EFFECT OF MINT INCORPORATION ON STORAGE STABILITY OF PAPAYA BASED READY TO SERVE (RTS) BEVERAGE

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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 ***Effect of Mint Incorporation on Storage Stability of Papaya Based Ready to Serve (RTS) Beverage*** by **Anurag Khadka** has been accepted as the partial fulfillment of the requirement for the **B. Tech degree in Food Technology.**

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Abstract

The main aim of this research was to prepare mint incorporated papaya RTS and study its storage stability. Papaya and mint were obtained from local market of Dharan city. After preparation of papaya juice, optimization using Design-Expert 13 software, with aqueous mint extract. Mint juice was then added to the RTS in varying ratios; A (94:6), B (95.5:4.5), C (96:4), D (97:3), E (98:2), F (98.5:1.5) and G (100:0). Sample A with 96:4 blend was determined to be the optimal choice through sensory evaluation. The selected blend then underwent pasteurization at 80°C for 30s and was stored for 45 days at refrigeration temperature (around 7°C) in pre-sterilized PET bottles.

Analysis at 15-day intervals showed significant changes in TSS, titrable acidity, reducing and non-reducing sugars, pH, and vitamin C during storage. The study also provides insights into the chemical and microbiological changes during a 45-day storage period, emphasizing the significance of storage conditions on the product's quality. The production cost for a 250 ml bottle of the final product was determined to be NRs.100.

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Abbreviations

Abbreviations	Full form
ANOVA	Analysis of Variance
CCT	Central Campus of Technology
DPPH	Diphenyl-1-picrylhydrazyl
FAO	Food and Agriculture Organization
LSD	Least Significance Difference
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
RTS	Ready to Serve
TPC	Total Plate Count
TSS	Total Soluble Solid
US	United States

Part I

Introduction

1.1 General Introduction

Papaya is also known as common man's fruit and belongs to Caricaceae family. Papayas contain soft, easily digestible flesh with a good amount of soluble dietary fiber that helps to have normal bowel movements; thereby reducing constipation problems. Papaya possess attractive colour, luscious taste, rich source of minerals like potassium and magnesium, nutrients such as carotenoids, vitamins C, E & flavonoids which acts as antioxidants; vitamins B, folate, pantothenic acid; and fiber. Papaya was selected for preparation of fruit drink due to its reasonable price, easy availability and high nutritive value (Panghal *et al.*, 2017). Sumaiya (2015) reported that fresh, ripe papaya is one of the fruits with the highest vitamin-C content 61.8 mg which has many important functions like free radicals scavenging, immune booster, and anti-inflammatory actions. Together, these nutrients are known to promote the health of the cardiovascular system and protect against colon cancer. Hence, for the present study, papaya was selected for preparation of functional fruit beverage. (Panghal *et al.*, 2017)

Fruits and vegetables tend to have very limited shelf life due to its perishable nature. In order to preserve, they are processed to Ready to Serve (RTS) beverages. RTS beverage is a non-fermented beverage prepared from fruits and vegetables of different concentrations in addition of sugar, water and additives. Fruits rich in sugar, vitamins and minerals are less explored due to astringency or bitterness. This can be solved by blending such fruits with other fruits and vegetables in order to increase flavor, nutrient properties and shelf life. These natural RTS beverages are valued for their nutritional content, refreshing quality, pleasant flavor and medicinal properties. Therefore, blending of natural RTS beverage is thought to be a good alternative for utilization and preservation of fruits (Rathinasamy *et al.*, 2021).

Ready to serve is a fruit drink which should have at least 10% fruit juice, 10% total dissolved solids, and about 0. 3% acidity. The beverage is consumed without dilution, hence called ready to serve (Divyasree *et al.*, 2018a). Productions of Ready-To-Serve (RTS) beverages have been increasingly gaining popularity throughout the country due to their health and nutritional benefits, apart from pleasant flavor and taste (Thamilselvi *et al.*, 2015). According to Rashid *et al.* (2018) natural RTS beverages made from blending and enrichment improves shelf life and has high sensory acceptability. Development of RTS beverages may reduce risk of health diseases and acts as a good appetizer. Physiochemical properties of RTS beverages like pH, acidity and total soluble solids were observed to change during storage. (Thamilselvi *et al.*, 2015)

In the past decade, alternative medicines have gained increasing interest among modern consumers who have adopted a natural perspective. Supporting these situation, herbals extracts have been used in several food products mainly beverages (Suna *et al.*, 2019). The medicinal and nutritional value of the fruit beverages can be enhanced by the incorporation of herbal extracts from mint, tulsi, ginger and many other herbs. They have been shown to possess antioxidant, anti-inflammatory, antimicrobial, antiviral, antiproliferative, immunomodulatory and antidiabetic properties due to their phenolic compounds. The extracts of the herbal plants can be added to enhance the taste and diversity of the products to the consumers in addition to their nutritional and medicinal values (Skąpska *et al.*, 2020).

Mint (Pudina), also known as *Mentha spicata* are familiar for their refreshing taste and strong aroma. They are commonly used in cooking and beverages. This herb is believed to stimulate and help with digestion and in reducing muscle spasms. This plant is commonly used to treat nausea during pregnancy, excessive emotional reactions, high body temperature, inflammation of the airways, and infections of the bronchial tubes. Mint leaves are a good source of β-carotene, calcium, and iron, vitamin C, riboflavin and thiamine (Majumdar *et al.*, 2012a).

1.2 Statement of the problem

The production and stability of ready-to-serve beverages containing mint (Mentha spicata) and papaya are the subjects of this study. The main goal is to find out how adding mint affects the produced beverages' sensory qualities, physicochemical characteristics, and microbiological quality over time. Specifically, the study aims to determine the optimal formulation parameters for achieving desirable flavor profiles without compromising stability, assess changes in pH, titratable acidity, color, and turbidity during storage, evaluate the microbial load and its evolution over time, and ascertain the shelf life under various storage conditions. By addressing these key aspects, the research aims to provide valuable insights into enhancing the quality and shelf stability of mint-incorporated papaya-based beverages, thus contributing to the development of natural and marketable ready-to-serve products.

1.3 Objectives

1.3.1 General Objectives

Effect of mint incorporation on storage stability of papaya based ready to serve (RTS) beverage.

1.3.2 Specific Objectives

The followings are the specific objectives of this work:

1. To analyze chemical composition papaya and mint juices.
2. To optimize the best proportion of mint leaf extract in the RTS.
3. To study the physicochemical properties of the prepared RTS.
4. To study the microbiological changes occurring during the storage of RTS.
5. To study the changes occurring during the storage of the RTS.
6. To evaluate the cost of the prepared RTS.

1.4 Significance of the work

The investigation into the preparation and storage stability of mint-incorporated papaya-based ready-to-serve beverages holds several significant implications. Firstly, this study contributes to the diversification and innovation within the beverage industry by introducing a novel product that combines the nutritional benefits of papaya with the refreshing taste and potential health benefits of mint. Such innovations cater to evolving consumer preferences for natural, healthy, and flavorful beverages, thereby potentially expanding market opportunities for manufacturers.

Secondly, by addressing the challenges associated with storage stability, this research addresses a critical aspect of product development and commercialization. Understanding how formulation parameters and storage conditions influence the quality and shelf life of the beverages is essential for ensuring product integrity and meeting regulatory requirements. The insights gained from this study can guide manufacturers in optimizing their production processes, enhancing product quality, and minimizing waste, thus contributing to improved economic viability and sustainability within the industry.

Moreover, the findings of this study have broader implications for the food and beverage sector, as they contribute to the knowledge base regarding the utilization of natural ingredients and preservation techniques. The identification of effective strategies for maintaining product quality and safety without resorting to synthetic additives aligns with the growing consumer demand for clean-label products and sustainable practices. Ultimately, the significance of this study lies in its potential to not only introduce a new and appealing beverage to the market but also to inspire further research and innovation in the development of natural, healthy, and shelf-stable food and beverage products.

1.5 Limitations of the work

1. The shelf life of the product was only studied for 50 days.
2. The physico-chemical properties like chlorophyll, glucose and fructose could not be studied due to time constraints.
3. Only specific packaging material was used for the storage stability.
4. Only papaya and mint were selected for the study.
5. The optimization was carried out based on sensory analysis only even though, antioxidant activity was also studied.
6. Sensory panelists employed were semi-skilled.

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 Ready to Serve (RTS) beverage

Fruit juices and drinks are primarily manufactured foods that are palatable and easily consumed by people of all ages. They also help you achieve your dietary requirements for vitamins and minerals in a balanced diet. There are a wide variety of items available on the market, including soda water, pulpy drinks, juice beverages with added sweetness, and carbonated soft drinks. Fruit juice-based beverages now make up a very minor portion of these non-alcoholic beverages when compared to artificially carbonated beverages. Natural fruit juice-based beverages are becoming more and more popular among consumers due to their superior quality, high calorific value, significant nutritional value, and potential medical benefits (Hemalatha *et al.*, 2018).

Ready to serve beverage is a non-fermented beverage made from variously concentrated fruits and vegetables, together with sugar, water, and additions (Rathinasamy *et al.*, 2021). RTS is a fruit drink which should have at least 10% fruit juice, 10% total dissolved solids, and about 0. 3% acidity. The advantage of a ready to serve (RTS) beverage is that there is no need to dilute it further with a required quantity of water, unlike other concentrated beverages such as squash, or syrup, which are diluted judiciously with water before consumption (Jain and Khurdiya, 2004). These organic RTS drinks are prized for their therapeutic qualities, nutritive value, and refreshing qualities (Rathinasamy *et al.*, 2021). However, processed juice suffers a wide range of biochemical changes during storage, thus it needs to be processed properly and preserved under appropriate conditions with suitable additives. Pasteurization, low temperature storage and use of preservatives are promising methods of fruit juice preservation. Preservatives are chemical agents intentionally added to food products to prevent or inhibit spoilage caused by molds, yeasts and bacteria. The most common preservatives which are being used in fruit processing industries are salts releasing sulphurdioxide and salts of benzoic acid (Singh and Sharma, 2017).

