein, carbohydrate, dietary fiber etc. Beetroot is being used for purpose of incorporation during preparation of different products such as ice-cream, cheese, flavor milk, value added cake, yoghurt, whey drinks, this result in increase in nutritional content of the product (Deshmukh, 2018).

Beetroot RTS is prepared from juice of beetroot with acidulants and sweetener. Sometimes beet is used to prepared mixed RTS, mixing along with carrot, apple, orange, lemon, tamarind, etc. This mixing can result in improve in both nutritive as well as sensory attributes of drink (Deshmukh, 2018).

2.3 Ginger

Ginger scientifically known as *Zingiber officinale* belongs to the family *Zingiberaceae* as given in below Table 2.3. Ginger has been widely used as spice and flavoring agents in foods and beverages. Ginger is one of the more commonly used herbal supplements. Ginger contains protein, carbohydrates, fiber, ash, and numbers of antioxidants like beta-carotene, terpenoids, ascorbic acid, alkaloids and polyphenols like flavonoids, flavones, glycosides and rutin (Aruoma *et al.*, 1997). Ginger may help in relieving joint pain from arthritis, and it also has blood thinning and cholesterol lowering effect which is useful for heart patients (Connell, 1969). The aroma of ginger is pleasurable and highly spiced which make it possible for food technologist to make a number of soft drinks like ginger cocktail, cordials, carbonated drinks, etc (Zeeshan *et al.*, 2018).

Table 2.3 Taxonomic classification of ginger

Kingdom	Plantae
Class	Monocotyledon
Order	Zingiberales
Family	Zingiberacea
Genus	Zingiber
Species	Zingiber officinale

Source: Kandasamy *et al.* (2020)

2.3.1 History of ginger cultivation

Ginger first appeared in the southern parts of the ancient China. From there, it spread to India, Maluku Islands (so-called Spice Islands), rest of the Asia and West Africa. Europe saw ginger for the first time in 1st century when the ancient Romans traded with India. When the Rome fell, Europe forgot about ginger until Marco Polo brought it to the East. (Anonymous, 2021b).

2.3.2 Soil and climate

It is cultivated in tropical, sub-tropical and humid climate. It can be grown up at an altitude of 1500 meters with well distributed rainfall. It requires high humidity throughout the growth. Soil should be rich in humus, light, loose, friable, well drained and at least 30 cm deep. Rhizome grows well in slightly acidic soil (Anonymous).

2.3.3 Nutrition of ginger

The major constituents in ginger rhizomes are carbohydrates, lipids, terpenes, and phenolic compounds. Proximate composition of ginger is shown in Table. 2.4.

Table 2.4 Proximate analysis of Ginger

Nutrients	Average value (%)
Moisture	87.4%
Total fat	0.8%
Total carbohydrate	10%
Protein	1.6%
Ash	1.0%
Ascorbic acid(mg/100g)	4mg/100g

Source: Ahammed *et al.* (2014)

2.3.4 Health benefits of use of ginger extract in beverage

One of the main benefits of the herbal ginger remedy is its ability to stimulate the circulatory system. The herb also helps in bringing an increased flow of blood to the surface of the skin; this singular property makes the ginger a very important herbal remedy for the treatment of condition such as chilblains and to treat impaired circulation along the hands and feet of patients. Perspiration in the body is increased by remedies made from ginger and at the same time, the herb helps in bringing about reduction in elevated body temperature during fevers consuming at least a combination of ginger based supplements is ideal for patients, so as to receive good amount of beneficial compound repeatedly confirmed by research, these include gingerols- found in fresh rhizome-based products and the shogaol which are found in dry products, it is reasonable to assume, the a combination of ginger product in the supplement to include sufficient quantities of these two beneficial compounds. It also aid in preservation effect on prepared beverage (Bastola, 2011).

2.4 Juice blending

Juice blending is one of the best methods to improve the nutritional quality of the juice. It can improve the vitamin and mineral content depending on the kind and quality of fruits and vegetables used (Afreen *et al.*, 2016). Two or more juice/pulp are blended in various

proportion for the preparation of nectar, RTS beverages, etc. This improves aroma, taste and nutrients of the beverages. Also there is increasing demand of consumers for new food products which should be nutritious and delicately flavored (Deka, 2000). The root plants like Beta vulgaris (beetroot) and Daucus carota (carrot) and Zingiber officinale (ginger) possesses wide range of compounds like flavonoids, phenolic acid, amino acid, ascorbic acid, tocopherol and pigments. Addition of spices to fruit juices improve flavor to make their flavor mare palatable. (Emelike *et al.,* 2016), (Hasani *et al.*, 2018) reports that increasing level of sugar syrup and acidity in the spiced treated beetroot juices enhanced the sensory attribute of color, flavor and general acceptability significantly higher than the control sample (Banigo *et al.*, 2015).

Sugar addition increases fruitiness, also it increases ripeness and decreases the green-leafy notes in apple flavors (Stampanoni, 1993). Increase in acidity increase sourness and tangy taste up to specific amount while too high negatively affect the sweetness (Bonnans and Noble, 1993). The overall organoleptic qualities are better in juice blended as kinnow, pomegranate juice and ginger than only kinnow and pomegranate (Bhardwaj, 2013) (Bhardwaj and Mukherjee, 2012).

2.5 Browning reaction

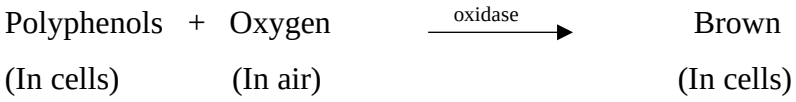
The browning reaction occurs widely in food. The colors produced range from light yellow to dark brown or black, depending on the type of product and the degree of reaction. In some foods, browning is considered desirable, such as honey, chocolate, the brown crust of baked goods, etc., while in other cases it is detrimental, such as browning of dehydrated fruits and vegetables (Srivastava and Kumar, 2007).

Browning reactions may be either enzymatic or no enzymatic. Many of the enzymatic reactions are seen in fruits and vegetables, and involve the oxidation of polyphenolic compound by oxidative enzyme in plant cells. The non-enzymatic browning reactions frequently involve sugars or sugar related compounds (Srivastava and Kumar, 2007).

2.5.1 Enzymatic browning

Many fruits and vegetables have the tendency to turn brown when damaged or when cut surfaces are exposed to air, e.g., apples, bananas, potatoes, etc., and this is due to enzymatic

reaction. The formation of brown color is due to action of enzyme phenolase also known as polyphenol oxidase, tyrosinase or catecholase) or phenolic substances. Normally the phenolic substrates are separated from phenolase in intact tissues and browning does not occur. When foods containing such substances are cut and exposed to air rapid browning of cut surfaces takes place.



The enzymatic reaction is due to oxidation of phenols into orthoquinones, which in turn rapidly polymerize to form melanin (the brown pigment). When the substrate is phenol, it is first converted by hydroxylation into orthodiphenol and then oxidized to orthoquinone. Tyrosine is the major phenolic substrate for phenolase action in foods. Other phenolic substances are caffeic acid, protocatechuic acid, and chlorogenic acid. The reaction occurs in several steps and are catalyzed by several enzymes e.g., phenolases, peroxidases and others (Srivastava and Kumar, 2007). Some fruits do not contain these enzymes and do not darken on exposure of cut surfaces to air (Srivastava and Kumar, 2007).

2.5.1.1 Prevention of enzymatic browning

Some of the methods for controlling browning are:

1. Use of chelating agents (e.g., EDTA) to bind and remove copper.
2. Vacuum or inert gas packaging to avoid the effects of oxygen.
3. Inactivation of polyophenolase by heat treatment (blanching).
4. Storage at low temperature to reduce rate of enzyme action.
5. Drying by physical or chemical (e.g., use of salts) means to reduce water activity below that required for enzyme action.
6. High pressure treatment and irradiation.
7. Use of browning inhibitors like SO2, ascorbic acid, phenolic antioxidants, acidulants (e.g., citric acid) (Rai, 2006).

2.5.2 Non enzymatic browning

The browning reaction that causes change in color and flavor of food that may be desirable (chocolate flavor of cocoa beans) and may be undesirable (dark brown of potato chips), not in involving any enzymes is known as non-enzymatic browning which includes roasting of

potatoes, baking of cake, and so on. The presence of reactive reducing sugars is responsible for browning in foods. On heating the sugars undergo ring opening, enolisation, dehydration and fragmentation. Heat-induced non enzymatic browning reaction can be divided into two groups: Maillard reaction and caramelization while others are ascorbic acid browning, lipid peroxidation etc (Srivastava and Kumar, 2007).

2.5.2.1 Maillard reaction

Maillard reaction also known as maillard browning is a color, flavor, odor and sometimes texture change which results from a chemical reaction between proteins and carbohydrates, It is named after Frenchman Maillard. The set of various reactions that sugar-amines undergo resulting in browning, maillard reaction (Srivastava and Kumar, 2007).

2.5.2.2 Caramelization

Caramelization is defined as the thermal degradation of sugars leading to the formation of volatiles (caramel aroma) and browned colored products (caramel colors), caramelization entails a series of complex reactions, some of which are still not well understood. The reactions, in sequence, include intramolecular arrangement of sugar molecules, dehydration, degradation, condensation, and polymerization (Rai, 2006).

2.5.2.3 Ascorbic acid browning

Ascorbic acid is a reductone and therefore can participate in the browning reaction as in maillard reaction. The molecule is first oxidized to dehydro-ascorbic acid and then transformed into di-ketogluconic acid. This acid is eventually decomposed to furfuraldehyde or related compounds which then polymerize or react with nitrogen (as in maillard reaction) to form brown pigments (Rai, 2007).

2.5.2.4 Lipid peroxidation

Lipid peroxidation occurs by the action of oxygen and reactive oxygen species on the fatty acids, especially unsaturated fatty acids. These are oxidized to form aldehydes and ketones which then react with amino acids to form brown pigments, as in the Maillard reaction. It is possible that peroxidation products induce the browning reaction of the Amadori products (Rai, 2007).

2.5.3 Browning Index

Browning index in the literature may mean one of two things: a simple indicator of a chemical change (often characterized by the optical density at a given wavelength or the ratio of the reflectance at 570 and 650 nm) or the color change due to oxidation of a freshly cut fruit or vegetable surface, during storage or drying, or the baking of bread (Hirschler, 2012). BI of any juices varies, from 0.29 to 1.72, among cranberry, cherry, grape, aronia and pomegranates depending upon the fruits also depends on nature of packaging materials and storage condition (Ponting *et al.,* 1960).

2.6 Fruit Juices

Fruit juice is defined as the fermentable but unfermented product obtained from the edible part of fruit which is sound and ripe, fresh or preserved by chilling or freezing of one or more kinds mixed together having the characteristic color, flavor and taste typical of the juice of the fruit from which it comes, i.e., the juice obtained directly from fruit. This product is often described as "direct juice" or "not from concentrate (NFC) juice" although these names are not controlled by the regulations (Mihalev *et al.*, 2018).

2.6.1 Types of fruit juices

According to their dispersion system composition, fruit juices can be divided into the following four main types

2.6.1.1 Clear/clarified (transparent) juice

This represents a water solution of the so-called soluble solids (sugars, organic acids, salts, free amino acids, water-soluble vitamins, and pigments, etc.) with particle sizes under 0.001 μm. It could be approximated with the cell sap found inside the plant cell vacuole. Clear juice is obtained by technological processing (clarification) of freshly pressed fruit juice (Mihalev *et al.*, 2018).

2.6.1.2 Opalescent (translucent) juice

In addition to the soluble solids (clear juice), contains colloidal substances with a distribution spectrum of 0.10-0.001μm. This dispersed phase includes pectin, hemicelluloses, proteins, protein polyphenol complexes, and dissolved starch (Mihalev *et al.*, 2018).

2.6.1.3 Cloudy (turbid) juice

This is actually an unclarified juice, i.e., pressed juice that is not subjected to clarification treatments. To improve cloud stability, coarse particles, which are generally unstable and prone to rapid sedimentation, can be partly removed (e.g., by centrifugation). With cloudy apple juice, 95% of all particles are of a smaller size than 2.5µm; the most frequent diameter is 0.60.8µm. These are relatively stable fine cloud particles, which consist of proteins, polysaccharides, lipids, and polyphenols. Fine cloud particles probably arise from cell membranes/walls, but appear not to be simply cell debris. There seems to be an association between cell membrane/ wall fragments and colloidally dissolved macromolecules, with native adsorbed pectin being an important factor for the cloud stability (Mihalev *et al.*, 2018).

2.6.1.4 Pulp-enriched juice

This contains a distinct amount of coarse cloud particles (sometimes termed pulp particles) with diameters of over 100µm, which are mostly fruit flesh fragments, e.g., juice sacs of the citrus fruit endocarp. Pulp-enriched juice can be obtained by blending of cloudy juice with fruit purée. Smoothies, comprising blended beverages of mashed fruits or purées and juices, could also be categorized under this type of dispersion system. The highest amount of coarse cloud particles can be found in fruit/vegetable purées (Mihalev *et al.*, 2018).