Many different types of RTS drinks are made from fruits like grapes, gooseberries, litchi, pineapple, orange, and more. These beverages can be mixed together, providing a cooling effect and offering additional advantages (Rathinasamy *et al.*, 2021).

General specifications for natural Ready-to-serve beverage are presented in table 2.1

Table 2.1 Ready-to-serve beverage (Natural)

Parameters	Value
Net volume juice content	
Lime	≥5%
Others	≥3.5%
Total soluble solids	≥10%
Sulphur dioxide	≤70 ppm
Benzoic acid	≤150 ppm
Acidity	0.3%
Synthetic sweetening agent	Not permitted
Added color	Permitted colors
Carbon dioxide	If aerated

Source: Pandey *et al.* (2017)

2.2.1 Materials used in preparation of RTS beverage

2.2.1.1 Added sugar

Added sugar is defined in a number of ways. The United States Department of Agriculture (USDA) defines "added sugars" as sugars added to food during processing. By that criterion, naturally occurring sugars present in food, such as the fructose in fruit and the lactose in milk, are not considered "added sugars". Sucrose, a disaccharide that is often known as table sugar, is composed of connected units of glucose and fructose bonded by a chemical link that is readily broken in the small intestine. Fruits and vegetables naturally contain sucrose. Sugars are desirable because they taste sweet and are easily palatable. Some ready-to-drink drinks contain sugar that has been broken down or exposed to an acidic environment (Guthrie and Morton, 2000).

2.2.1.2 Citric acid

Anhydrous citric acid, a tricarboxylic acid with the chemical formula $C_6H_8O_7$, is found in citrus fruits. Citric acid is used to pharmaceutical formulations because of its antioxidant properties. It maintains the stability of the active ingredients and has preservation properties. By chelating blood calcium, it also serves as an acidulant, an anticoagulant, and a pH regulator (Grewal and Kalra, 1995).

2.2.1.3 Colors and flavors

To improve visual appeal and restore colours lost during preparation, food is given certain tastes and colours. Additives are used to change the desired colour or enhance flavour. Food flavour is enhanced by a variety of spices as well as artificial and natural flavours. In a similar vein, colours enhance some products' appearances to meet consumer expectations. Flavour decreases with storage because volatile flavour components are lost (Byanna and Gowda, 2012).

2.3 Papaya

Carica Papaya belonging to family Caricaceae is commonly known as papaya in English, Papita in Hindi and Erandakarkati in Sanskrit. The plant is native to tropical America and was introduced to India in 16th century. The plant is recognised by its weak and usually unbranched soft stem yielding copious white latex and crowded by a terminal cluster of large and long stalked leaves, is rapidly growing and can grow up to 20m tall (Yogiraj *et al.*, 2014).

Traditionally leaves have been used for treatment of a wide range of ailments, like in treatment of malaria, dengue, jaundice, immunomodulatory and antiviral activity. Young leaves are rich in flavonoids (kaempferol and myricetin), alkaloids (carpaine, pseudocarpaine, dehydrocarpaine I and II), phenolic compounds (ferulic acid, caffeic acid, chlorogenic acid), the cynogenetic compounds (benzylglucosinolate) found in leaves. Both leaf and fruit of the papaya possess carotenoids namely β- carotene, lycopene, anthraquinones glycoside, as compared to matured leaves and hence possess medicinal properties like anti-inflammatory hypoglycaemic, anti-fertility, abortifacient, hepatoprotective, wound healing, recently its antihypertensive and antitumor activities have also been established. Leaves being an important part of several traditional formulations are undertaken for standardization for various parameters like moisture content, extractive values, ash values, swelling index, etc (Anjum *et al.*, 2013).

Worldwide over 6.8 million tonnes (MT) of fruit were produced in 2004 on about 389,990 Ha (FAO 2004). Of this volume, 47% was produced in Central and South America (mainly in Brazil), 30% in Asia, and 20% in Africa. The papaya industry in Brazil is one of the largest worldwide that continues to show rapid growth. Although papaya is mainly grown (>90%) and consumed in developing countries, it is fast becoming an important fruit internationally, both as a fresh fruit and as processed products (Silva *et al.*, 2007).

Fresh papaya fruits are prone to mechanical damage and microbial infections, thus limiting their transport and shelf life. Processed papaya products such as puree may provide all-season supply with shelf-stable and highly nutritive fruit products. However, the off-flavour emerging during processing limits their industrial utilisation. Exceptions are blends with fruits other than papaya to mask the unpleasant flavour (Lieb *et al.*, 2018).

2.3.1 Chemical Constituents of *papaya* Linn

papaya is one of the valuable plant used for various purposes in medicinal field. Leaves, fruit and seeds of the papaya are used as ethno medicine.

Chemical composition of various part of papaya plant are described in table 2.2.

Table 2.2 Chemical composition of various part of papaya plant

Part	Constituents
Fruit	Protein, fat, fibre, carbohydrates, minerals, calcium, phosphorus, iron, vitamin C, thiamine, riboflavin, niacin, and caroxene, amino acid, citric acids and molic acid (green fruits), volatile compounds : linalol, benzylisothiocynate, cis and trans 2, 6-dimethyl-3,6 expoxy-7 octen-2-ol. Alkaloid, α; carpaine, benzyl-β-d glucoside, 2-phenylethl-β-D-glucoside, 4-hydroxyl -phenyl-2 ethyl-B-D glucoside and four isomeric malonated benzyl-β-D glucosides
Juice	N-butyric, n-hexanoic and n-octanoic acids, lipids; myristic, palmitic, stearic, linoleic, linolenic acids-vaccenic acid and oleic acids

*Source:*Yogiraj *et al.* (2014)

2.3.3 Nutritional Value of Papaya.

Papaya is common's man fruit, which is reasonably priced and has a high nutritive value. It is low in calories and rich in natural vitamins, and minerals. The comparative low calories content (32 Kcal / 100 g of ripe fruit) makes this a favorite fruit of obese people who are into weight reducing regime. Papaya has low carotene compared to other fruit such as apples, guava, sitaphal and plantains, which helps to prevent damage by free radicals. Unripe green papaya is used as vegetable, it does not contain carotene but also all other nutrients are present. The fruit is a rich source for different types of enzymes. Papain, vegetable pepsin present in good amount in unripe fruit is an excellent aid to digestion, which helps to digest the protein in food at acid, alkaline and neutral medium. The celiac disease patients, who cannot digest the wheat protein gliandin, can tolerate it, if it is treated with crude papain, papaya has the property of tenderizing meat. This knowledge is being put to use by cooking meat with raw papaya to make it tender and digestible (Chakre, 2010). The fermented papaya fruit is a promising nutraceutical as an antioxidant. It improves the antioxidant defence in elderly patients even without any overt antioxidant deficiency state at the dose of 9 g/day orally. The papaya lipase, a hydrolase enzyme tightly bonded to the water insoluble fraction of crude papain, is considered as a "naturally immobilized" biocatalyst (Marotta *et al.*, 2006) .Papaya markedly increases iron (Fe) absorption from rice meal, which was measured in parous Indian women, using the erythrocyte utilization of radioactive Fe method. The black seeds edible and have a sharp, spicy taste. They are sometimes ground up and used as asubstitute for black pepper. In some parts of Asia the young leaves of papaya are steamed and eaten like spinach (de María *et al.*, 2006).

Nutritive value of 100 gm of papaya fruit are described in Table 2.3.

Table 2.3 Nutritive value of 100gm of papaya fruit.

Constituents	Ripe Papaya	Green Papaya
Protein	0.6 g	0.7 g
Minerals	0.5 g	0.5 g
Fibre	0.8 g	0.9 g
Fat	0.1 g	0.2 g
Carbohydrates	7.2 g	5.7 g
Energy	32 Kcal	27 Kcal
Total	2,740 µm	0
Carotene	888	0

Source: Yogiraj *et al.* (2014)

2.4 Mint

Medicinal plants are an important source of life-saving drugs for humans, especially in developing countries. According to the World Health Organization estimates, more than 80 % of the world's population in developing countries depends primarily on herbal medicine for basic health care due to the issues associated with the use of synthetic drugs and antibiotics and the renewal of interest in the use of plant-based drugs (Mahendran *et al.*, 2021). Research on medicinal plants and their traditional medicinal use has increased in different regions of the world over the past few decades. It is important to document indigenous traditional knowledge through ethnobotanical studies for the conservation and utilization of biological resources. Therefore, it is acknowledged that plants can be used in their original or advanced form. Numerous biologically active substances are known to contain medicinal plants that have been isolated from plants and applied based on ethnobotanical expertise and approved drugs from medicinal plants (Carney *et al.*, 1999).

The genus Mentha belongs to the Lamiaceae family and can be found in Europe, Africa, Asia, Australia, and North America. The genus consists of approximately 25 species and rather fewer hybrids. The high number of different taxonomic names attributed by the taxonomists during the past 200 years to the mint plants reflects a great morphological variation (Kokkini, 1991). In Nepal, three species of Mentha (*M. arvensis, M. longifolia and M. spicata*) are reported to occur. These plants provide starting material for perfumes and other essential oil products. *Mentha spicata L.* locally known as Pudina and Simbabari, is widely distributed throughout Nepal especially on the moist and shady places e.g. bank of river, stream & escape lands. It is a glabrous, perennial herb, 30-90 cm high, with creeping rhizome. Leaves are smooth or nearly so, upper sessile lower petiolate, lanceolate to ovate in shape, coarsely dentate, smooth above, glandular below with round tip and 2-3.5 cm by 1.5-2.5 cm in size with aromatic smell. Flowers are in spike, sessile and white in color (Paudel and Pant, 2006).

Mentha is an industrial crop that is widely cultivated for its essential oil, the major constituent of which is l-menthol, a monocyclic monoterpenic alcohol. The essential oil, menthol and other chemical constituents of Mentha are used for a variety of purposes in the food, perfumery and pharmaceutical industries (Taneja and Chandra, 2012). Fresh and dried leaves from spearmint are used to make teas and aromatic agents (Ali-Shtayeh *et al.*, 2019). The herb is considered stimulant, carminative, antispasmodic, tonic, stomachic, sudorific, anthelmintic and antiseptic. Leaves are given in fever and bronchitis and decoction of leaves is used as lotion on aphthae. Seeds are mucilaginous. The soft plants are used as pickle and also as spices. A sweetened infusion of the herb is given as a remedy for infantile troubles, vomiting in pregnancy and hysteria (Paudel and Pant, 2006). *Mentha spicata* is used to treat gastrointestinal, respiratory, bad breath, carminative, anti-spasmodic, diuretic and sedative agents. Different modes of preparation (decoctions, tincture and tablets) of spearmint have been used to treat flatulence disorders in traditional Iranian medicine (Mahboubi, 2021). In traditional Iranian remedies, spearmint leaves are used to strengthen the stomach and are helpful for symptoms of dyspepsia (Babaeian *et al.*, 2015). Spearmint oil is a flavoring agent used in the preparation of chewing gum, cosmetics and toothpaste (Mahboubi, 2021).

2.4.1 Taxonomy and botanical characterization

Mentha spicata L belongs to the family Lamiaceae (Mint family), a family consisting of 260 genera and 7000 species that grow under a wide range of agro climatic conditions (Brahmi *et al.*, 2017). The genus Mentha L. includes 42 species, hundreds of sub-species, 15 hybrids, cultivars and varieties (Brahmi *et al.*, 2017; Salehi *et al.*, 2018; Silva, 2020).

Mentha spicata is a perennial rhizomatous herb growing up to 30 to 100 cm in height. Stems are erect, four-angled, branched and glabrous. The leaves are ovate to lanceolate, 2-7 cm long with toothed margins. Inflorescences are dense, terminal, 3 to 12 cm long, and 5 to 10 mm wide. Pedicellate flowers in slender spikes are interrupted, pink or white (Klinkenberg, 2003). The species spearmint (*M. spicata*) is commercially grown worldwide to be used as flavoring foods and medicines (Abbaszadeh *et al.*, 2009).

Taxonomic classification of *Mentha spicata*

Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiosperm
Class	Dicotyledonae
Family	Lamiaceae
Genus	*Mentha*
Species	*Spicata* (spearmint)

Source: (Mahendran *et al.*, 2021)

2.4.2 Chemical composition of mint

The chemical composition of mint is shown in Table 2.4 below.

Table 2.4 Chemical composition of fresh mint leaves

Parameter	Value(%)
Moisture (% wb)	76.01
Ash (%)	3.48
Protein (% wb)	1.75
Fat (%)	2.20
Fiber (%)	6.2
Carbohydrate (%)	10.39

Source: (Zheljazkov *et al.*, 2010)

2.4.3 Varieties in mint

The taxonomy of mints, genus Mentha from the Lamiaceae family, is a complex problem and several classifications varying in the number of recognized species have been proposed in the past (Tucker *et al.*, 1980). Mentha is of worldwide distribution and comprises according to the latest taxonomic treatment, 18 species and additional 11 hybrids placed into the four sections Pulegium, Tubulosae, Eriodontes, and Mentha. More than 3,000 names, from species to formae, have been published for the genus Mentha since Linné (1753).

The systematics of section Mentha is especially difficult because of frequent hybridization occurring both in wild populations and in cultivation (Harley and Brighton, 1977). Outcrossing is favored by genodioecy and the taxonomy of this hybrid complex is complicated by concomitant polyploidy and stabilization of novel forms by ease of vegetative propagation (Tucker *et al.*, 1980). Within section Mentha it has been suggested that the five basic *species Mentha arvensis L., Mentha aquatica L., Mentha spicata L., Mentha longifolia (L.) Huds*, and *Mentha suaveolens* have given rise to eleven naturally occurring and named hybrids (Tucker and Naczi, 2007), However, *M. spicata* and possibly *M. longifolia* are also of hybrid origin and incongruence of nuclear and plastid DNA based phylogenies indicated that all species of this section may have experienced some extend of reticulate gene flow during their evolution (Gobert *et al.*, 2006).

The present literature (Šarić-Kundalić *et al.*, 2009) suggests a differentiation of section Mentha into the three basic lines, capitatae, spicatae, and verticillatae, based on inflorescence characters. The line 'capitatae' includes all species with compact, head-like inflorescence; the type species is *M. aquatica*. The 'spicatae' species have a spike as shown by *M. spicata, M. longifolia,* and *M. suaveolens*. The third line is represented by *M. arvensis* having a inflorescence vertically partitioned into whorls.

2.4.4 Origin, history, distribution and production of mint

If the current diversity of mint is correlated with its origin, then Southwest or Central Asia (former Laurasia core area) can be associated with the Lamiaceae, with three directions of proliferation; along the Mediterranean, to the Southern Africa via the East African mountains and to the Northwest of Asia. It was then distributed to Western North America, Southeast

Asia and Australasia. The time when Mentha was involved in this distribution and dispersal of Lamiaceae is unknown but a Mentha like fossil is known from the Eocene in North America; *Menthites eocenicus*, which is one of the oldest known fossil in the Lamiaceae (Lawrence, 2006).

There are many different species, hybrids and special selections of mints that are grown all over the world, most of which will thrive in cool, moist locations with some shade. They can generally tolerate a wide range of conditions including direct sunlight. Mints grow quickly, extending a network of runners above and below the ground; consequently, one mint plant should provide enough mint for one household. Japan started commercial production of mint around 1870 AD. During that time, the product was called Japanese mint and Japan was the only commercial producer. After the Second World War, Brazil started producing mint commercially as it was found in the country's forests. Later on, the production of mint spread to other South American countries. The cultivation of mint also began in other countries such as China and India in around 1960. Initially, India was an importer of menthol but, after the green revolution in 1986, mint took off as an agricultural commodity (Taneja and Chandra, 2012).

India is currently the world's largest consumer, producer and exporter of menthol and related products. Other major producers of mint oil are China, Japan, Brazil and the USA (Taneja and Chandra, 2012). According to figures from the Multi-Commodity Exchange (MCX) of India, the total world production of Mentha oil in 2007–8 was nearly 32 000 tonnes, covering about 0.29 million hectares of land, with average productivity of about 110 kg/ha. As India is the world leader in production and consumption, any change in trends in this country directly affects the international prices of mint oil and related products. Some of the mint products in high demand include *Mentha arvensis* oil, deterpenated oil, l-menthol crystals, menthol flakes, menthol powder, neo-menthol, iso-menthol, peppermint oil, liquid-menthol, l-menthone, cis-3-hexenol, cis-3-hexenyl acetate, menthyl acetate, mint terpenes, 3-octanol, 3-octanyl acetate and l-limonene. According to the MCX, India contributes 75–80 % of total production, with the rest contributed by China (9 %) followed by Brazil and the USA (Taneja and Chandra, 2012).

2.4.5 Uses and health benefits of mint

The most common traditional use of spearmint was to prevent diarrhea. An ethnobotanical survey in Iran reported that leaf, aerial part, flower and stem had been used to treat diarrhea, stomach ache, digestive and Anthelmintic. Similarly, in Nepal traditional herbal medicine, the whole plant of *M. spicata* is used for the treatment of diarrhea, stomach ache, dysentery, urine retention and indigestion (Mahendran *et al.*, 2021).

Spearmint is described by the South African traditional system to be used leaf decoction for the management of coughs, colds and asthma. The traditional use of aerial decoction and infusion of *M. spicata* to treat colds has been reported in Turkey. Likewise, in Pakistan traditional medicine, *M. spicata* leaves decoction is used for healing digestive problems. In Iran, *M. spicata* aerial parts, leaves and essence reported to treat diabetes. In traditional Ayurvedic systems in India, *M. spicata* leaves are reported to treat jaundice. In another ethnobotanical study, it was reported that seed and oil of *M. spicata* are used to treat arthritis (Mahendran *et al.*, 2021).

The successful utilization of plants by its agribusiness, food and pharmaceutical industries is based on completely understating of their biologically active secondary metabolites (Koblovská *et al.*, 2008). Phenolic acids are one of the most important active compounds in the entire plant. Previous research on *M. spicata* has suggested that the presence of rosmarinic acid, flavonoids, lignans and caffeic acid as the main metabolites (Mahendran *et al.*, 2021).

This herb can be used for various therapeutic purposes. Several experiments have focused on the antibacterial activities of spearmint. The essential oil of *M. spicata* was investigated as an anti-bacterial study against *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella paratyphi*, *Acinetobacter spp*. and *Klebsiella pneumoniae.* The antifungal properties of essential oil extracted from *M. spicata* was studied against 17 fungi like *Aspergillus niger, Candida albacans, Trichophyton rubrum* and others. The antibacterial and antifungal activities are the result of antibacterial and antifungal response of essential oils present in mint leaves (Mahendran *et al.*, 2021).

The polyphenols present in mint leaves also show significant antioxidant property along with larvicidal activity, anti-diabetic activity (by reduction in blood glucose level), anticancer activity (by inhibition of different cancer cells), anti-inflammatory activity, hepatoprotective activity, antipyretic activity and improvement of learning and memory effects (Mahendran *et al.*, 2021). Herrlinger *et al.* (2018) reported the effects of spearmint (*M. spicata*) capsules on, sleep, mood and cognitive performance in men and women with age-associated memory impairment (AAMI). Oral administration of spearmint aqueous extract at 900 mg/day (two capsules) for 90 days displayed significantly enhanced mood and working memory scores.