2.6.2 Ready To Serve

This type of beverages is not diluted before serving, hence called RTS (ready-to-serve). Generally, it is prepared from juice or pulp or both by mixing the sweeteners (sugar), acidulants (citric acid) and colorings and flavorings materials are optional. Ready-to- drink beverages are carbonated. The bottle is then filled with carbonated water and closed. The degree of carbonation employed varies according to the product. RTS are specified with having fruits parts not less than 10%, TSS not less than 10%, acidity not less than 0.3%,etc as given in Table No: 2.5 (FSSAI, 2011).

Table 2.5 Specifications for Ready-To-Serve juice

Parameters	Value
Fruit content	Not less than 10%
Total soluble solid	Not less than 10%
Acidity	0.2-0.3%
Sulphur dioxide	Not more than 70ppm

Source: FSSAI (2011)

2.6.3 Juice processing

The perishable nature of fruit juices poses significant challenges associated with production and preservation. The fermentation of juice soon after squeezing was the biggest challenge until preservation techniques were developed (Correa *et al.*, 2010). Studies have shown that the amounts of nutraceuticals in fruit juices are dependent on how they are produced, processed, and preserved. Thus, there is a need not only to document the traditional ways of extracting and preserving juices, but also to explore how novel processes can help to reduce the challenges encountered by the juice industry. Traditional techniques including canning, pasteurization, concentrating, freezing, evaporation, and spray drying have resulted in extensions in shelf life also the high-pressure processing has significant effect on microbial, physical and chemical properties of fruit juice (Bull *et al.*, 2004) but at the cost of nutritional or health attributes.

Thermal treatments sometimes fail to produce a quality, high-nutrition, and microbiologically stable product. In recent decades, the emphasis has been on employing novel approaches to enhance the safety and shelf life whilst retaining the nutritional quality of fruit juices. Numerous emerging technologies including high-pressure processing, pulsed electric field (PEF) processing, ultrasound, ozone processing, light-based technologies, irradiation, and non-thermal plasma have been applied for fruit juice preservation, processing, and packaging. These novel techniques are rapidly acquiring the juice market as they are efficient in shelf-life extension, enzymatic activity reduction, and microorganism inactivation, while maintaining the quality of the original, fresh pressed produce (Eissa and Mohmed, 2012). Drinking water must be of government standard as reported by Haldar *et al.* (2016) for preparation of RTS.

Juice extraction from beetroot involves series of process including cleaning in tap water, peeling, cutting in water to prevent browning, blanching followed by immediate cooling and grinding in electric grinder which is filtered through muslin cloth to obtain clear juice (Ansari *et al.*, 2017). Ginger juice from ginger rhizome by washing properly with water followed by peeling, and grinding using blender and filtered with cloth by pressing (Gaur *et al.*, 2019). Processes for preparation of blended RTS is shown in Fig 2.2 given below includes mixing as per requirements followed by pasteurization, bottling, cooling, labeling and finally storage.

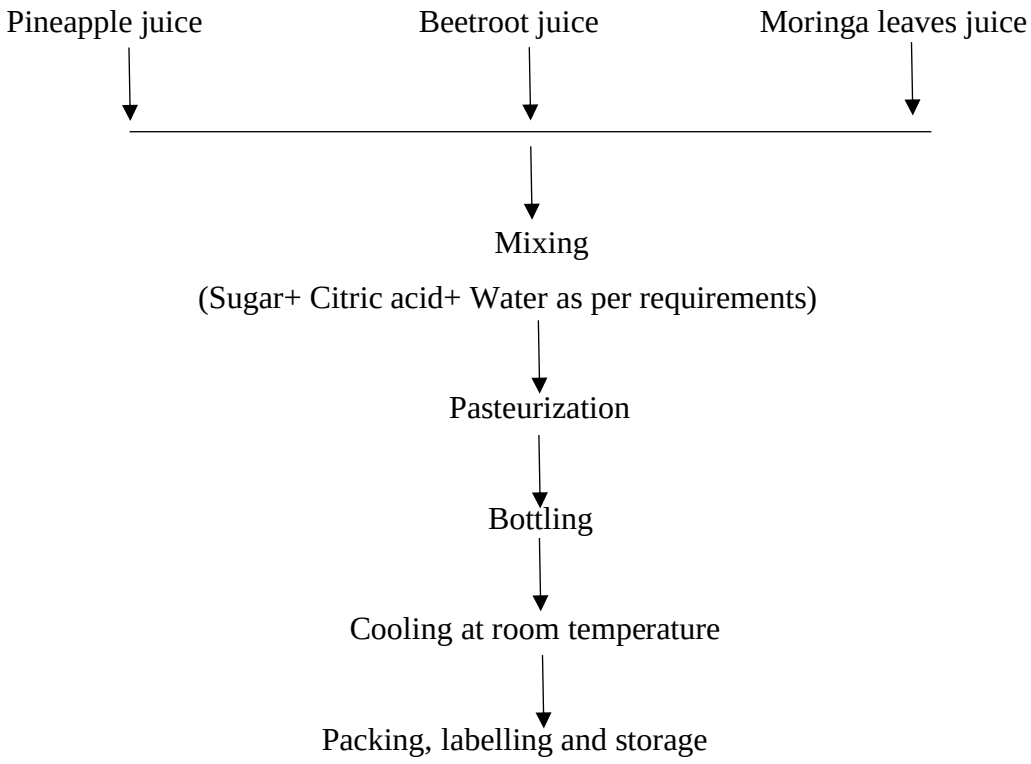


Fig 2.2 preparation of blended health drink

Source: Kharode *et al.* (2019)

2.6.4 Challenges associated with fruit juices production

As fruit juices are a healthier choice among consumers, the quality and the safety of juice products are always a worry, and they are always subject to very detailed legislation ensuring all necessary information on their nutritional benefits and compositions (Rajauria and Tiwari, 2017).

Apart from strict regulations, there are some other factors that pose challenges in the production of fruit juices and inhibit the growth of the global juice market. One of the main

challenges is associated with the constant supply of fruits, as most of fruits are seasonal and this affects the overall production. Other challenges include: manufacturing challenges (homogenization, extraction, filtration, processing, preservation, packaging, and storage); ingredients challenges (fruit components, sweeteners, flavors, colors, preservatives, nutraceutical ingredients, and miscellaneous additives); quality issues (color and flavor deterioration, appearance changes, packaging material, storage conditions, microbiological problems, shelf life, water quality and bottling issues); and most recent are new product development and marketing challenges (cost constraints, marketing brief, consumer assessment and complaints) However, availability of substitutes such as carbonated soft drinks, sports and energy drinks, and other hybrid drinks pose the prime challenges to the juice industry and inhibit the growth of the global fruit juice market (Rajauria and Tiwari, 2017).

2.6.5 Microbiological background and target microorganisms of fruit juices

2.6.5.1 *Escherichia coli, Salmonella,* and *Listeria monocytogenes*

Pasteurization is a treatment that can increase the safety of fruit juices. In choosing the target microorganism to calculate the lethality of a pasteurization treatment, juice processors may consider either *E. coli* O157:H7 or *Salmonella,* due to the numerous outbreaks that have been associated with them in unpasteurized juices, or *L. monocytogenes* due to its ubiquitous nature. The target microorganism should be the most heat-resistant pathogen likely to occur in the juice because, in inactivation conditions that are applied for the most heat-resistant pathogen, other microorganisms are also eliminated (Agcam *et al.*, 2018).

Some food borne pathogens can develop acid adaptation systems that induce cross-protection, and make them more resistant against other environmental stresses, thus increasing their ability to survive in juice. *E. coli* O157:H7, *L. monocytogenes*, *Salmonella spp*., and *C. parvum* can tolerate low pH values and survive in fruit juices and juice concentrates longer than cells that are unable to adapt. The acid adaptation of *Salmonella* spp., *L. monocytogenes*, and, *E. coli* O157:H7 also increases the heat resistance of these bacteria in apple, orange, white grape juices, apple cider, juice blends, cantaloupe, and watermelon juice (Agcam *et al.*, 2018).

2.6.5.2 *Alicyclobacillus*

Presently, more than 20 species have been described to belong to *Alicyclobacillus* genus but only four species (*A. acidoterrestris, A. pomorum*, *A. herbarius,* and *A. acidiphillus*) have been reported to be responsible for fruit juice or beverage spoilage. However, *A. acidoterrestris* is considered to be the most important, due to the number of spoilage episodes and its incidence. *Alicyclobacillus* spoilage is characterized by a phenolic off flavor as a result of its ability to produce guaiacol, 2,6-dibromophenol and 2,6-dichlorophenol (Agcam *et al.*, 2018).

2.6.5.3 Molds and yeasts

The growth in fruit juices of the fungus that can produce mycotoxins should be prevented for public health. Spores and vegetative cells of most molds are inactivated upon exposure to 60ºC for 5 min to avoid fungal growth and mycotoxin formation in foods. Notable exceptions are the ascospores of certain strains of the molds *Byssochlamys nivea, Byssochlamys fulva, Neosartorya fischeri, Talaromyces flavus,* and *Eupenicillium javanicum* in high-acid fruit pulps/juices (Agcam *et al.*, 2018).

Fermentation caused by yeasts and molds can be a problem in the juice industry, but the main problem in apple juice is patulin, a mycotoxin produced by various species of mold. Patulin have been reported as mutagenic, carcinogenic, and teratogenic (Agcam *et al.*, 2018).

According to a survey on the yeast flora of frozen fruit juice concentrates, the isolates recovered represented 12 genera and 21 species of yeast. The five most frequently isolated yeast species included *S. cerevisiae* (24.7%), *Candida stellata* (22.1%), *Z. rouxii* (14.3%), *T. delbrueckii,* and *R. mucilaginosa* (Agcam *et al.*, 2018). The maximum permitted limit for total viable microbial count in any fruit juices is found to be $1\times10^{4.}$ The microbial contamination can occur at any step from production to consumption but the microbial growth during storage depends on quality of packaging, storage temperature and preservatives added (Rahman *et al.*, 2011).

2.6.5.4 Preservation of juice

Freshly extracted juices are highly attractive in appearance and possess good taste and aroma but deteriorates rapidly, if kept for some time. To retain the natural taste and aroma of juice it

is necessary to preserve it soon after extraction without allowing it to stand for any length of time. Various method of preservation are employed and each has its own merits (Parajuli, 2010) . The methods generally used are:

a. Pasteurization
b. Addition of chemicals or sugar.
c. Drying and freezing
d. Filtration.

2.6.5.5 Pasteurization

The term “pasteurization“ was originally named after the French scientist, Louis Pasteur, who invented the process of heating at a mild temperature for a short time to extend the shelf life of liquids (wine and beer). Thermal pasteurization is a relatively mild form of heat treatment that is used to inactivate relatively heat-sensitive microorganisms, such as vegetative bacteria, yeasts, and molds, which are responsible for food spoilage or food poisoning. (Agcam *et al.*, 2018).

Fruit juices are pasteurized at such temperatures and for such periods as would render them sterile, without impairing their flavor. Usually, the juices are pasteurized according to the nature of juice and the size of the container. Acid fruit juice requires lower temperature and less time for pasteurization than the less acid ones. Pectin enzymes, which cause changes in the flavor and also bring about the clotting of particles in the juice, can be destroyed by heating the juice at the temperature mentioned above also enzymes requires air for their action and can, therefore be destroyed at a moderate temperature by removing the air from the juice. To obtain satisfactory results, it is essential to keep all equipment perfectly clean and to carry out the work under hygienic conditions. There are a number of methods used for the pasteurization of fruit juice. Some of the common methods are described in the following paragraphs (Parajuli, 2010). Minimum processing condition with the purpose of pasteurization for different foods are illustrated in the Table**:** 2.6

Table 2.6 Purpose of pasteurization for different foods

Food	Main purpose	Subsidiary purpose	Minimum processing condition
pH <4.5 (Fruit juices)	Enzyme inactivation (pectin esterase and polygalacturonate)	Destruction of spoilage microorganism (yeast, fungi)	65ºC for 30 min; 77ºC for 1 min 88ºC for 30 sec
Beer	Destruction of spoilage micro-organisms (wild yeast, *Lactobacillus* species and residual yeasts)		65°C-68°C for 20 min (in bottle); 72-75°C for 1-4 min at 900-1000 kPa
pH> 4.5 (Milk)	Destruction of pathogens (*Mycobacterium tuberculosis*, *Coxiella burnetti*)	Destruction of spoilage organisms, enzymes.	63°C for 30 min; 71.5°C for 15 seconds
Ice cream	Destruction of pathogens	Destruction of spoilage organisms.	65°C for 30 min; 71°C for 10 min; 80°C for 15 seconds

Source: Fellows (2000)

2.6.5.6 Bottle method or 'holding' pasteurization

This is used commonly home scale. The extracted juice is strained, filtered, as required and filled into bottles leaving proper head spaces for the expansion of the juice during heating. The bottles are then sealed airtight and pasteurized (Parajuli, 2010).

2.6.5.7 Pasteurization by over flow method

In this method the juice is heated to temperature about 2.5°C higher than the pasteurization temperature and filled into hot sterilized bottles up to the rim, taking care to see that during

filling and sealing, the temperature of the juice does not fall below the pasteurization temperature. The bottles should be hot at the time of filling to safeguard against fall of temperature of the juice and to prevent breakage of bottles are pasteurized at a temperature 2.5°C lower than the filling and sealing temperature. After pasteurization, the bottles are cooled. On cooling, juice contracts leaving a small head space, which does not contain any air (Parajuli, 2010).