2.5 Pasteurization

The term "pasteurization" was coined after Louis Pasteur, a French scientist who pioneered the procedure of heating liquids at a low temperature for a brief time to extend their shelf life (wine and beer). Thermal pasteurization is a mild form of heat treatment that is used to inactivate heat-sensitive microorganisms that cause food deterioration or food poisoning, such as vegetative bacteria, yeasts, and molds. Thermal pasteurization has been shown to successfully inactivate fruit juice enzymes such as polyphenol oxidase (PPO), lipoxygenase (LOX), peroxidase (POD), and pectinmethylesterase (PME), which are responsible for quality deterioration. As a result, the shelf life of thermally processed fruit juices can be extended for several months without posing any safety risks or causing significant quality degradation (Ağçam *et al.*, 2018).

Pasteurization is relatively mild heat treatment, in which food is heated to below 100ºC. In low acid foods it is used to minimize possible health hazards from pathogenic micro-organisms and to extend the shelf life of food for several days. In acidic foods ($pH < 4.5$, for example bottled fruit juice) it is used to extend the shelf life for several months by destruction of spoilage micro-organism (yeasts or molds) and/or enzyme inactivation. In both type of food, minimal changes are caused to the sensory characteristics of nutritive value. The main purpose of pasteurization in fruit juices is the inactivation of spoilage enzymes (pectin esterase and polygalacturanase) with destruction of spoilage microorganisms (yeast, fungi) as a subsidiary purpose. Processing conditions of 65°C for 30 min, 77°C for 1 min or 88°C for 15 s can be sufficient to achieve the desires outcome in fruit juices (Fellows, 2022).

Fruit juices are pasteurized at temperatures and for lengths of time that render them sterile while preserving their flavor. Juices are usually pasteurized based on the type of juice and the size of the container. Pasteurization of acid fruit juice requires a lower temperature and less time than pasteurization of less acidic fruit juice. Pectin enzymes, which produce flavor changes and particle clotting in juice, can be removed by heating the juice to the temperatures listed above. Additionally, enzymes require air to function, thus they can be destroyed at a moderate temperature by eliminating the air from the juice (Parajuli, 2010).

2.6 Juice packaging

The traditional packaging procedure for fruit juices involves heating of the deaerated juice around 90–95°C in either plate- or tubular-type heat exchanger, then filling of the hot juice in metal cans followed by sealing, then inverting the cans and holding at that temperature for 10–20 min and finally cooling. This hot-filled/hold/cool process guaranteed that the juice was commercially sterile, the seams were of high-quality, the cans had an acid-resistant lacquer, the juice had been accurately deaerated, and a shelf life of at least 1–2 years was attainable. However, because of the acidic nature of fruit juices, numerous defects or scratches in the tin layer resulted in quick corrosion, dissolution of metal into the juice, production of hydrogen gas, and container breakdown due to swelling. The uses of glass container eliminate these problems provided that the container closure (typically metal) was resistant to attack by the juice. The use of glass bottles for the packaging of fruit juices was also widespread, though the hot-filled/hold/cool process had to be applied with lot of care to circumvent breakage of the glass containers. Glass is still the preferred packaging medium for high-quality fruit juices (Ghoshal, 2019).

However, over the recent years a rising percentage of fruit juices and concentrates has been packaged aseptically, generally, into laminates of plastic film/aluminum foil/paperboard. These products are stored at room temperature and the keeping quality in terms of nutrient compositions, shelf life, etc., are significantly affected by the barrier properties of the carton, the interactions of the juice with the carton, and the outside storage environment. At the end of shelf life typically after 4–6months and is associated with parameters like the extent of non-enzymatic browning and the sorption of the key aroma and flavor compounds by the plastic in contact with the juices, the latter process being referred to as scalping. Because of its lipophilic nature, the oil fraction of citrus juices will be engrossed by many nonpolar packaging polymers (Ghoshal, 2019).

2.7 Microbiological background and target microorganisms of fruit juices

In choosing target microorganisms to calculate the lethality of a pasteurization treatment, juice processors may consider either *E. coli* or *Salmonella*, due to the numerous outbreaks that has 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 inactivation conditions that are applied for the most heat resistant pathogen, eliminates other microorganism (Ağçam *et al.*, 2018). In the industry, the aim of thermal pasteurization is not to kill all microorganisms in foods; the target is to destroy pertinent pathogens and lower levels of spoilage organisms that may grow during storage and distribution. A 5-log reduction can be considered for all processes that aim to reduce the microbial count. The process needs to consider the "pertinent pathogen", determined according to the type of juice. *Salmonella spp*. is considered a good target for orange juice, *Escherichia.coli* and *Cryptosporidium* are considered good targets for apple juice, and *Listeria monocytoge* is considered a good target for various juices that have never been involved in outbreaks (Lima Tribst *et al.*, 2009).

In the past, the association of fruit juices with foodborne disease outbreaks was unlikely to have occurred, mainly because of their acidic pH values (2.2<pH<4.5). However, outbreak occurrences, mainly since the 1980s, resulted in more attention being given to acidic fruit juices (Lima Tribst *et al.*, 2009). In recent years, different research groups all over the world have reported that microorganisms are able to improve thermal tolerance by a mechanism called acid adaptation. In other words, acid adaptation or acid tolerance is a phenomenon by which microorganisms show an increased resistance to environmental stress after exposure to a moderate acid environment. Some food borne pathogens can develop acid adaptation systems that include cross protection and make them more resistant against other environmental stress, increasing their ability to survive in juice. *E. coli, L. monocytogenes, Salmonella spp*. and *C. parvum* can tolerate low pH values and survive in fruit juices and concentrates longer than cells that are unable to adapt. The acid adaptation of *Salmonella spp., L. monocytogenes* and *E. coli* also increases the heat resistance of these bacteria in apple, orange, white grape juices, apple cider, juice blends, cantaloupe and watermelon juice (Ağçam *et al.*, 2018).

Besides bacterial and protozoan hazards, mycotoxins represent another hazard to the safety of fruit juices. Among several mycotoxins found in foods, patulin and ochratoxin A produced by a variety of molds can be considered as the most important fruit juice-associated mycotoxins. In recent years, a number of studies have been carried out into the occurrence of patulin and ochratoxin A in apple and grape juices respectively (Lima Tribst *et al.*, 2009).

Part III

Materials and methods

3.1 Raw materials

3.1.1 Papaya (*Carica papaya*)

Ripe Papayas were bought from local market of Dharan. They were taken on the same day to the Central Campus of Technology laboratory. They were sorted, graded and washed thoroughly in water to remove foreign materials. They were then peeled and cut into small pieces before grinding. Thereafter juice was extracted by passing through three folds of muslin cloth.

3.1.2 Mint (Mentha spicata)

Mint leaves (pudina) were collected from local market of Dharan. They were thoroughly washed in water after sorting. Then they were coarsely ground with equal parts water to facilitate extraction. Mint leaves were used as a flavoring and antioxidant agent.

3.1.3 Sugar

Sugar required for the preparation of the product was bought from the markets of Dharan as required.

3.1.4 Packaging Materials

The plastic bottles used for primary packaging is a type of PET bottle and cap-seal used for sealing is plastic cap wrapped with aluminum foil.

3.1.5 Chemicals and equipments

All the chemicals required during the dissertation were provided by Central Campus of Technology. List of chemicals used for this work are mentioned in Appendix B.1. The required equipment and glassware were obtained from the laboratory of Central Campus of Technology. List of equipment used for this work is shown in Appendix B.2.

3.1.6 Other materials

Other materials were bought from local market of Dharan. List of materials used for this work is given as; Muslin cloth, Plastic bottles (PET), Plastic cups, Aluminum foil.

3.2 Methods

The total work was based on preparation and storage stability study of RTS using Papaya and mint juice.

3.2.1 Preparation of papaya and mint juice

The work was based on preparation and study of storage stability of RTS using papaya and mint.

3.2.1.1 Selection of raw materials

Fresh and healthy ripe papaya and mint leaves were selected.

3.2.1.2 Washing raw materials

Washing was done in the stainless-steel vessel full of water. Papayas were submerged inside the vessel and the outer surface containing soil particles were washed out by rubbing with the help of running water. Mint was washed by simply dipping in the bucket full of clean water for 2/3 times.

3.2.1.3 Peeling and extraction

Papaya fruit was peeled manually using hands and knife. The top and core were removed from papaya. About 10 % of the total weight of papaya was discarded as peel. For the extraction of papay a juice, electric mixer grinder was used. Papaya was cut into small pieces for the convenience of grinding. Mint leaves were grinded with the help of motor and pestle but with pure water in 1:1 ratio.

3.2.1.4 Filtration

The extracted juice of above materials were filtrated in muslin cloth for 3 times to get juice of less residues as shown in figure 3.1.

Ripe papaya fruit

↓

Washing

↓

Peeling and cutting

↓

Grinding

↓

Squeezing and filtration

↓

Papaya juice

Source: Debnath *et al.* (2022) with slight modification

Fig 3.1 Preparation of papaya juice

3.2.1.5 Preparation of mint leaves extract

Mint leaves were collected, washed thoroughly and cut into small pieces. Then those mint leaves along with distilled water were taken into motor and pestle for grinding at 1:1 ratio. The grinded liquid mixture was filtered through muslin cloth 3 times and the leaf extract was obtained as shown in figure 3.2.

Fresh mint

↓

Washing with clean water

↓

Cutting into small pieces

↓

Grinding with water at 1:1 ratio

↓

Squeezing and filtration

↓

Mint juice

Source: Ali (2018) with slight modifications

Fig 3.2 Preparation of mint leaves extract

3.2.1.6 Experimental design

Design Expert 13 was used to create the recipe. Mixture design was used to formulate the recipe. The final product (RTS papaya and mint) was prepared in single steps by optimizing mint as a flavoring agent. Hence, for the design independent variables were mint juice and the papaya RTS. The experimental design is shown in the table 3.1.

Table 3.1 Mixture design of mixed RTS of mint, papaya parts per 100 ml

Sample	Papaya	Mint
A	94	6
B	95.5	4.5
C	96	4
D	97	3
E	98	2
F	98.5	1.5
G	100	0

3.2.1.7 Optimization of mint extract as a flavoring agent in papaya RTS

Mint juice was added as a refreshing flavoring agent. Different proportions of mint juice were added to the RTS sample. The best flavored RTS drink was selected on the basis of sensory evaluation (color, appearance, aroma, taste and overall acceptance).

The flowchart of papaya RTS blended with mint is given in fig 3.3.

Fresh Papaya

↓

Papaya pulp ← Addition of 0.3% Citric acid and adjusting TSS to 15^0Bx

↓

Ready to Serve (RTS) of Papaya ← Aqueous mint

↓

Blending with mint juice in different proportions

↓

Pasteurization at 80°C for 30 s,

↓

Cooling, filling & capping

↓

Storage

Source: Ali (2018) with slight modifications

Fig 3.3 Flowchart of papaya RTS blended with mint

3.2.1.8 Pasteurization, filling, capping and cooling

Pasteurization of the prepared RTS was performed in a glass vessel at 80°C. When the temperature reached 80°C, holding of juice was carried out for 30 s in the vessel. After holding, the juice was cooled down for few minutes in a sterilized environment before being filled into pre-sterilized PET bottles. The bottles were pre-cleaned and disinfected by dipping in 2% sodium hypochlorite solution, held for 10 minutes and rinsed with hot water before capping. Then the bottles were held inverted while they cooled down to room temperature. Later the filled bottles were stored at refrigeration temperature (7ºC).

3.2.1.9 Chemical, microbiological and storage stability study of the RTS

The pasteurized RTS was aseptically filled into pre-sterilized PET bottles (250 ml) and was stored in refrigeration temperature (7°C). Then the storage stability according to the change in terms of TSS, acidity, pH, reducing sugar, total sugar, retention of vitamin C and microbiological changes were analyzed at the interval of 15 days upon 45 days of storage.

3.2.2 Analytical procedure

Although, numerous writers have detailed various methods and parameters for analyzing juice, this study only determined those factors and related procedures that were viable in the laboratory. The test was carried out in triplicates. This ensures accuracy and reproducibility of the results. Each sample was analyzed under identical conditions, and the mean value was calculated to represent the findings.

3.2.2.1 Determination of total soluble solids (TSS)

Total soluble solids were determined with the help of hand refractometer (0-30) and the observed values were expressed as °Brix according to (Ranganna, 1986)

3.2.2.2 Determination of titrable acidity

The titrable acidity was determined as per (Ranganna, 1986).

3.2.2.3 Determination of reducing sugar and total sugar

The reducing sugar and total sugar of RTS were determined as per Lane and Enyon method as described in (Ranganna, 1986).

3.2.2.4 Determination of vitamin C

Vitamin C or ascorbic acid was determined by 2-6-dichloro-indophenol visual titration as per (Ranganna, 1986).

3.2.2.5 Determination of pH

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

3.2.2.6 Determination of non-reducing sugar

It was determined as per Lane and Enyon method as described in (Ranganna, 1986).

3.2.2.7 Determination of protein

Protein was determined by micro-kjeldahl method using conversion factor of 6.25 as described in (Ranganna, 1986).

3.2.2.8 Determination of ash content

Ash content was determined as described in (Ranganna, 1986) by dry ashing method.

3.2.2.9 Determination of crude fiber content

Crude fiber content was determined as described in (AOAC, 2005).

3.2.2.10 Determination of antioxidant activity

The antioxidant activity papaya and mint juice was determined as per Panico *et al.* (2009) with slight modifications. The control sample (A control) was made by adding 0.28 mL of DPPH solution (0.1 mM, in 95% methanol) to a 10 mL conical flask, and then diluting it with methanol to the necessary volume. 0.28 mL of the DPPH solution and 0.28 mL of the test sample (A sample) were used in the preparation and poured into a 10 mL conical flask. The mixture was then diluted with methanol to the necessary level. Following repeated inversions, the mixture was incubated for 30 minutes at ambient temperature in a darkened area. The absorbance was calculated with the aid of a spectrophotometer set at 517 nm, in comparison to the control sample. The radical scavenging activity was estimated as a decrease in DPPH absorbance and was calculated using the following equation:

$$DPPH\ Radical\ Scavenging\ Activity(\%) = \frac{(Ac - As)}{Ac} \times 100$$

Where, Ac= Control reaction absorbance & As= testing sample absorbance

3.2.3 Microbiological analysis

Total Plate Count (TPC) was determined by pour plate technique on Plate Count Agar (PCA) medium (incubated at 37°C/48 h). Yeast and molds count was determined by pour plate technique on Potato Dextrose Agar (PDA) medium incubated at 37°C/48 h (AOAC, 2005).

3.2.4 Sensory analysis

The sensory analysis was conducted in 9 point's hedonic score basis among 9 semi-trained panelists of Central Campus of Technology. The panelists were asked to taste the samples individually without verbal communication with each other. The format for sensory score card is presented in Appendix A.

3.2.5 Statistical method

The data were analyzed for two-way ANOVA, mean ANOVA (No blocking at 5% level of significance), LSD and interaction effects using GenStat Release 12.1 software (copyright 2009, VSN International Limited) at 5% significance level were obtained to determine whether the samples were significantly different from each other and to determine which one is superior among them. The mean is compared using LSD method. Standard deviation and means were also analyzed form the same statistical tool.

Part IV

Results and discussion

The work was carried out for the preparation of mint incorporated papaya based RTS beverage and study on its storage stability. Papaya and mint juice were the major ingredients along with different amounts of sugar and citric acid for maintaining TSS of 15°Bx and 0.3% acidity. The RTS was then blended with mint juice at different proportions. The selected best sample was studied for storage stability by storing at refrigeration temperature for 45 days.

4.1 Chemical composition of fruit

The chemical composition of papaya pulp was determined and presented in the table 4.1.

Table 4.1 Chemical composition of papaya pulp

Parameters	Value(%)
Moisture content (wb)	89.78±0.6
Protein (wb)	1.12±0.09
Carbohydrates	5.34±0.07
Ash (db)	0.31±0.02
Crude fiber (db)	1.48±0.04
Antioxidant activity (db)	55±0.5
TSS(^{0}Brix)	8±0.1

*The values are the mean ± standard deviation of triplicate analysis

According to Annegowda and Bhat (2016)moisture content of papaya pulp showed 90.32 %, protein 1.9 %, Crude fiber 1.3 %, ash content 0.35 % and 58.37% antioxidant activity. Thus, the variation in composition can be due to seasonal variation, different maturity stages, origin and various other factors.

4.2 Chemical composition of fresh mint

Table 4.2 represents the chemical composition of fresh mint leaves.

Table 4.2 Chemical composition of mint*

Parameters	Value(%)
Moisture content (%)	82.72±0.7
Acidity (% as citric acid)	0.11±0.01
Vitamin C (mg ascorbic acid/100 g)	13.5±0.23
Antioxidant activity (%)	73±0.5

*Values are means of triplicates, figures in the parenthesis are the standard deviations

The moisture content of mint leaves were found to be 83.85% as per reported by (Kripanand and Guruguntla, 2015), 0.119% acidity as per (Gonare *et al.*, 2021), antioxidant activity 84.43% according to (Grzeszczuk and Jadczak, 2009), and Vitamin C was found to be 14.52 mg ascorbic acid/ 100g as reported by (Rahman *et al.*, 2007). Similar findings were obtained during this dissertation while some variations were seen due to different possible factors.

The essential oil of mint; methanol was not determined during the dissertation since mint was used only as a flavoring agent without taking in its preservative activity into account.

4.3 Optimization of the best proportion of mint extract in papaya RTS

The RTS obtained from papaya juice was subjected for optimization with mint as a flavoring agent in different proportions. The effect of blending of mint juice on sensory quality of papaya RTS is shown in the fig 4.1-4.4.

4.3.1 Color

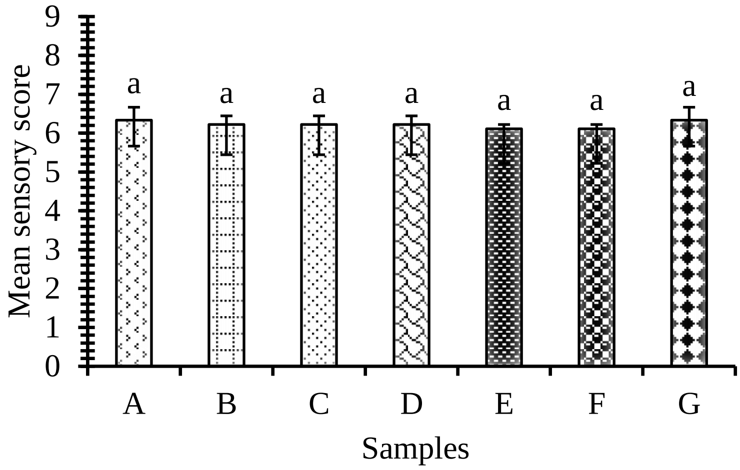

Fig. 4.1 Mean color scores of different papaya and mint RTS

The mean color scores for RTS samples A, B, C, D, E, F and G (control) were found to be 6.333, 6.222, 6.222, 6.222, 6.111, 6.111 and 6.333 respectively. Post-hoc analysis (Tukey test) showed that there was no significant effect ($p<0.05$) of variation of papaya and mint RTS of different proportions at 5% level of significance.