2.6.5.8 Flash pasteurization

This is a process in which the fruit is heated for only a short time at a temperature higher than the pasteurization temperature for the juice. The juice is heated to a temperature 5.5°C higher than the pasteurization temperature and is held at that temperature for about a minute and then filled into container, which are sealed airtight under cover of stream to sterilize the seal, and then cooled (Parajuli, 2010).

2.6.6 Relation of fruit juice acidity and thermal treatment

The hydrogen-ion concentration of a food is a controlling factor in regulating many chemicals, biochemical, and microbiological reactions, and is symbolized by the term pH. Hydrogen-ion concentration is expressed in moles and pH is the negative log ion concentration. The pH value of foods is a deterministic factor of growth and activity of microorganisms. Thus, pH is also important in determining adequate heating requirements.

The most important factor affecting microbial spoilage is acidity, and thermal processing requirements for various foods depend mainly on pH. For example, the main purpose of thermal treatment is destruction of pathogenic bacteria in low-acid foods (pH. 4.5) such as mango, banana, or watermelon, and destruction of spoilage microorganisms or inactivation of specific enzymes for protecting food quality in medium- or high-acid foods (pH, 4.5) such as orange, lemon, or apple juice. The growth or presence of spore-bearing bacteria is not the key risk in acidic foods and killing the spore bearing microorganisms is not the target of the pasteurization process. Thus, pasteurization is applicable for highly acidic foods. The spoilage can be caused by generally non spore forming *Lactobacillus* and *Leuconostoc*, yeast, or molds, in high acid foods. On the other hand, in acidic products such as tomatoes (pH 4.0-4.4), spore-forming bacteria can be a risk factor, especially *Bacillus coagulans*, *Clostridium pasteurianum*, and *Clostridium thermosaccharolyticum*. High-acid fruits contain many

enzymes such as catalase, POD, PPO, and some of them (mainly PODs) have higher resistance to heat than the spoilage organisms. Thus, enzyme inactivation can be the target of pasteurization in some cases especially in canned fruit products (Agcam *et al.*, 2018).

2.6.7 Types of pasteurization according to intensity

Nowadays, people tend to consume not only safe and shelf-stable foods, but also foods that are rich in nutrients and are favorable in appearance, while food processors demand high speed, minimum cost and energy lost through food processing techniques. For these reasons, different pasteurization types are developed by researchers. According to intensity of applied heat treatment, there are four groups of conventional pasteurization:

- High-temperature long time (HTLT);
- High-temperature short time (HTST);
- Mild temperature-long time (MTLT); and
- Mild temperature-short time (MTST) (Petruzzi *et al.*, 2017).

HTLT pasteurization with temperatures in the range of 80°C-100°C and duration of less than 30 sec is the most commonly used method in the processing of juices. Low-acid juices with pH 4.5 need stronger treatments to have protected food quality. This treatment type could affect some bioactive compounds (phenolic compounds, flavonoids, and anthocyanins) in a positive way, and can reduce the activity of some enzymes, while other bioactive compounds with health benefits are affected negatively (Petruzzi *et al.*, 2017).

The pasteurization process with a combination of temperature less than 80°C and duration greater than 30 sec is called MTLT pasteurization. It is a process that is applied for improving minimally processed food products with longer shelf life (Petruzzi *et al.*, 2017).

2.6.8 Aseptic Packaging

Aseptic packaging can be defined as the filling of a commercially sterile product into a sterile container under aseptic conditions and hermetically sealing the containers so that reinfection is prevented (David 2012). This results in a product, which is shelf-stable at ambient conditions. The term “aseptic” is derived from the Greek word “septicos” which means the absence of putrefactive micro-organisms.

2.6.8.1 Aseptic Processing – Methodology

Aseptic processing comprises the following as shown in the Fig. 2.3:

1. Sterilization of the products before filling.
2. Sterilization of packaging materials or containers and closures before filling.
3. Sterilization of aseptic installations before operation (UHT unit, lines for products, sterile.
4. Production of hermetic packages (Ansari and Datta, 2003).

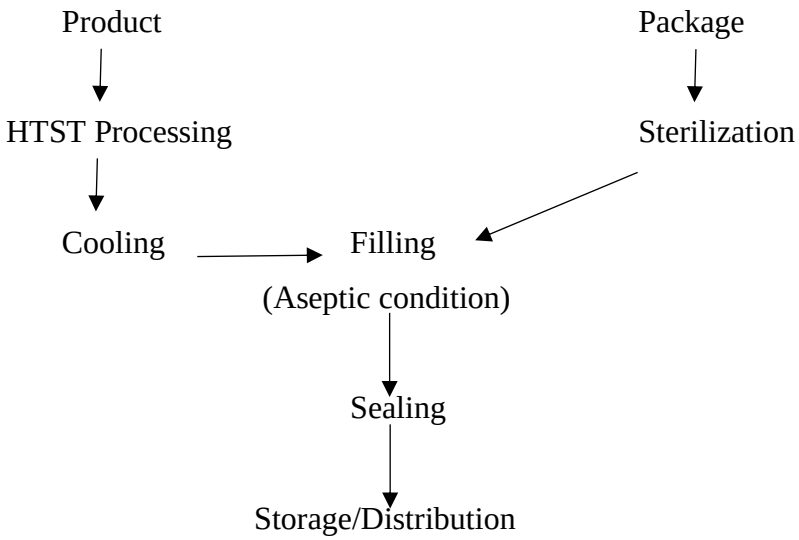


Fig. 2.3 Flow sheet for aseptic Packaging

Source: David (2012)

Besides the features mentioned above, additional advantages are that the HTST process utilizes less energy, as part of the process-heat is recovered through the heat exchangers and the aseptic process is a modern continuous flow process needing fewer operators (Ansari and Datta, 2003).

2.6.9 Storage stability of fruit juices

All food products are inherently unstable and quality retention depends upon a number of factors, including storage time and temperature. This is recognized in all new product work, in changing or improving existing products, and in modifying process. Storage stability technologies have come a long way in a few short years. This can be seen visually in the evolution of systems for holding products to study storage stability, first in desiccators, to

programming storage cabinets, to storage rooms which can be programmed to duplicate the climate of any place on earth (Desrosier and Desrosier, 1977).

2.6.9.1 Change in color during storage of RTS

The product kept at -17°C or -28°C has a slightly lighter appearance whereas the product stored at 21°C and 37°C tend to darken. The degradation in color may be due to Millard reaction or may be some other reasons as well (Li et al., 2018). Fruit juices appear darker due to browning. Browning of fruit juice during storage is result of a non-enzymatic chemical reaction between amino acids and reducing sugars called as Maillard reaction. HMF (hydroxymethylfurfural), formed in the Maillard reaction as well as during caramelization (pyrolysis of sugar), is the main product during storage which cause browning of food (Singh and Sharma, 2017).

2.6.9.2 Change in TSS during storage of RTS

A gradual rise in TSS value during storage of fruit juice has been reported under all storage conditions which might be associated with continuous increase in hydrolysis of polysaccharides and acids (Bhardwaj and Pandey, 2011). Bhardwaj (2013) proposed about the gradual passage of storage time as a function of increase in TSS which may be due to greater hydrolysis of polysaccharides. Also in preparation of mixed fruit RTS the slight increase in TSS was observed (Deka and Sethi, 2001). However, this rise in TSS is functional to storage temperature and a direct relation has been reported between increase in TSS and storage temperature. This might be correlated with lower rate of hydrolysis of sugars, polysaccharides and organic acids at lower temperature following the La Chatelier Principles of chemical reactions (Singh and Sharma, 2017).

2.6.9.3 Change in ascorbic acid (vitamin C) during storage of RTS

Vitamin C is important nutrient that possesses antioxidant ability and provides the protection against free radicals (Esteve et al., 2005). Ascorbic acid degradation is common in all consumable items during storage and can occur aerobically as well as anaerobically. However, rate of aerobic degradation is 100 to 1000 times faster than anaerobic degradation (Krishnaveni et al., 2001). Vitamin C is light and heat sensitive (Davey et al., 2000) , the concentration of vitamin C follows first order kinetics and thus storage time affects vitamin C

content (Singh and Sharma, 2017). On normal room temperature storage, the degradation of thermo sensible nutrients present in fruit juices such as, ascorbic acid occurs which when may oxidize may result in off flavor (Oliveira et al., 2012).

2.6.9.4 Change in acidity during storage of RTS

The titratable acidity of fruits or fruit juice includes the organic acids predominantly present in fruits. These organic acids are of high nutritional values and are useful in extending shelf life of fruit juice during storage. However, these are highly sensitive to temperature, storage condition and duration. The organic acids undergo degradation during storage which might be due to conversion of acids into sugar and salt by invertase enzymes (Singh and Sharma, 2017). Acidity can also increase due to the formation of acid by sugars or the breakdown of polysaccharides and oxidation of reducing sugars or by breakdown of pectic substances and similar pattern was also found in a juice blend of bottle guard and basil leaves juice by Majumdar *et al.* (2011).

2.6.9.5 Change in betalains during storage of RTS

Besides their potential beneficial effect on human health, betalains are the pigments responsible for the vivid color of beetroot. A significant decrease in betalains (betacyanins and betaxanthins) content of drink was recorded during the storage and more retention was observed under refrigerated storage conditions than ambient conditions. Loss of betalains in drink might be due to their high susceptibility to auto oxidative degradation and poor stability during storage. The possible changes that betalains may undergo during degeneration such as breakdown of the aldimine bond, dehydrogenation de-glycosylation and isomerization, which leads to decrease in the betalains content during storage (Khan, 2016). Similar observations have been reported by Herbach *et al.* (2007) in purple pitaya (*Hylocereus polyrhizus*) juice. Less decrease in color units of drink packed in glass bottle than PET bottle was observed because of slower rate of chemical reactions in product packed in glass bottle as a result of difference in their thermal conductance properties. Similar decreasing trend of red, yellow and blue color units has been reported by Thakur *et al.* (2017) in box myrtle drink and Hamid *et al.* (2017) in mulberry drink during storage. The characteristics color of raw food is due to natural pigment present in the plant material. Sometime, artificial coloring matter is added during food preparation to achieve the desirable color and acceptability (Cantwell and Kader, 2006).

Part III

Materials and methods

3.1 Materials

3.1.1 Raw materials

- Beetroot: Beetroot of good quality was bought from local market of Dharan, Nepal. Beetroot of variety crimson globe was use for juice preparation.
- Ginger: Ginger was brought from local market of Dharan, Nepal.
- Table sugar: The table sugar was purchased from the local market of Dharan, Nepal.
- Citric acid: Citric acid was provided from Central Campus of Technology laboratory.
- Water: A pure drinking water from the campus drinking water source was used for the dilution and volume make of the ready to serve juice.
- Bottle: Bottles of 250 ml capacity was purchased from the local market of Dharan.

3.1.2 Chemicals and equipment required

Equipment and chemicals used were available in Central Campus of Technology are given in appendix B.1 and B.2.

3.2 Methodology

The total work was based on preparation and study of storage stability or beetroot- ginger RTS.

3.2.1 Preparation of Beetroot and ginger for juice extraction

A fresh, ripened and healthy beetroots were sorted and selected for further processing. After washing, peeling was done manually using a knife and it was submerged in clean water in order to control browning. The small pieces were dipped in hot water at 80℃ for 3-5 min after blanching and washed with tap water to prevent further cooking. Juice was extracted with the help of electrical mixer grinder followed by filtration using muslin cloth as shown in Fig. 3.1.

Ginger rhizomes were sorted and followed by washing in running tap water, peeled manually then grinded. The paste was filtered through two folds of muslin cloth to get clear juice.

The Beetroot RTS with variation in juice content A (10%), B (12.5%), C (15%), D (17.5%) and E (20%) keeping TSS of juice fixed at 13ºBx and acidity 0.3% was prepared and best proportion of juice was selected by sensory analysis from panelist and further it was subjected for sensory analysis varying TSS of RTS to A (9%), B (11%), C (13%), D (15%) and E (17%) keeping acidity and juice content fixed at 0.3% and 15%. After optimization of TSS and juice content, sensory analysis performed for acidity in varying percentage as A (0.20%), B (0.25%), C (0.30%), D (0.35%) and E (0.40%). Sensory analysis for optimization of ginger juice content with variation of juice content A (0%), B (0.5%), C (1.0%), D (1.5%) and E (2.0%) with already optimized TSS and acidity.

3.2.2 Preparation of blended RTS

After analysis and optimization of RTS, the final optimized blended RTS was prepared. The RTS of beetroot juice content 15%, TSS 15ºBx, acidity 0.3% and added 1.5% ginger juice content was finalized by sensory analysis. Pasteurization of RTS was performed in a stainless-steel vessel at 88ºC, when the temperature reached to 88ºC, holding of the juice was done for 30 sec in the vessel.

Soon after holding, juice was then filled into the sterilized bottles, which was dipped into 0.2 % KMS solution and rinsed with hot water. Then the bottles were inverted and again held for 5 min and cooled to room temperature before subjected to storage.

3.2.3 Storage of juice

Pasteurized RTS was filled in pre-sterilized bottles of 250 ml capacity and some samples (optimized) were stored in room temperature (25±3°C) whereas others in refrigeration temperature (5±1°C). For the storage stability samples were subjected to chemical analysis and microbiological changes was studied at 7 days of interval for 28 days of storage in the laboratory.

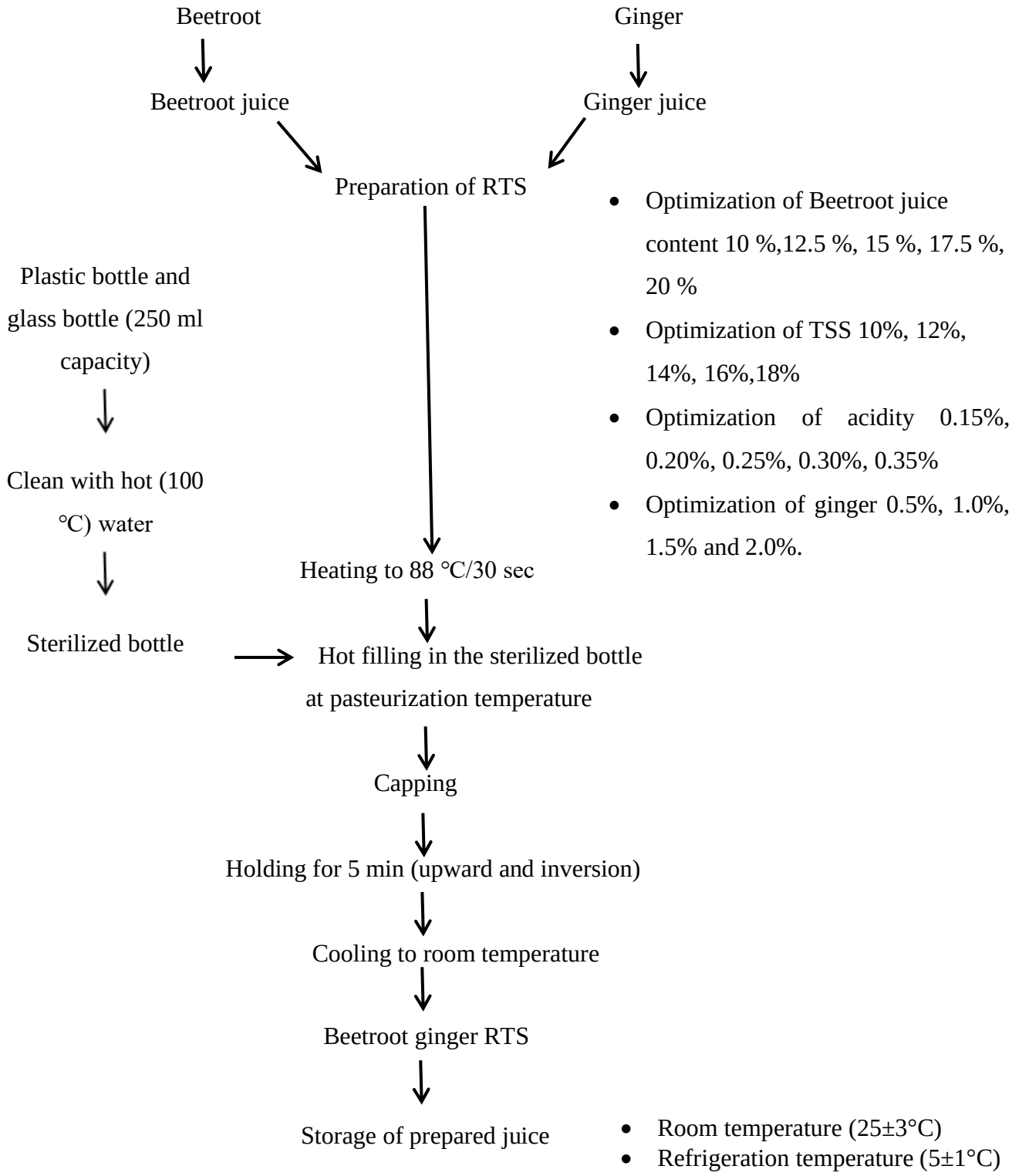


Fig: 3.1. Self-explanatory flow sheet of preparation of beetroot RTS

3.3 Analytical procedure

TSS, pH, acidity, reducing sugar, total sugar and ash of optimized product were analyzed. The final product was then kept at room temperature for 28 days and analysis was

conducted on every 7 days which included determination of TSS, acidity, vitamin C, browning index and microbial analysis including Total plate count (TPC) and Coliform count.

3.3.1 Sensory evaluation of formulated products

Among 20 prepared samples of RTS, the best one was determined by the sensory evaluation method. For sensory evaluation, 9-point hedonic scale as per Ranganna (1986) was used. The panelist members consisted of research students and teachers of CCT, Hattisar who had some previous experience (semi-trained) in the sensory evaluation.

The parameters for sensory evaluation were taken to be appearance/color, smell/flavor, taste, mouth feel, and overall acceptability. Panelist were requested to give the points from 1 to 9, 1 for extremely disliked and 9 for extremely liked sample. Sensory evaluation was carried out in individual booth with adequate light and free from obnoxious odors. Each panelist was provided with coded samples with random numbers and evaluation card (Appendix A).

3.3.2 Chemical Analysis

3.3.2.1 Titrable acidity

Titration was carried out by titrating 10 ml clear juice with standard N/10 NaOH and result was expressed as percentage citric acid.

$$\% \text{ Acidity} = \frac{\text{vol. of titrant X strength of NaOH X eqv. wt of citric acid}}{\text{wt of sample X1000}} \text{X } 100$$

3.3.2.2 Total soluble solid

Total soluble solid was determined with hand refractometer (0-30 ºBx) and values were expressed as degree brix according to Ranganna (1986).

3.3.2.3 pH

pH was directly measured by using pH meter which was standardized by using buffer solution of pH 7 and 4 at the temperature required.

3.3.2.4 Reducing sugar

Reducing sugar and total sugar of RTS was determined by using Lane and Eynon as described in Ranganna (1986).

3.3.2.5 Vitamin C

Ascorbic acid was determined by 2-6-dichloro-indophenol titration method as given in Ranganna (1986).

3.3.2.6 Moisture content

Moisture content of sample was determined by evaporating the water from the known juice sample by placing in a petri plate in an incubator according to Ranganna (1986).

3.3.2.7 Betalain

Light absorption measured at 538 nm and 476 nm was used to calculate the betanin and betaxanthin concentrations, respectively. In addition, the absorption at 600 nm was measured and used to correct for small amounts of impurities. The results were expressed as betacyanin (calculated in terms of betanin) and betaxanthin (calculated in terms of vulgaxanthin-I). The total betalain concentration is expressed as the sum of the betacyanins and betaxanthins.

3.3.2.8 Browning index

Browning extent was measured by the increase in absorbance of a sample extract at 440 nm using spectrophotometer (Labtronics, India). A clear extract of the sample was prepared by the addition and thorough mixing of 30 ml alcohol to 20 ml of centrifugate of the sample, then filtering through Whatman No. 1 paper as described in (Ranganna, 1986).

3.3.2.9 Hardness

Total hardness of water was determined by titrating with EDTA using Eriochrome black T as an indicator and titrating till sky blue end point (Rai and KC, 2007).

3.3.2.10 Alkalinity

Total alkalinity of water was determined by titration with HCl using methyl orange to pink end point as given by (Rai and KC, 2007).

3.3.3 Microbiological analysis

Total plate count (TPC) was determined by pour plate technique on Plate Count Agar (PCA) medium (incubated at 30°C/48h). Coliform count was determined by pour plate technique on MacConkey medium (incubated at 37°C/48h) (AOAC, 2005).

3.3.4 Data analysis

Analysis of variance (ANOVA) was carried out for data from sensory evaluation. The data were analyzed for two-way ANOVA, mean ANOVA (No blocking at 5% level of significance), LSD and interaction effects using Genstat (Genstat Discovery Edition 12, 2009) at 5% significance level were obtained to determine whether the sample were significantly different from each other and to determine which one is superior among them. The specimen evaluation card used for the sensory test appears in Appendix A. The mean was compared using LSD method. Standard deviation and means were also analyzed from the same statistical tool.

3.3.5 Storage studies

Blended RTS juice was filled in sterilized plastic and glass bottles of 250 ml capacity which were further subjected to two different conditions i.e., room temperature (25±1°C) and refrigeration temperature (5±1°C) for 28 days. The samples were drawn at interval of 7 days and evaluated for chemical properties (TSS, acidity, ascorbic acid, browning index and betalain) and microbiological qualities (TPC and coliform).

Part IV

Results and Discussions

4.1 Chemical analysis of raw juice.

Chemical composition of raw ginger and beetroot juice is given in Table 4.1.

Table 4.1: Chemical composition of (raw) juice

Parameter	Beetroot juice	Ginger juice
Moisture %	88.71(0.04)	94.97(0.1)
TSS (ºBx)	9.0	4.0
Total acidity (% as citric acid)	0.015(0.01)	0.12(0.01)
pH	6.5(0.13)	5.0(0.12)
Juice yield (% total fresh weight)	64.2(2.33)	57.61 (4.5)
Betalain (mg/l)	489.53(4.3)	-

* Values are the means of three determinations. Figures in the parentheses are the standard deviations.

The chemical composition of beetroot juice were analyzed and result revealed that moisture content was 88.71%, TSS was 9.0ºBx, acidity was 0.015% juice yield 64.2% and pH was 6.5, respectively results observed were similar with Kale *et al.* (2018). Similarly, chemical composition of ginger juice was observed as moisture 94.97%, TSS 4ºBx, total acidity 0.12%, pH 5.0 and juice yield was 57.61 respectively. The results reported were in close agreement with Ahammed *et al.* (2014); Bhardwaj and Pandey (2011).

4.2 Analysis of the drinking water

The drinking water we used for the dilution and preparation of RTS was analyzed before the use and the results are shown in the Table 4.2. Chemical composition of water shows pH 6.8, hardness 56 ppm, alkalinity 85 ppm and coliform was absent, was under the specification of Indian standard for drinking water as mention by Haldar *et al.* (2016).

Table 4.2 Composition of drinking water

Parameter	Value
pH	6.8
Hardness ($mgCaCO_3/H_2O$)	56 ppm
Alkalinity	85 ppm
Coliform	Nil

4.3 Optimization of beetroot juice content

Five different samples were prepared by varying juice content at constant acidity and TSS. The five different samples A, B, C, D and E with beetroot juice content of 10%, 12.5%, 15%, 17.5% and 20% respectively were subjected to sensory evaluation mean sensory score are shown in Fig**.** 4.1.

Here,

Sample A=10% Sample B=12.5%, Sample C=15%, Sample D=17.5% Sample E= 20%

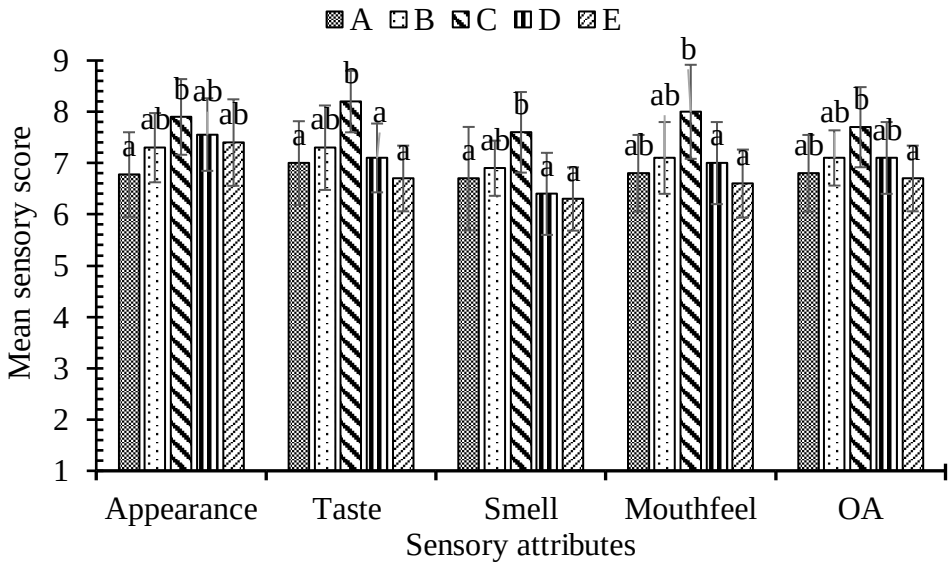


Fig 4.1 Mean sensory evaluation for different proportion of beetroot juice content

*Levels not connected by same letter are significantly different.

Appearance

From the sensory evaluation for appearance of the five different samples A, B, C, D and E of beetroot juice content of 10 %, 12.5 %, 15 %, 17.5% and 20 % mean sensory score were found to be 6.77, 7.3, 7.9, 7.56 and 7.4 respectively. Statistical analysis showed significant effect ($p<0.05$) of variation of proportion of beetroot juice in appearance of blend at 5% level of significance. Sample A&C were found significantly different while the rest samples were not significant different in appearance on variation with juice content. Sample C was found superior in appearance due to optimum acceptance of panelist.