4.3.2 Aroma

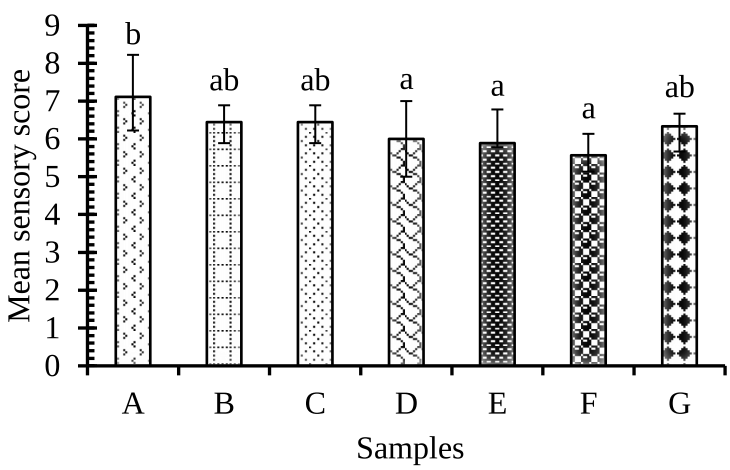

Fig. 4.2 Mean aroma scores for different papaya and mint RTS

The mean aroma scores for RTS samples A, B, C, D, E, F and G were found to be 7.111, 6.444, 6.444, 6, 5.889, 5.567 and 6.333 respectively. Statistical analysis showed that there was significant effect ($p<0.05$) of variation of papaya and mint RTS at 5% level of significance. Sample A (7.111) had the highest mean score among other RTS samples. Post-hoc analysis (Tukey test) indicated that mean aroma score between samples A, B, C, G and B, C, D, E, F, G were not significantly different but the aroma score of sample F was the lowest among all. Therefore, based on aroma, sample A was found to be best among all RTS.

4.3.3 Taste

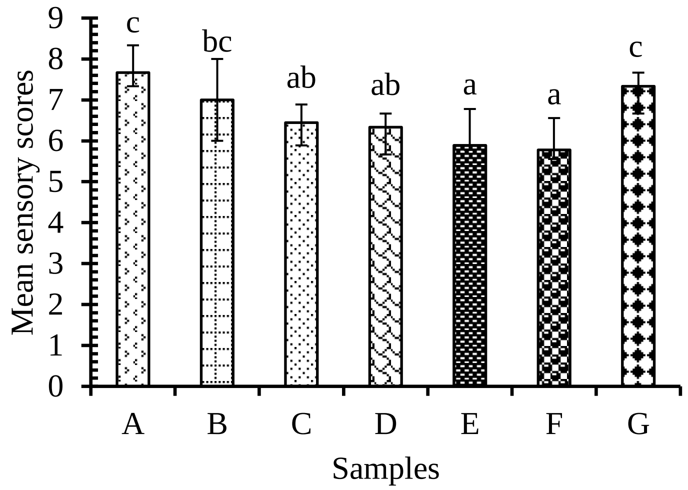

Fig. 4.3 Mean taste scores of different papaya and mint RTS samples

The mean taste scores for RTS samples A, B, C, D, E, F and G were found to be 7.667, 7, 6.444, 6.333, 5.889, 5.778 and 5.893 respectively. Statistical analysis showed that there was significant effect ($p <0.001$) of variation of papaya and mint RTS at 5% level of significance, while no significant difference between the panelists were observed ($p=0.256$). Samples with higher mint percentage were rated higher. Post-hoc analysis (Tukey test) reveled similar performance between the samples A and B. Also, Samples B-D and D-G performed similarly. Based on the result, sample with the highest mint percentage gave the best taste.

4.3.4 Overall acceptance

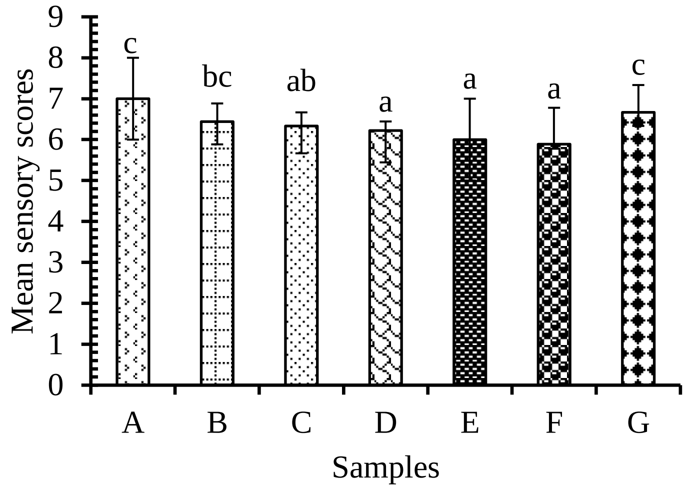

Fig. 4.4 Comparison of the mean score of overall acceptance between the samples

The mean overall scores for RTS samples A, B, C, D, E, F and G were found to be 7, 6.444, 6.333, 6.222, 6, 5.889 and 6.667 respectively. Statistical analysis showed that there was significant effect ($p<0.05$) of variation of papaya and mint RTS at 5% level of significance. Therefore, based on overall acceptance, sample A (7) was found best among all the samples. Based on Post-hoc analysis (Tukey test), the mean sensory score of the best sample was 7 and that of control (sample G) was 6.667 which showed that the sensory score on overall acceptability of the best sample was higher than that of the control sample.

4.4 Physio-chemical analysis

The data pertaining to the various chemical characteristics of papaya RTS blended with mint juice is presented in the table 4.3. The best sample (papaya and mint RTS) was compared with the control sample (papaya RTS). TSS and acidity were maintained constant in both best sample and control sample. The best sample was higher in pH and Vitamin C while the control sample was higher in reducing sugar, non- reducing sugar and total sugar.

Table 4.4 Chemical composition of best (sample A) and control sample*

Parameters	Best sample	Control sample
TSS	15±0	15±0
pH	4.1±0.1	4.1±0.01
Reducing sugar (mg/100)	3.46±0.13	3.57±0.17
Non reducing sugar (mg/100)	5.46±0.42	5.53±0.34
Total sugar (mg/100)	9.21±0.23	9.4±0.12
Vitamin C (mg/100)	9.1±0.2	9.21±0.15
Acidity (%)	0.3±0.01	0.3±0.0

*The values are the mean ± standard deviation of triplicate analysis

The TSS content of both best and control samples were similar to that reported by Mahar (2021), while acidity, pH, reducing sugar, non-reducing sugar, ascorbic acid content and total sugar were slightly lower than that reported by Mahar (2021).

4.5 Storage stability study of mint leaf extract incorporated RTS (best sample)

The final mint leaves extract incorporated RTS (best sample) and was pasteurized at 80°C for 30 s, hot filled in PET bottles and stored refrigeration temperature for a period of 45 days. The best sample was analyzed at 15, 30 and 45 days for its TSS, acidity, pH, reducing sugar, non-reducing sugar, total sugar, ascorbic acid content, total plate count and yeast, mold. The effect of storage intervals on the physiochemical and microbiological characteristics of RTS drink is presented in Appendix C.

4.5.1 TSS

The relationship between the TSS of best sample with storage period and storage temperature is shown in figure 4.5 below.

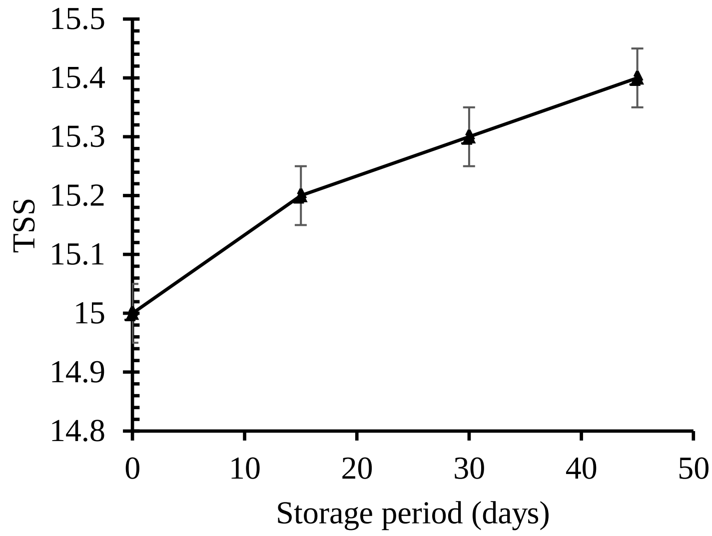

Fig 4.5 Effect of storage time and temperature on TSS of RTS

*Values are the means of three determinations. Vertical bars represent ± standard deviation.

A slight increase in the TSS of the beverage during 45 days of storage at refrigeration temperature was observed as shown in fig 4.5. TSS of the beverage were found to be 15, 15.2, 15.3, 15.4 ºBx for refrigeration sample (7 ºC) during 0, 15, 30 and 45 days of storage respectively. Statistical analysis showed that the storage time had significant effect ($p<0.05$) on the TSS of the beverage. Thus, LSD indicated that the values were significantly different to each other.

The fruit juice contains various reducing and non-reducing sugars which tend to change during storage due to various interconversion processes. The gradual transformation of other sugars and acids into reducing sugars during storage results in the generation of more reducing sugar over time (Singh and Sharma, 2017). Retention or minimum increase in total soluble solids content of juice during storage is desirable for the preservation of good juice quality. The TSS value of fruit juice shows a slow, steady rise under all storage conditions. This could be due to gradual degradation of the polysaccharides and acids in the juice over a period of time (Bhardwaj and Pandey, 2011). Complex carbohydrates breaking down over time can lead to an increase in the sugar content of a product. Higher temperatures are associated with elevated TSS levels, causing them to increase as well. This might be connected to the slower breakdown of sugars, starches, and organic acids at lower temperatures, in accordance with the La Chatelier Principle in chemistry.