Taste

Five sample of varying beetroot juice content 10%, 12.5%, 15%, 17.5% and 20% subjected to sensory evaluation the mean sensory score was found to be 7, 7.3, 8.2, 7.1 and 6.7 respectively. Statistical analysis shows significant effect on taste with variation of juice content at 5% level of significance. Sample A&C, C&D, and C&E were found significantly different while the rest samples were not significantly different in taste on variation with beetroot juice content. Highest mean score was observed with sample C due to optimum acceptance of panelist.

Smell

From the sensory evaluation for smell of the five different samples A, B, C, D and E the mean sensory score was found to be 6.8, 6.9, 7.6, 6.4 and 6.3 respectively. Statistical analysis showed significant effect ($p<0.05$) of variation of proportion of beetroot juice in smell of blend at 5% level of significance. Sample A&C, C&D, and C&E were found significantly different while the rest samples were not significantly different in smell on variation with juice content. The highest mean score was observed with sample C, due to optimum acceptance of panelist.

Mouthfeel

From the sensory evaluation for smell of the five different samples A, B, C, D and E the mean sensory score was found to be 6.8, 7.1, 8, 7 and 6.6 respectively. Statistical analysis showed significant effect ($p<0.05$) of variation of proportion of beetroot juice in mouthfeel of blend at 5% level of significance. Sample C&D, and C&E were found significantly different while the rest samples were not significantly different in taste on variation with

juice content. Among 5 samples sample C got highest sensory score that might be due to beetroot juice content increases earthy flavor of beetroot RTS when increase more than certain level.

Overall acceptability

From the sensory evaluation for smell of the five different samples A, B, C, D and E the mean sensory score was found to be 6.8, 7.1, 7.7, 7.1 and 6.7 respectively. Statistical analysis showed significant effect ($p<0.05$) of variation of proportion of beetroot juice in overall acceptability of blend at 5% level of significance. The highest mean score was observed with sample C, due to optimum acceptance of panelist.

From sensory mean score it can be concluded that with increasing juice content all the sensory parameters including appearance, taste, smell and mouthfeel were found to be improved that may be due to increasing juice content increase real juiciness similar results was reported by Gunathilake (2011) in his palmyrah RTS, from different treatment of different pulp concentration from 8-16 % best sample was of 12% from sensory panelist score. Gunathilake (2011) reported that with increasing pulp percentage acceptability of panelist was increase this may be due to pulp impart improve color flavor along with taste to RTS.

4.4 Optimization of TSS

Five different samples were prepared by varying TSS at constant acidity after optimizing juice content. The samples A(9˚Bx), B(11˚Bx), C(13˚Bx, D(15˚Bx) and E(17˚Bx) were subjected to sensory evaluation mean sensory score are shown in Fig**.** 4.2

Here,

Sample A=9˚Bx Sample B=11˚Bx, Sample C=13˚Bx, Sample D=15˚Bx Sample E= 17˚Bx

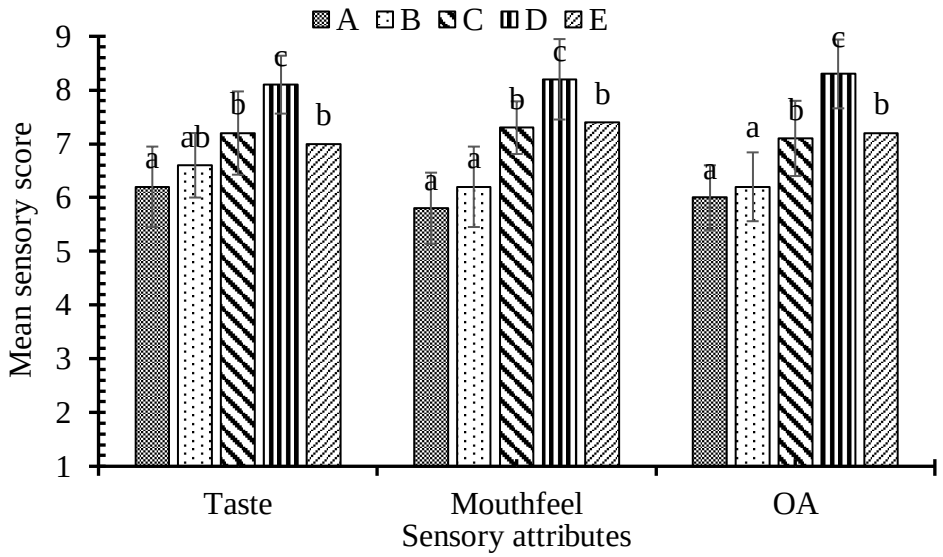


Fig 4.2 Mean sensory evaluation for different proportion of sugar content.

*Levels not connected by same letter are significantly different

Taste

The five different sample A, B, C, D and E of variation of TSS were subjected to sensory analysis. Statistical analysis showed that there was significant effect ($P<0.05$) of variation of TSS in the taste of juice at 5% level of significance. Samples A&C, A&D, A&E, C&D and D&E were significant different in taste at variation of TSS which may be by difference in sugar ratio. Sweetened juice sensory score seemed to be increase from 9 to 15˚Bx. Sensory score depreciate at 15˚Bx or higher similar results was reported by Emelike *et al.* (2016). Sample with higher sugar level was preferred by panelist similar observation was made by Hasani *et al.* (2018). The highest mean score was observed with sample D, due to optimum acceptance of panelist.

Mouthfeel

Five samples A, B, C, D and E got the mean sensory score of mouthfeels 5.8, 6.2, 7.3, 8.2 and 7.4 respectively. Statistical analysis showed significant effect ($P<0.05$) of variation of TSS in the taste of juice at 5% level of significance. Samples A&C, A&D, A&E, B&C, B&D, B&E and C&D were significantly different in mouthfeel at varying TSS, but the rest samples were not significantly different with each other. Sample D got high value due to optimum acceptance by panelist.

This shows that mouthfeel of juice was observed to increase at increasing sugar concentration leading to better acceptance by panelist similar to results reported by Banigo *et al.* (2015) in his blend of soy/carrot/beetroot drink.

Overall acceptability

Overall acceptance is the main factor for accepting the sample. The mean sensory score for the overall acceptance of all five samples A, B, C, D and E were found to be 6, 6.2, 7.1, 8.3 and 7.2 respectively. Statistical analysis showed significant effect ($P<0.05$) of variation of TSS in the taste of juice at 5% level of significance. Samples A&C, A&D, A&E, B&C, B&D, B&E and C&D were significantly different in overall acceptance at varying TSS, but the rest samples were not significantly different with each other. In overall, sample D having TSS 15˚Bx got high mean score due to optimum sugar to acid ratio. Sugar addition increases fruitiness, also it increases ripeness and decreases the green-leafy notes in apple flavors was reported by Stampanoni (1993) in his drink. Increase in sugar treatment increases overall acceptability up to 15 ˚Bx, results were in line with Emelike *et al.* (2016).

4.5 Optimization of acidity

By keeping the beetroot juice 15 % and TSS 15 ˚Bx constant, five different samples A, B, C, D and E were prepared of varying acidity 0.15%, 0.2%, 0.25%, 0.30% and 0.35% respectively and were subjected to sensory evaluation. The mean sensory score obtained of different samples from sensory evaluation is shown is Fig**.** 4.3.

Here, Sample A=0.15% Sample B=0.2%, Sample C=0.25%, Sample D=0.3% Sample E= 0.35%

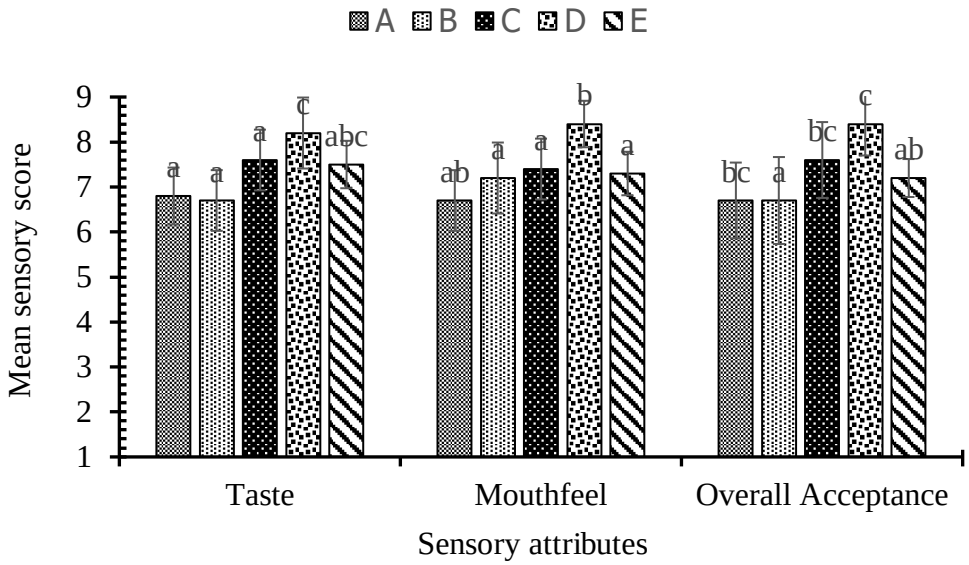


Fig. 4.3 Mean sensory score for different acidity of beetroot RTS.

*Levels not connected by same letter are significantly different.

Taste

The five different samples A, B, C, D and E of acidity variation 0.15%, 0.2%, 0.25%, 0.30% and 0.35% got mean sensory score for taste 6.8, 6.7, 7.6, 8.2 and 7.5 respectively. Statistical analysis showed significant effect ($P<0.05$) of variation of acidity in the taste of juice at 5% level of significance. Samples A&D, B&D and C&D were significantly different in taste at varying acidity. Among the five samples, sample D got the high mean score, due to optimum acceptance of panelist.

With increase in acidic content in sample increases sourness and increase tangy taste up to specific amount while too high negatively affect the sweetness as reported by Bonnans and Noble (1993).

Mouthfeel

Samples A, B, C, D and E were subjected for sensory analysis for mouthfeel and mean sensory score were found to be 6.7, 7.2, 7.4, 8.4 and 7.3. Statistical analysis showed significant effect ($P<0.05$) of variation of acidity in the mouth feel of juice at 5% level of significance. Samples A&D, B&D, C&D and D&E were significantly different in mouthfeel at varying acidity. Among the five samples, sample D got the high mean score, due to optimum acceptance of panelist.

Increase in acid content impart increase in fruity flavor and decrease in muddy flavor of beetroot juice while too high acid affect sweetness as reported by Bonnans and Noble (1993). Thus, sample D was found to be optimal as per sensory panelist.

Overall acceptance

Samples A, B, C, D and E were subjected for sensory analysis for overall acceptance and mean sensory score were found to be 6.7, 6.7, 7.6, 8.4 and 7.2. Statistical analysis showed significant effect ($P<0.05$) of variation of acidity in the overall acceptance of juice at 5% level of significance. Samples A&B, A&D, B&C, B&D and D&E were significantly different in overall acceptance. Among five samples sample D got the high mean score. With addition of citric acid, the overall acceptability was score higher this may be due to increase in taste and mouthfeel of juice compared to control. The results were in line with Emelike *et al.* (2016).

4.6 Optimization of ginger content

By keeping the beetroot 15 parts, TSS 15 ˚Bx and acidity 0.3% constant five different samples A, B, C, D and E were prepared of varying ginger juice content 0%, 0.5%, 1. %, 1.5% and 2. % respectively and were subjected to sensory evaluation. The mean sensory score obtained of different samples from sensory evaluation is shown is **Figure** 4.4

Sample A=0 % Sample B=0.5%, Sample C=1. 0%, Sample D=1.5% Sample E= 2.0 %

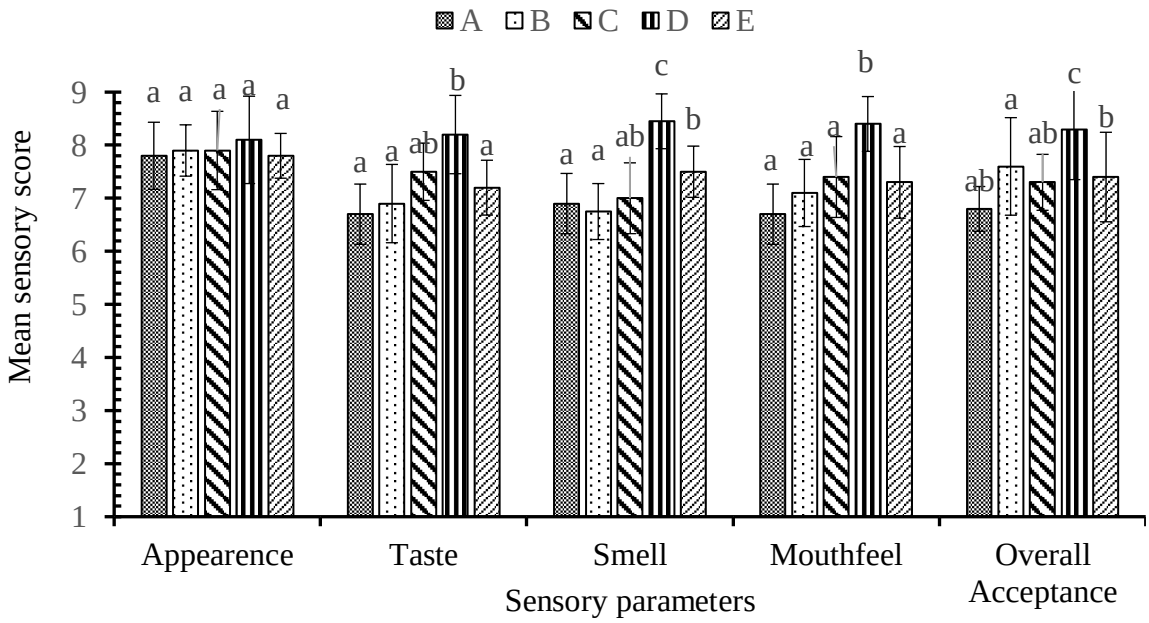


Fig 4.4 Mean sensory for different proportion of ginger juice content

*Levels not connected by same letter are significantly different

Appearance

The five different samples A, B, C, D and E of ginger juice content 0%, 0.5%, 1.0%, 1.5% and 2.0% respectively are subjected to sensory analysis and mean score of appearance of sample A, B, C, D and E were found to be 7.8, 7.9, 7.9, 8.1 and 7.8. Statistical analysis showed that there is no significant effect ($P<0.05$) of variation of acidity in the overall acceptance of juice at 5% level of significance. Due to intense red color of beetroot juice, there is no significant difference in appearance. Among five samples, sample D got the high mean score, due to optimum acceptance of panelist. Emelike *et al.* (2016) stated that addition of spice blend significantly improved the sensory score for color of juice compare to control.