4.5.2 Titrable acidity

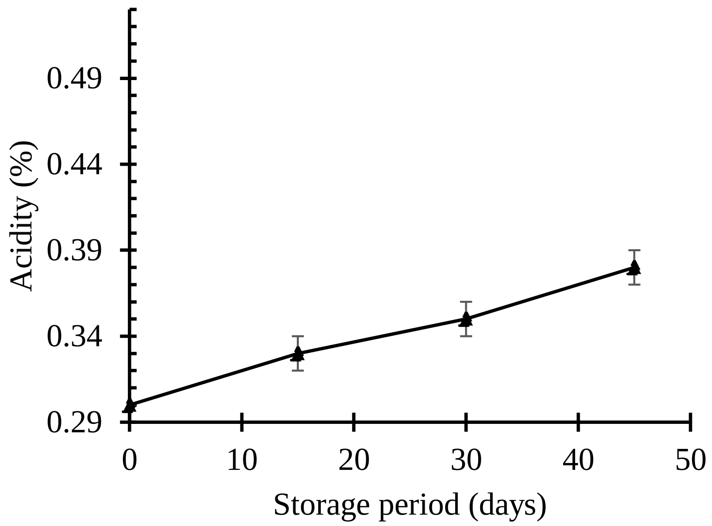

Fig 4.6 Effect of storage time and temperature on acidity of the RTS

*Values are the means of three determinations. Vertical bars represent ± standard deviation.

The steady increase in the titratable acidity of the beverage during 45 days of storage at refrigeration temperatures were observed as shown in fig 4.6. Acidity of the beverage were found to be 0.3, 0.33, 0.35, 0.38 during 0, 15, 30 and 45 days of storage respectively.

The acidity of the RTS increased following a 45-days storage period. One possible reason for this is the breakdown of ascorbic acid into organic acids, along with the breakdown of pectic substances. This could also occur due to the conversion or breakdown of polysaccharides and oxidation of reducing sugars to acids (Divyasree *et al.*, 2018b).

4.5.3 pH

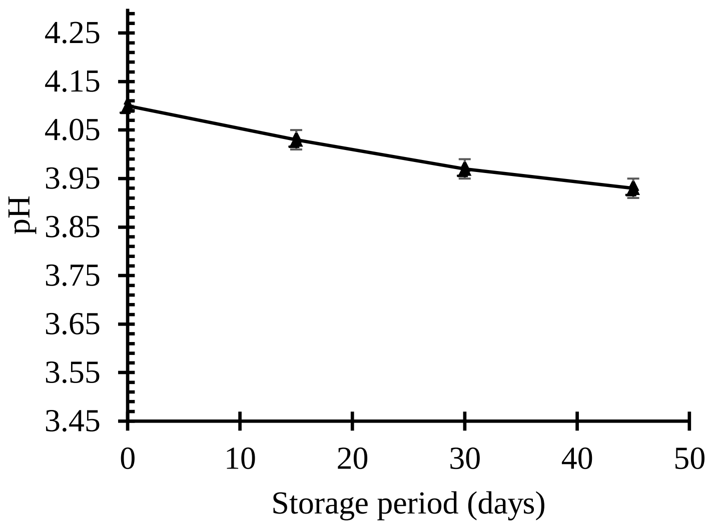

Fig 4.7 Effect of storage time and temperature on pH of the RTS

*Values are the means of three determinations. Vertical bars represent ± standard deviation.

A slight decrease in the pH of the beverage during 45 days of storage at refrigeration temperatures were observed as shown in fig 4.7. pH of the beverage were found to be 4.1, 4.03, 3.97, 3.93 for refrigeration sample (7 ºC) during 0, 15, 30 and 45 days of storage respectively.

The decrease in pH might be due to increase in titrable acidity, as acidity and pH are inversely proportional to each other. Similar results were reported for a juice blend of bottle guard and basil leaves by (Majumdar *et al.*, 2012b).

4.5.4 Reducing sugar

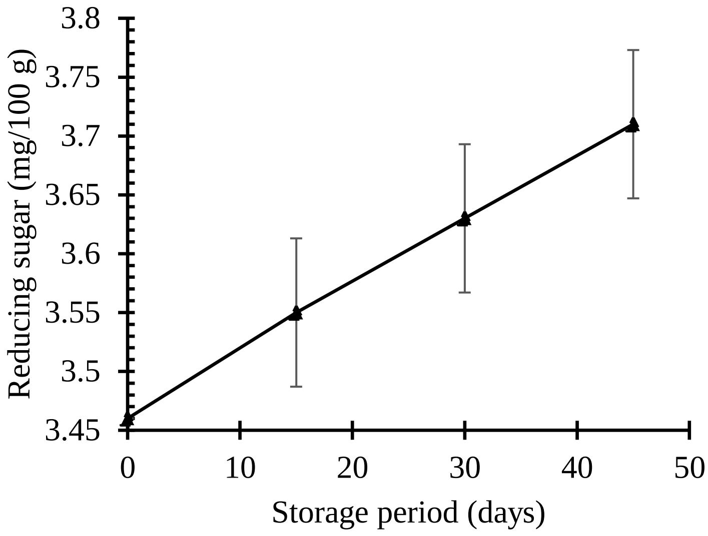

Fig 4.8 Effect of storage time and temperature on reducing sugar of the RTS

*Values are the means of three determinations. Vertical bars represent ± standard deviation.

Gradual increase in the reducing of the beverage during 45 days of storage at refrigeration temperatures was observed as shown in fig 4.8. Reducing sugar of the beverage were found to be 3.46, 3.55, 3.63, 3.71 for refrigeration sample (7 ºC) during 0, 15, 30 and 45 days of storage respectively.

The fruit juice contains various reducing and non-reducing sugars which tend to change during storage due to various interconversion processes. The gradual transformation of other sugars and acids into reducing sugars during storage results in the generation of more reducing sugar over time (Singh and Sharma, 2017). Similar results were reported for a juice blend of bottle guard and basil leaves by (Majumdar *et al.,* 2012b).

4.5.5 Total sugar

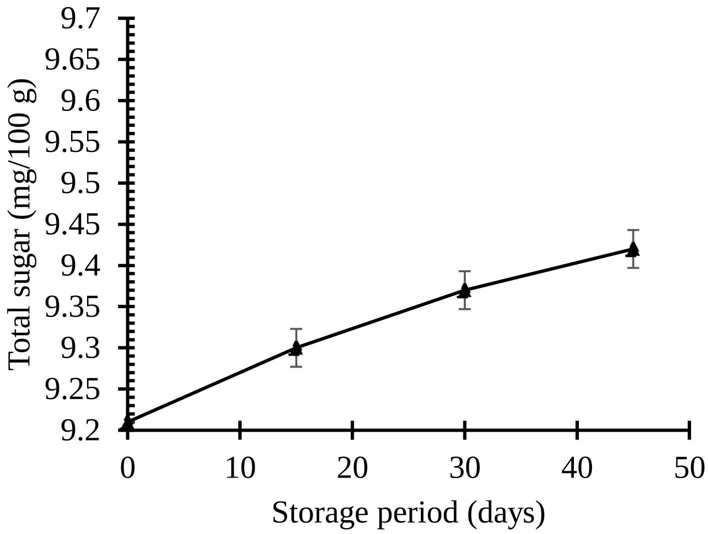

Fig 4.9 Effect of storage time on total sugar of the RTS

*Values are the means of three determinations. Vertical bars represent ± standard deviation.

An increase in total sugar content of the beverage during 45 days of storage at refrigeration temperatures was observed as shown in fig 4.9. Total sugar of the beverage were found to be 9.1, 9.3, 9.37, 9.42 for refrigeration sample (7 ºC) during 0, 15, 30 and 45 days of storage respectively.

Sugars are one of the most important constituents of fruit products, essential for and also act as a natural food preservative (Bhardwaj and Pandey, 2011). The increase in total sugars during storage could be result of hydrolysis of polysaccharides like pectin, cellulose and starch into simple sugars as reported by (Singh and Sharma, 2017).

4.5.6 Non-reducing sugar

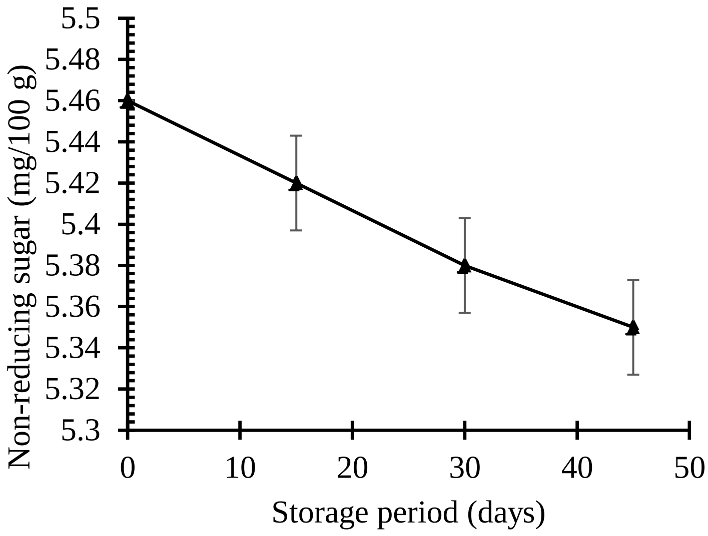

Fig 4.10 Effect of storage time and temperature on non-reducing content of the RTS

*Values are the means of three determinations. Vertical bars represent ± standard deviation.

Decrease in non-reducing sugar of the beverage during 45 days of storage refrigeration temperatures were observed as shown in fig 4.10. Non-reducing sugar of the beverage were found to be 5.46, 5.42, 5.38, 5.35 for refrigeration sample (7 ºC) during 0, 15, 30 and 45 days of storage respectively.

This could occur due to continued enzymatic activity, microbial fermentation, chemical reactions, and physical processes like crystallization, all being at a slower rate than at warmer temperatures (Singh and Sharma, 2017).