Taste

Samples A, B, C, D and E were subjected for sensory analysis for taste and mean sensory score were found to be 6.7, 6.9, 7.5, 8.2 and 7.2. Statistical analysis showed that there is significant effect ($P<0.05$) of variation of ginger juice content in the taste of juice at 5% level of significance. Samples A&D, B&D, and D&E are significantly different in taste. Among five samples sample D got the high mean score, due to optimum acceptance of panelist. Treatment of juice with ginger significantly increase the taste of beetroot juice similar trend was observe by Emelike *et al.* (2016) in beetroot blend with ginger/ehuru.

Smell

Samples A, B, C, D and E were subjected for sensory analysis for smell and mean sensory score were found to be 6.9, 6.75, 7, 8.45 and 7.5. Statistical analysis showed that there is significant effect ($P<0.05$) of variation of ginger juice content in the smell of juice at 5% level of significance. Samples A&D, A&E, B&D, B&E, C&D and D&E are significantly different in smell. Nice (1995) stated that the spices are added to juices and fruit to improve their flavor makes more palatable. Emelike *et al.* (2016) state increasing ginger juice content somehow mask the muddy flavor of beetroot result in increased sensory score in terms of smell. Among five samples sample D got the high mean score, due to optimum acceptance of panelist.

Mouth feel

Samples A, B, C, D and E were subjected for sensory analysis for mouthfeel and mean sensory score were found to be 6.7, 7.1, 7.4, 8.4 and 7.4. Statistical analysis showed that there is significant effect ($P<0.05$) of variation of ginger juice content in the taste of juice at 5% level of significance. Samples A&D, B&D, C&D and D&E are significantly

different in taste. Among five samples sample D got the high mean score in mouth feel, due to optimum acceptance of panelist. Emelike *et al.* (2016) stated spiced juice results increase in mouth feel and general acceptability compared with without ginger.

Overall acceptance

Samples A, B, C, D and E were subjected for sensory analysis for overall acceptance and mean sensory score were found to be 6.8, 7.6, 7 .3, 8.3 and 7.4. Statistical analysis showed that there is significant effect ($P<0.05$) of variation of ginger juice content in the taste of juice at 5% level of significance. Samples A&D, B&D, B&E, C&D and D&E are significantly different in taste. Among five samples sample D got the high mean score, due to optimum acceptance of panelist.

Fruit juices have been found to be more acceptable or more effective when two or more fruits are blended together to produce mixed fruit juices (Banigo *et al.*, 2015).Bhardwaj and Mukherjee (2012) stated that the overall organoleptic qualities were better in juice blended as kinnow, pomegranate juice and ginger than only kinnow and pomegranate.

4.7 Analysis of final juice

According to sensory score the beetroot juice with fruit content 15 % with 1.5 % of ginger and 85 % of water, TSS of 15˚Bx and acidity 0.3%. The final product was further analyzed as in Table.4.3

Table 4.3 Chemical composition of blended juice.

Parameters	Values
Total Soluble solid (˚Bx)	15
Acidity (% citric acid)	0.3(0.02)
Reducing sugar %	1.38(0.18)
Total sugar %	4.16(0.17)
pH	3.87(0.02)
Vitamin C as ascorbic acid (mg/100g)	4.11(0.15)
Ash %	0.35(0.02)
Betalain (mg/l)	85.41(5.4)

*Values are the means of three determinations. Figures in the parentheses are the standard deviations.

4.8 Storage stability of the final product

Prepared beetroot ginger RTS was stored at 2 different storage conditions (room temperature 25±3℃ and refrigeration temperature 5±1℃) in glass bottle and PET bottle of 250ml capacity for 28 days' time period. The chemical and microbiological changes during storage were studied at every week for four weeks.

4.9 Microbial analysis

In microbiological analysis TPC and coliform were performed and the changes in microbial counts during storage are given in Fig 4.4. Four different samples were taken and coded as A, B, C and D where A is for glass bottle in room temperature, B is for PET bottle in room temperature, C is for glass bottle in refrigeration temperature and D is for PET bottle in refrigeration temperature.

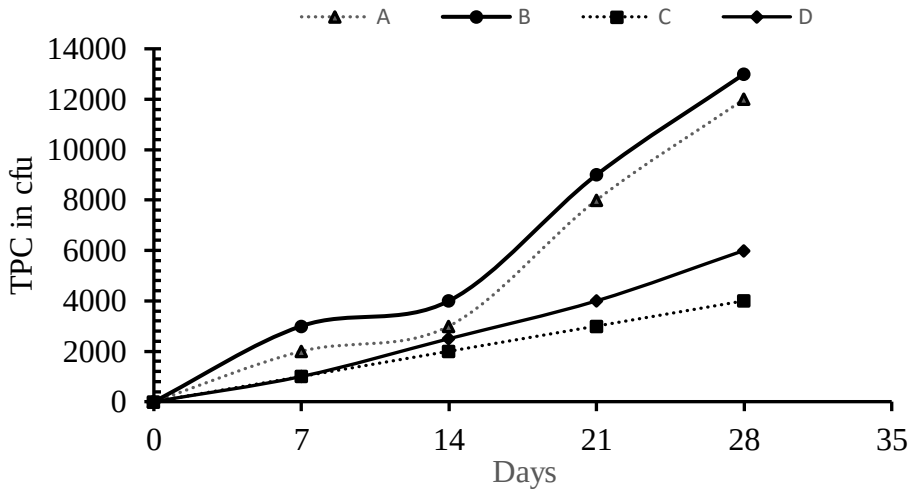


Fig 4.5 Change in TPC during storage of beetroot-ginger RTS

The results shows that no coliform was seen till 28 days but total plate count was found to be increasing with time of storage. Also, temperature and storage material had slight effect in increase number of microbial load with time, glass bottle at refrigeration had lower microbial load increasing rate compared to plastic bottle at room temperature. Growth of microbial load during storage depends upon the packaging material, storage temperature and use of preservatives (Rahman *et al.*, 2011). Rahman *et al.* (2011) had reported maximum permitted count in juice is 1×10^4cfu/ml thus, sample at room temperature exceed the limit after 28 days.

4.10 Chemical changes during storage of beetroot- ginger RTS

Four different samples of beetroot-ginger juice were taken and coded as A, B, C, D respectively. The chemical characteristics of the Beetroot- ginger RTS were analyzed in terms of TSS, acidity, Vitamin C, browning index and betalain (as betaxanthin and betacyanin) at 7 days intervals and the results are shown in Fig. 4.6 to 4.11.

4.10.1 Changes on TSS during storage

A slight increase in TSS of beetroot- ginger RTS during 28 days of storage at different storage condition was observed (Fig 4.6). TSS for the beverage range from 15 ˚Bx – 15.24 ˚Bx (glass bottle at room temperature), 15 ˚Bx- 15.3 ˚Bx (PET bottle at room temperature), 15 ˚Bx-15.17 ˚Bx (glass bottle at room temperature), and 15 ˚Bx -15.2 ˚Bx (PET bottle at

refrigeration temperature). The effect of storage time on TSS of beverage is shown in Fig 4.6

Here,

A= Glass bottle in room temperature B = PET bottle in room temperature

C= Glass bottle in refrigeration temperature D= PET bottle in refrigeration temperature

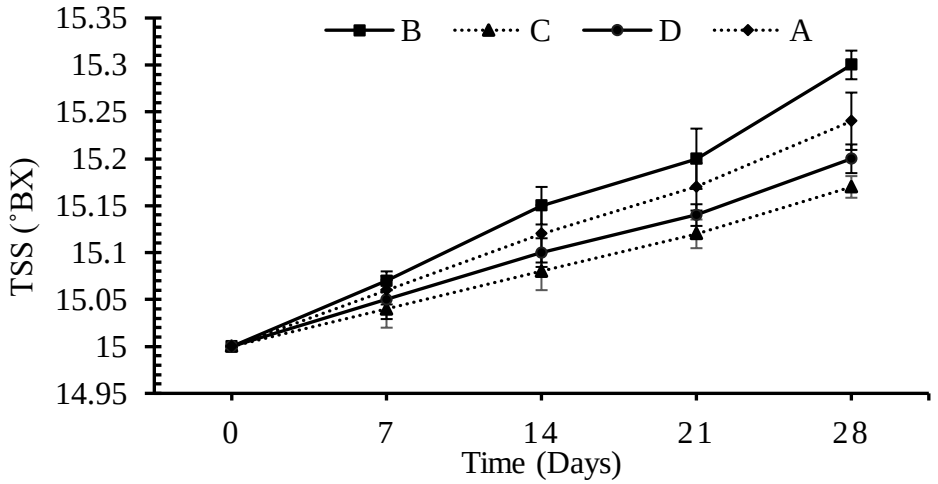


Fig 4.6 Effect of storage condition and temperature on TSS of beetroot-ginger RTS juice*

*Values are the mean of three determinations.

Retention or minimum increase in TSS content of juice during storage is desirable for preservation of good juice quality (Bhardwaj and Pandey, 2011). Above graph shows that increase in trend of TSS with time similar results were observed in the preparation of mixed fruit RTS by Bull *et al.* (2004) Deka and Sethi (2001). A gradual rise in TSS value during storage of fruit juice has been reported under all storage conditions which might be associated with continuous increase in hydrolysis of polysaccharides and acids. The gradual passage of storage time as a function of increase in TSS may be due to greater hydrolysis of polysaccharides. However, this rise in TSS was functional to storage temperature and a direct relation has been reported between increase in TSS and storage temperature. This might be correlated with lower rate of hydrolysis of sugars, polysaccharides and organic acids at lower temperature following the La Chatelier Principles of chemical reaction (Sharma *et al.*, 2011).

As regards the packaging material, it was observed that the RTS beverage stored in glass bottled showed less increase in TSS content during storage than RTS beverage in PET bottles, it might be due to chemically inert nature impermeability and capacity of bottle to withstand high temperature (Ganai, 2004).

4.10.2 Changes on titrable acidity during storage

The effect of storage time on the acidity of the beverage is shown in Fig. 4.7.

A= Glass bottle in room temperature B = PET bottle in room temperature

C= Glass bottle in refrigeration temperature D= PET bottle in refrigeration temperature

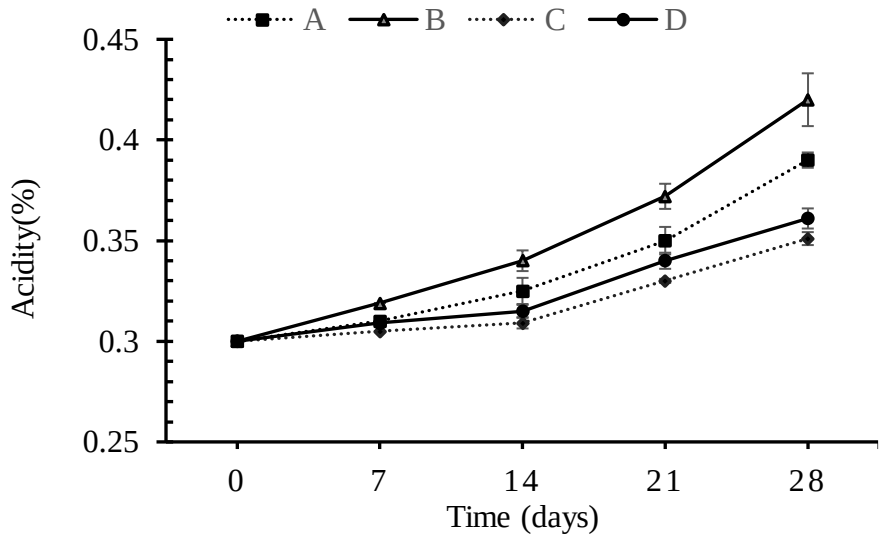


Fig. 4.7 Effect of storage condition and temperature on acidity of beetroot-ginger RTS juice*

*Values are the means of three determinations.

It was observed that beverage stored in glass bottle showed slower increase in acidity during storage as compared to PET bottles. Increase in acidity with storage time was observed in juices stored at refrigeration temperature both in PET and glass bottles. This increase may be attributed to the content of organic acids, pH, storage time and temperature as it was observed in study carried out on pH and titratable acidity (Dusabumuremyi *et al.*, 2021). The increasing trend in Fig**.** 4.7 might be due to the formation of acid by sugar or by breakdown of polysaccharides and oxidation of reducing sugar or by breakdown of pectic substances. Similar results were reported by Majumdar *et al.* (2011) in his blended bottle guard and basil leaves juice.

4.10.3 Changes on vitamin C during storage

The effect of storage time on the vitamin C of the beverage is shown in Fig. 4.8.

Here, A= Glass bottle in room temperature B = PET bottle in room temperature

C= Glass bottle in refrigeration temperature D= PET bottle in refrigeration temperature

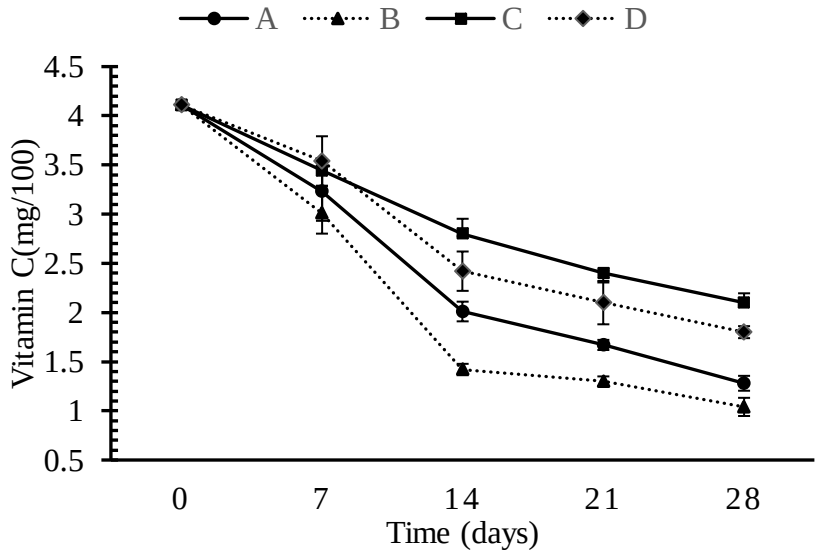


Fig. 4.8 Effect of storage condition and temperature on Vitamin C content of beetroot-ginger RTS juice.

*Values are the means of three determinations.

The vitamin C content of RTS were found to be slight decrease during 28 days of storage at 4 different storage condition was observed (Fig 4.8). Vitamin C for the beverage range from 4.11-2.1 mg/100ml (glass bottle at room temperature), 4.11-1.80mg/100ml (PET bottle at room temperature), 4.11-1.28mg/100ml (glass bottle at refrigeration temperature), and 4.11-1.04mg/100ml (PET bottle at refrigeration temperature) Similar trends have been reported by Thakur *et al.* (2018) in wild aonla fruits juice.

Vitamin C is an important nutrient that possesses antioxidant ability and provides the protection against free radicals (Esteve *et al.*, 2005). It is also considered as an indicator of the nutritional quality of juices. Storage temperature, type of processing and packaging materials affect the rate of ascorbic acid degradation during storage (Bull *et al.*, 2004). This decrease was probably due to the fact that ascorbic acid being sensitive to oxygen, light and heat was easily oxidized in presence of oxygen by both enzymatic and non-enzymatic catalyst (Davey *et al.*, 2000). Similar results of decrease in ascorbic acid content

with the increase in storage intervals in storage stability of jack fruit RTS beverage was reported by Krishnaveni *et al.* (2001). Storage temperature is one of the measures contributing factors for ascorbic acid degradation during storage as it is highly thermal sensitive. Pasteurization of fruit juice produces p-vinylguaiacol (PVG) and induces ascorbic acid degradation Sharma *et al.* (2011).

The decrease was significantly lower under refrigerated conditions as compared to ambient conditions. Slower rate of loss of phenols and ascorbic acid in refrigerated storage might be due to slower reaction rate in refrigerated conditions as compared to ambient. However, retention of more ascorbic acid of drink in glass bottle may also be because of slower reaction rates in glass bottle, as glass material absorb heat at slower rate as compared to PET. Similar reports are reported by Thakur *et al.* (2018).

4.10.4 Changes on browning index (BI) during storage

Effect of storage condition on browning index of beetroot-ginger RTS is shown in Fig 4.9.
Here,

A= Glass bottle in room temperature B = PET bottle in room temperature

C= Glass bottle in refrigeration temperature D= PET bottle in refrigeration temperature

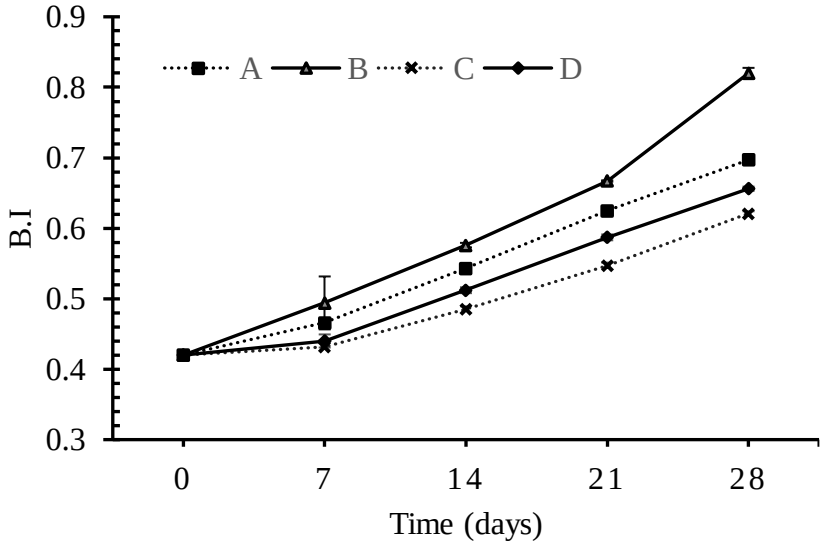


Fig. 4.9 Effect of storage condition and storage time on browning index (BI) of beetroot-ginger RTS juice*

*Values are the means of three determinations.

Initially at zero-day BI was 0.42 while that of 28th day were 0.697, 0.82, 0.621 and 0.656 for sample A, B, C and D respectively. Similar patterns were observed by Berlinet *et al.* (2004); Rizk *et al.* (2009). BI of any juices varies, from 0.29 to 1.72, among cranberry, cherry, grape, aronia and pomegranates depending upon the fruits. The BI was also found to be highly dependent upon nature of packaging material and storage temperature (Ponting *et al.*, 1960).

Browning of fruit juice during storage is result of a non-enzymatic chemical reaction between amino acids and reducing sugars called as Maillard reaction. HMF (hydroxymethylfurfural), formed in the Maillard reaction as well as during caramelization (pyrolysis of sugar), is the main product during storage which cause browning of food. The formation of HMF accelerated with storage time and temperature. Bhardwaj (2013) had reported a liner increase in non-enzymatic browning during storage of blended juice of Kinnow for 6 months.

4.10.5 Changes on Betalain during storage

Effect of storage condition on betalain content of beetroot-ginger RTS is shown in Fig 4.5 and Fig 4.10. Betalain is a pigment composed of beta-cyanin and betaxanthin. Initially at zero-day betacyanin was 64.167 mg/100ml while that of 28th day were 3.28 mg/100ml, 3.025mg/100ml, 26.115 mg/100ml and 20.14 mg/100ml for sample A, B, C and D respectively. Similarly, betaxanthin change from 19.98 mg/100ml at zero day to 4.41 mg/100ml, 2.89 mg/100ml, 12.47 mg/100ml and 11.98 mg/100ml. Similar result was observed in Thakur *et al.* (2020) in his betalain rich drink. The effect of storage time on betalain (betaxanthin and betacyanin) of beverage is shown in Fig 4.10 and Fig 4.11.

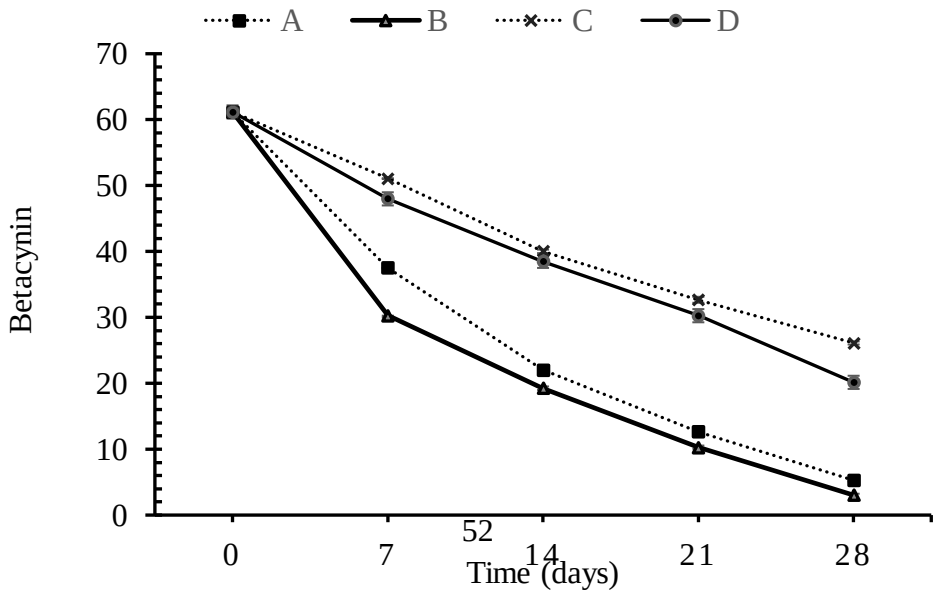


Fig 4.10 Effect of storage condition and storage time on betacyanins of beetroot-ginger RTS juice*

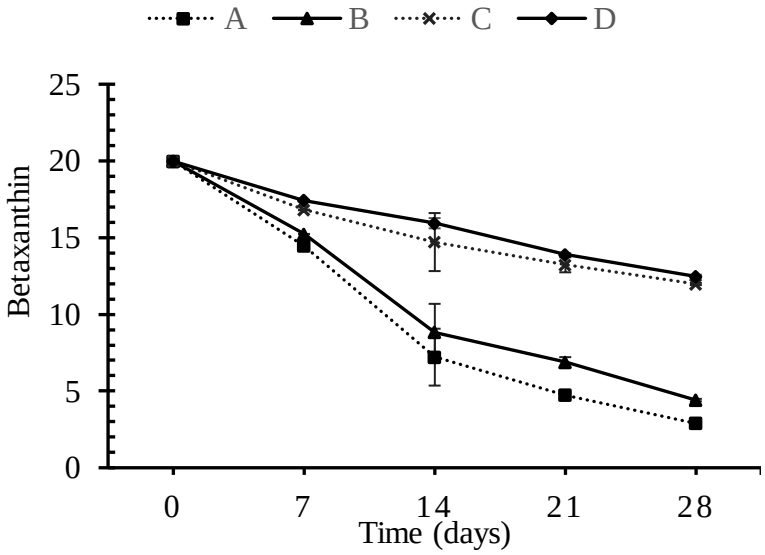


Fig 4.11 Effect of storage condition and storage time on betaxanthin of beetroot-ginger RTS juice*

*Values are the means of three determinations.

A significant decrease in betalains (betacyanin and betaxanthin) content of drink was recorded during the storage which was more under refrigerated storage conditions than ambient conditions. Loss of betalains in drink might be due to their high susceptibility to auto-oxidative degradation and poor stability during storage. The possible changes that betalains may undergo during degeneration such as breakdown of the aldimine bond, dehydrogenation de-glycosylation and isomerization, which leads to decrease in the betalains content during storage (Khan, 2016). Similar observations have been reported by Herbach *et al.* (2007) in his purple pitaya (*Hylocereus polyrhizus*) juice. Less decrease in color units of drink packed in glass bottle than PET bottle was observed because of slower rate of chemical reactions in product packed in glass bottle as a result of difference in their thermal conductance properties. Similar decreasing trend of red, yellow and blue color units has been reported by Thakur *et al.* (2017) in box myrtle drink and Hamid *et al.* (2017) in mulberry drink during storage.

Part V

Conclusion and Recommendations

5.1 Conclusions

Based on results and discussion of the study, following conclusions were drawn:

1. Among different juice proportion RTS prepared from 15% of beetroot juice and 1.5% of ginger juice were found to be superior from sensory evaluation.
2. Suitable TSS and acidity of product was selected as 15 ºBx and 0.3% respectively from sensory analysis.
3. The TSS, acidity, browning index and microbial load increased while ascorbic acid and betalain decreased during storage period of 28 days.
4. Product stored at the refrigeration temperature (5±1°C) retained the desired quality attributes better than product stored at room temperature (25±3°C). Glass bottles gave greater protection against degradation of the chemical attributes of the fruit juices.