4.5.7 Ascorbic acid

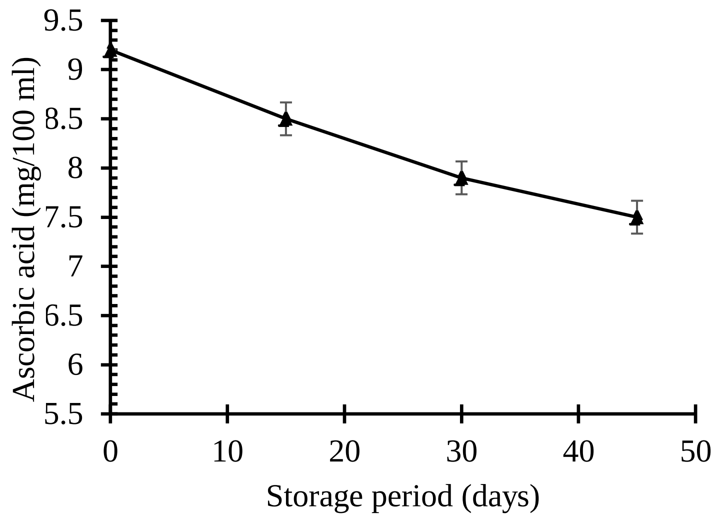

Fig 4.11 Effect of storage time and temperature on ascorbic acid content of the RTS

*Values are the means of three determinations. Vertical bars represent ± standard deviation.

The gradual decrease in the ascorbic content of the beverage during 45 days of storage at refrigeration temperature were observed as shown in fig 4.11. Ascorbic acid content of the beverage were found to be 9.2, 8.5, 7.9, 7.5 for refrigeration sample (7 ºC) during 0, 15, 30 and 45 days of storage respectively. Similar results of decrease in ascorbic acid content with the increase in storage time was reported by (Pavithra and Mini, 2023).

Ascorbic acid degradation is common in all consumable items during storage and can occur aerobically as well as anaerobically. In presence of light and temperature ascorbic acid is oxidized to dehydroascorbic acid so causing significant loss in beverages and nectars (Mohamed *et al.*, 2014). Storage temperature is one of the measure contributing factors for ascorbic acid degradation during storage as it is highly thermal sensitive (Singh and Sharma, 2017).

4.5.8 Microbiological analysis

In microbiological analysis, Total Plate Count(TPC) and Yeast and Mold Count (YMC) were performed and the changes in microbial counts during storage is given in table 4.4.

Table 4.4 Microbial changes during storage

Parameters/Days	TPC (cfu/ml)	YMC (cfu/ml)
Day 0	1×10^3	1×10^3
Day 15		
C	2×10^3	1×10^3
R	4×10^3	2×10^3
Day 30		
C	6×10^3	4×10^3
R	1.3×10^4	9×10^3
Day 45		
C	8×10^3	5×10^3
R	2.1×10^4	1.2×10^4

Fruit juices are frequently contaminated with bacteria that deteriorate the quality and impose safety concerns for consumer acceptance with microbial safety limit of 10^4 cfu/ml (Mohamed *et al.*, 2014). RTS stored in refrigeration condition was well under the microbial safety limits till the end of 45 days of storage. In the above table C and R refers to sample stored at ambient and refrigeration condition respectively.

4.6 Cost evaluation of the product

The market price of mixed fruit Ready to serve beverage ranges from NRs 150 to 200 for 1000 ml. More specifically RTS of papaya costs NRs 50 per 250ml, compared to our which cost around Nrs100 per 250ml.

The cost associated with production of this RTS is nearly 100 % higher than that of market as of today. With increased farming of papaya and mint for juice processing purpose, (when their potential for juice production and valuable by products production is realized) the production price is expected to go down.

Part V

Conclusions and recommendations

5.1 Conclusions

The study was carried out in on controlled condition using equipment like pH meter, spectrophotometer and refractometer to obtain a valid result in pH, ascorbic acid content and TSS. The conclusions are given on this research based on the obtained results and discussions made. From the above result and discussion, the following conclusions were drawn from this research work.

1. The best among different variations of mint incorporated papaya RTS beverage was sample A (94:6) maintaining constant TSS and acidity of 15ºBx and 0.3%.
2. Juice stored at refrigeration temperature was found to be superior to the RTS stored at room temperature with respect to microbial load.
3. The juice can be stored at refrigeration temperature without adding any chemical preservatives with desirable acceptability up to 45 days.

5.2 Recommendations

Based on present study, the following recommendations can be made.

1. Other micro-nutrients could be studied in this formulation, using modern instrument.
2. Shelf-life study of best product in other packaging materials and using suitable preservatives could be studied.
3. Different pasteurization time and temperature combination or aseptic condition can be studied during filling and packaging.

Part VI

Summary

RTS beverage is produced by blending one or more juices with addition of sugar, citric acid, color and flavor. Papaya juice was obtained by following process. Papaya after sorting and washing with normal water were peeled and cut for juice extraction. They were separately blended, and filtered through muslin cloth after the extraction to obtain clear juice. While mint juice was obtained by grinding with half parts water followed by passing through muslin cloth for filtration. The process of optimization of dragon fruit and pineapple juice was designed with the help of a software Design-Expert 13.

Thereafter, mint juice was incorporated in the RTS and seven samples were prepared. A (94:6), B (96:4), C (100:0), D (97:3), E (98:2), F (95.5:4.5) and G (98.5:1.5) were prepared maintaining 15 °Bx TSS and 0.3% acidity. Based on its sensory analysis, sample A was found to be the best option. Subsequently, distributed the selected sample A (94:6) was distributed into three PET bottles that had been treated with a 2% hypochlorite solution for cleaning. The fruit drink then underwent a 30 s heating process at 80°C to eliminate bacteria before being bottled and sealed.

The juices were kept for 45 days in the fridge (7°C). Any chemical or bacterial changes on a 15 days basis was carried out. From statistical analysis, it was found that the storage period had changes in TSS, titrable acidity, reducing sugar, non-reducing sugar, pH and vitamin C of mint incorporated RTS drink.

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Appendices

Appendix A

Specimen card for sensory evaluation

Name of panelist: Date: 2079/03/21

Product: Mint incorporated dragon fruit based RTS blended with pineapple

Dear panelist, you are provided with six samples of mint incorporated dragon fruit based RTS blended with pineapple in varying pulp proportion. Please taste the samples of RTS and check how much you prefer for each of the samples. Give the point for your degree of preference for each sample as shown below.

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

Sensory parameters	Samples					
	A	B	C	D	E	F
Color						
Aroma						
Taste						
Overall acceptance						

Comments:……………………………………………………………………………...........
....
....
....

Signature: ……………

Appendix B

B.1 Chemicals

All the 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
Boric acid	Fehling's solution
Ascorbic acid	Dextrose solution
Oxalic acid	Petroleum ether
Lead acetate	Hydrochloric acid
Sodium hydroxide pellets	Methanolic KOH
Sodium carbonate	Phenolphthalein
Methylene blue	Buffer solution
Meta phosphoric acid	Sodium alginate
Methyl orange	Acetic acid
Citric acid	2,6-dichlorophenol Indophenol dye
Carrez solution I and II	Bromocresol green
Ethanol	Methyl red
Sodium bicarbonate	PCA
Sulphuric acid	PDA
DPPH	Methanol

B.2 Equipment

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

Table B.2 List of equipment used

Equipment	Equipment
Grinder machine	Juice extractor
Volumetric flask	Beaker
Test tube and pipette	Weighing machine
Refractometer	Conical flask
Electronic balance	Measuring cylinder
Spectrophotometer	pH meter
Knives	Thermometer
Chopping board	Round bottom flask
Burette	Kjeldahl apparatus

ANOVA (two way) result for sensory analysis of papaya-mint RTS

Table C.2.1 ANOVA (no blocking) for color of papaya-mint RTS

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr
Samples	6	3.0794	0.5132	0.52	<0.001
Panelists	8	16.5079	2.0635	2.09	0.056
Residual	48	47.4921	0.9894		
Total	62	67.0794			

Table C.2.2 ANOVA (no blocking) for aroma of papaya-mint RTS

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr
Samples	6	12.0000	2.0000	4.15	0.002
Panelists	8	29.7460	3.7183	7.71	<0.001
Residual	48	23.1429	0.4821		
Total	62	64.8889			

Table C.2.3 ANOVA (no blocking) for taste of papaya-mint RTS

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr
Samples	6	29.7778	4.9630	13.72	<0.001
Panelists	8	14.8571	1.8571	5.13	0.256
Residual	48	17.3651	0.3618		
Total	62	62.0000			

Table C.2.4 ANOVA (no blocking) for overall acceptance of papaya-mint RTS

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr
Samples	6	11.3333	1.8889	4.21	0.002
Panelists	8	4.6984	0.5873	1.31	0.262
Residual	48	21.5238	0.4484		
Total	62	37.5556			

Table D.1 Mean and standard deviation of observed parameters

Parametres	Days	Mean	Standard deviation
TSS	0	15.00	0.01
	15	15.20	0.03
	30	15.30	0.03
	45	15.40	0.03
Titratable Acidity	0	0.30	0.01
	15	0.33	0.02
	30	0.35	0.02
	45	0.38	0.02

pH	0	4.10	0.01
	15	4.03	0.01
	30	3.97	0.01
	45	3.93	0.01
Reducing sugar	0	3.46	0.01
	15	3.55	0.13
	30	3.63	0.13
	45	3.71	0.13
Total sugar	0	9.10	0.01
	15	9.30	0.04
	30	9.37	0.04
	45	9.42	0.04
Non reducing sugar	0	5.46	0.01
	15	5.42	0.02

	30	5.38	0.02
	45	5.35	0.02
Ascorbic acid	0	9.20	0.01
	15	8.50	0.02
	30	7.90	0.02
	45	7.50	0.02

Photo Gallery