5.2 Recommendations

Based on the present study, the following recommendations had made:

1. Long term study for shelf- life of beetroot-ginger blended RTS can be done.
2. Shelf-life of RTS (storage stability) can be studied using different preservatives and different color packaging materials.
3. Study of all the component of beetroot juice including bioactive component, anti-nutritional factors and so on.

Part VI

Summary

Beetroot (*beta vulgaris)* is lesser-known root crop found in Nepal. Beetroot contains some muddy flavor which was found to be unpleasing for people thus ginger was added to mask the flavor and add some spice tang to our juice. Thus, blending can improve nutritional as well as appealing value of juice than single juice. In this study, beetroot and ginger was taken from local market of Dharan. And other essential materials, chemicals and apparatus were taken from central campus of Technology laboratory respectively. First, beetroot and ginger were subjected to preliminary operations for juice extracted. The chemical composition of raw beetroot juice was found to be 88.71% moisture, 9˚Bx TSS, 0.014% total acidity (% citric acid), 6.5 pH, similarly of ginger juice was 94.97% moisture, 4˚Bx TSS, 0.12% acidity, pH 5, respectively.

RTS was optimized by serial sensory analysis first of all beetroot juice was optimized followed by TSS, acidity. Obtained product was further mixed with ginger and subjected to sensory evaluation to get optimum amount of ginger juice. Final product obtained was with 15% fruit juice 15 TSS, 0.3% acidity and 1.5% ginger juice were subjected to storage analysis for 28 days. Sample was analyzed weekly for change in TSS, titrable acidity, vitamin C, browning index, Betalain content (betacyanin and betaxanthin), total plate count and coliform. Zero-day analysis was carried out in order to find the changes in chemical composition of product. The chemical analysis of the fresh product showed 15˚Bx TSS, 0.3% acidity as citric acid, 4.11% vitamin C, 61.167mg/l betacyanin, 19.98mg/l betaxanthin, 0.42 Browning index, 0 coliform and total plate count was not detected. TSS, acidity, browning index and total plate count was found to be increase gradually whereas vitamin C, betacyanin and betaxanthin are found to be decrease gradually. Packaging materials shows slight reduction in rate of change in properties packed in glass bottles as compare to PET bottle. Fruit juice stored at the refrigeration temperature (5°C) retained the desired quality attributes in juices better than juices stored at room temperature (22-27°C). Glass bottles gave greater protection against degradation of the chemical attributes of the fruit juices.

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Appendices

Appendix A

SPECIMEN CARD FOR SENSORY EVALUATION

Name of the panelist: …… Date: ……

Product: Ready to serve juice (Beetroot- ginger)

Observe the product by tasting. Use appropriate scale to show your attitude by checking at the point best described your feeling of products. Write any of defects present described below. An honest expression of your personnel feeling will help me;

Parameter	Sample code				
	Sample A	Sample B	Sample C	Sample D	Sample E
Appearance/ Color					
Taste/ Flavor					
Aroma/ Smell					
Mouthfeel					
Overall acceptance					

Judge the characteristics on the 1-9 scale as below:

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

Any comments:

Signature:

Appendix B

B.1 Chemicals

All chemicals required for the experiment were obtained from laboratory of Central Campus of Technology. List of chemicals used for this work is shown in Table B.1

Table B.1 list of chemicals used

Chemicals	Chemicals
Sodium benzoate	Sodium hydroxide
Oxalic acid	Hydrochloric acid
Buffer solution (7 and 4)	2-6-dichloro-indophenol(dye)
Metaphosphoric acid (HPO_3)	Ascorbic acid
Carrez I solution	Carrez II solution
Fehling A solution	Fehling B solution
Copper sulphate	Methylene blue indicator
60% ethanol	Sodium carbonate

B.2 Equipments

All the eqipments required for the experiment were obtained from laboratory of Central Campus of Technology. List of equipments used for this work is shown in Table B.2

Table B.2 List of equipment

Physical apparatus	Physical apparatus
Stainless steel vessels	Hand refractometer (0-30ºBx)
Ph meter	Titration apparatus
Autoclave	Weighing arrangement
Mixer grinder	Knives
Heating arrangement	Thermometer
Other routine glassware	Centrifuge (Remi R 24)
Spectrophotometer (Labtronics, India)	

Appendix C

Table. C Cost evaluation (for every 100ml bottle)

S.N.	Particulars	Quantity	Rate (Rs)	Amount (NRs)
1	Beetroot	15ml	130/KG	3.12
2	Ginger	1.5ml	60/KG	0.18
3	Sugar	15gm	100/KG	15
	Raw material cost			18.3
	Processing cost and labor cost (10% of raw material cost)			1.83
	Profit 10%			2.013
	Grand total cost			22.143

Total cost of Beetroot orange RTS is found to be 22.143 per 100 ml.

Appendix D

ANOVA result for sensory analysis of beetroot juice content

Table D.1 ANOVA (no blocking) for appearance of beetroot juice optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	7.5200	1.8800	3.24	0.023
Panelist	9	5.1200	0.5689	0.98	0.472
Residual	36	20.8800	0.5800		
Total	49	33.5200			

Table D.2 ANOVA (no blocking) for mouthfeel of beetroot juice optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	11.6000	2.9000	5.12	0.002
Panelist	9	6.5000	0.7222	1.27	0.284
Residual	36	20.4000	0.5667		
Total	49	38.500			

Table **D.3** ANOVA (no blocking) for smell of beetroot juice optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	10.6800	2.6700	3.74	0.012
Panelist	9	6.1800	0.6867	0.96	0.487
Residual	36	25.7200	0.7144		
Total	49	42.5800			

Table **D.4** ANOVA (no blocking) for taste of beetroot juice optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	12.9200	3.2300	5.85	<.001
Panelist	9	4.8200	0.5356	0	0.059
Residual	36	19.8800	0.5522		
Total	49	37.6200			

Table D.5 ANOVA (no blocking) for overall acceptance of beetroot juice optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	6.0800	1.5200	2.80	0.040
Panelist	9	4.0800	0.4533	0.84	0.588
Residual	36	19.5200	0.5422		
Total	49	29.6800			

Appendix E

Table E.1 Two-way ANOVA (no blocking) for taste in Sugar optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	20.4800	5.1200	16.00	<.001
Panelist	9	8.9800	0.9978	3.12	0.007
Residual	36	11.5200	0.3200		
Total	49	40.9800			

Table E.2 Two-way ANOVA (no blocking) for mouthfeel in Sugar optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	37.6800	9.4200	28.45	<.001
Panelist	9	11.3800	1.2644	3.82	0.002
Residual	36	11.9200	0.3311		
Total	49	60.9800			

Table E.3 Two-way ANOVA (no blocking) for overall acceptance in Sugar optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	31.0000	7.7500	29.68	<.001
Panelist	9	11.6000	1.2889	4.94	<.001
Residual	36	9.4000	0.2611		
Total	49	52.000			

Appendix F

Table F.1 Two-way ANOVA (no blocking) for taste in acidity optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	15.3200	3.8300	8.47	<.001
Panelist	9	5.9200	0.6578	1.45	0.202
Residual	36	16.2800	0.4522		
Total	49	37.5200			

Table F.2 Two-way ANOVA (no blocking) for mouthfeel in acidity optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	15.4000	3.8500	9.49	<.001
Panelist	9	8.000	0.8889	2.19	0.046
Residual	36	14.6000	0.4056		
Total	49	38.000			

Table F.3 Two-way ANOVA (no blocking) for overall acceptance in acidity optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	20.2800	5.0700	12.57	<.001
Panelist	9	6.0800	0.6756	1.67	0.131
Residual	36	14.5200	0.4033		
Total	49	40.8800			

Appendix G

Table F.1 Two-way ANOVA (no blocking) for appearance in ginger juice optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	2.7200	0.680	1.07	0.386
Panelist	9	5.9200	0.6578	1.03	0.432
Residual	36	22.8800	0.6356		
Total	49	31.5200			

Table G.2 Two-way ANOVA (no blocking) for smell in ginger juice optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	14.5000	3.62500	12.92	<.001
Panelist	9	7.5250	0.8361	2.98	0.009
Residual	36	10.1000	0.2806		
Total	49	32.1250			

Table G.3 Two-way ANOVA (no blocking) for taste in ginger juice optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	9.9700	2.4925	7.16	<.001
Panelist	9	8.3200	0.9244	2.66	0.018
Residual	36	12.5300	0.3481		
Total	49	30.8200			

Table G.4 Two-way ANOVA (no blocking) for mouthfeel in ginger juice optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	10.000	2.5000	6.82	<.001
Panelist	9	17.925	1.9917	5.43	<.001
Residual	36	13.200	0.3667		
Total	49	41.1250			

Table G.5 Two-way ANOVA (no blocking) for overall acceptance in ginger juice optimization

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	11.8800	2.9700	10.16	<.001
Panelist	9	6.0800	0.6756	2.31	0.036
Residual	36	10.5200	0.2922		
Total	49	28.4800			

Appendix H

Table H.1 Two-way ANOVA (no blocking) for changes in TSS during storage.

Source of variation	d.f	s.s	m.s	v.r	F pr.
Days	4	0.011911	0.00390	0.57	0.643
Samples	3	669.473916	167.368479	24189.08	<.001
Residual	12	0.083030	0.006919		
Total	19	669.588857			

Table H.2 Two-way ANOVA (no blocking) for changes in Browning Index during storage

Source of variation	d.f	s.s	m.s	v.r	F pr.
Days	4	0.203043	0.050761	48.00	<.001
Samples	3	0.024801	0.008267	7.82	0.004
Residual	12	0.012689	0.001057		
Total	19	0.240533			

Table H.3 Two-way ANOVA (no blocking) changes in acidity during storage

Source of variation	d.f	s.s	m.s	v.r	F pr.
Days	4	0.0027894	0.0009298	6.43	0.008
Samples	3	0.024801	0.008267	7.82	0.004
Residual	12	0.0017351	0.001057		
Total	19	0.0212382			

Table H.4 Two-way ANOVA (no blocking) changes in Vitamin C during storage

Source of variation	d.f	s.s	m.s	v.r	F pr.
Days	4	18.38845	4.59711	74.51	<.001
Samples	3	1.86956	0.62319	10.10	0.001
Residual	12	0.74039	0.06170		
Total	19	20.99840			

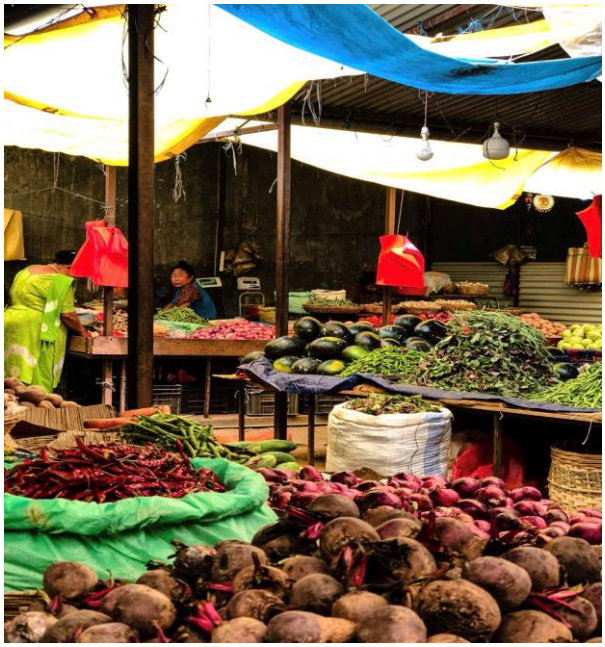
Table H.5 Two-way ANOVA (no blocking) changes in betacynin during storage

Source of variation	d.f	s.s	m.s	v.r	F pr.
Days	4	5538.57	1384.64	54.92	<.001
Samples	3	1111.06	370.35	14.69	<.001
Total	12	302.57	25.21		
Total	19	6952.20			

Table H.6 Two-way ANOVA (no blocking) changes in betaxanthin during storage

Source of variation	d.f	s.s	m.s	v.r	F pr.
Days	4	383.754	95.938	19.29	<.001
Samples	3	138.736	46.245	9.30	0.002
Total	12	59.674	59.674		
Total	19	582.164			

Color plates



P1. Beetroot from local market of Dharan

P2. Sensory evaluation

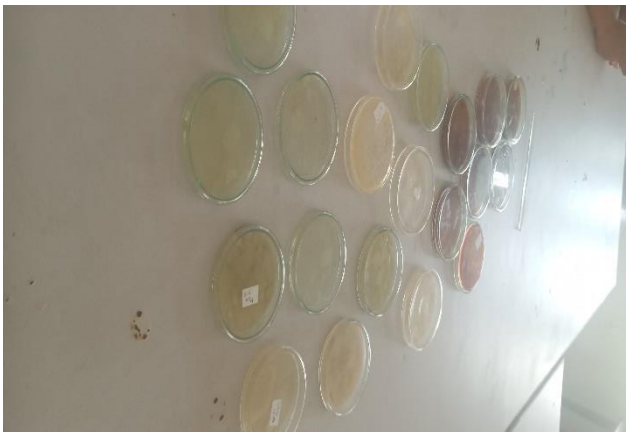
P3. Samples for analysis

P4. Samples for analysis

P5 Samples for betalain determination

P6 Spectrometric analysis of sample

P7 Microbial analysis



P8 Results of microbial plating