PREPARATION AND STUDY ON STORAGE STABILITY OF MIXED RTS BEVERAGE OF DRAGON FRUIT AND PINEAPPLE INCORPORATED WITH MINT

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Preparation and Study on Storage Stability of Mixed RTS Beverage of Dragon Fruit and Pineapple Incorporated with Mint

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 and Study on Storage Stability of Mixed RTS Beverage of Dragon Fruit and Pineapple Incorporated with Mint*** by **Lochan Mishra** 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 dragon fruit based RTS blended with pineapple and study its storage stability. Dragon fruit (white variety), pineapple and mint were obtained from local market. After preparation of their respective juices, optimization using Design-Expert 13 software resulted in seven dragon fruit to pineapple ratios; A(50:50), B (62.5:37.5), C (66.67:33.33), D (75:25), E (83.33:16.67), F (87.5:12.5) and G (100:0). Sample A with 50:50 blend was identified to be the best through sensory evaluation. This blend further served as the control for further experiment. 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 30 s and was stored for 45 days at three different temperatures (room, refrigeration, and frozen temperature) 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. Statistical analysis using one-way and two-way ANOVA demonstrated the significant effect of the storage period on these parameters ($p<0.05$). 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.73.

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List of 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

The dragon fruit, which is a type of tropical cactus, develops in a triangular form and has flesh-filled segments. It is a perennial plant that grows on other plants. Belonging to the *Hylocerous* genus, these plants fall under the *Cactaceae* family (Panchal *et al.*, 2018). Due to its color, nutritional content, and other characteristics, dragon fruit has drawn interest from the general public in recent years, primarily in Asian countries (Harivaindaran *et al.*, 2008). It is a sizable source of antioxidants, which is a quality that adds value to any food crop (Rebecca *et al.*, 2010). Due to the fruit's inclusion of black, crunchy seeds, its texture is occasionally compared to that of the kiwifruit. The flesh is pleasantly sweet and low in calories, and it can be eaten uncooked. Having a nutty flavor and being high in lipids, the seeds are consumed along with the flesh. Intense shape, color, and amazing blossoms are just a few of the mouthwatering qualities of dragon fruit. This lovely fruit is not only delicious and cooling, but it also contains a lot of water, other essential minerals, and a variety of nutritional components (Ariffin *et al.*, 2009) .

The freshness of any kind of food can diminish as time passes and is influenced by the duration and storage conditions. Processing of food products can be carried out to aid in the durability of that product. Rarely are processed dragon fruit goods sold in our markets, and very little processing of dragon fruit has been done in our nation (Ariffin *et al.*, 2009). If high-quality dragon fruit products like RTS, jellies, jams are created, they may be well-received by year-round dragon fruit enthusiasts (M. Islam *et al.*, 2012). This measure also promotes the development of processing industries in the growing areas of the country (Begum *et al.*, 2018).

There is increasing demand for healthy beverages and consumer awareness about the health benefits of consuming juices in recent years. This development can be attributed to several factors including changing dietary preferences and advancements in juice processing technology (Kahraman and Feng, 2021). People of all ages enjoy drinking fruits drinks that are already prepared and can be consumed without any additional steps. These foods are easy to digest, very refreshing, quench your thirst, taste good, and are much healthier nutritionally in comparison to many fizzy and artificial drinks (Divyasree *et al.*, 2018).

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.*, 2018). 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). Mixed fruit juices furthermore attract the attention of consumers due to superior quality of the juice, both in terms of nutritional and organoleptic quality. Mixtures of fruit juices can be made from a variety of fruits, such as oranges, pineapples, and others, to ensure that all essential nutrients are present in the various components (Akusu *et al.*, 2016).

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).

Pineapple (*Ananas Comosus*) is one of the most important commercial fruits grown in the world. Only pineapples, among the plants in the *Bromeliacae* family, are cultivated for their delectable and beneficial fruits, which we savor and procure. Unlike most fruits, pineapples do not ripen further once they are harvested. They must be selected at the point of ripeness to ensure they are suitable for consumption. For the fruit to be enjoyable for customers, transformation in color from green to yellow must occur in the lower section of the fruit, it must contain a minimum of 12% soluble solids and a maximum of 1% acidity. This yummy tropical fruit can be eaten fresh, dried, or in canned juices and jams. It has fiber, bromelain, manganese, copper, vitamin C, vitamin B complex, calcium, zinc, and b-carotene. The reason why people enjoy it so much is due to its delicious taste and the perfect balance of sweetness and sourness. This fruit provides numerous advantages when included in your diet as it helps our immune system, aids in digestion of proteins, alleviates our symptoms of cold, and makes our bones stronger (Lobo and Paull, 2017).

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.*, 2012).

1.2 Statement of the problem

Growing number of individuals from various countries are engaging in commercial agriculture of dragon fruit due to its ability to grow at wide range of conditions and the profitable opportunity it presents to sell their produce at reasonable prices (F. M. Hossain *et al.*, 2021). Its cultivation is increasing rapidly due to its high nutritional and market value, thus the need of manufacturing processed dragon fruit products must also be realized, taking into consideration the perishable property of the fruit. It also serves in providing the benefits of the fruit to the consumers all-round the year. Hence the research on this very topic "Preparation and study on the storage stability of mixed RTS beverage of dragon fruit and pineapple incorporated with mint" was selected for carrying out the dissertation work.

Since the flavor and texture of dragon fruit is not exactly in accordance with the Nepalese palate, a well favored and highly nutritious fruit; pineapple was utilized to produce a blended fruit juice to overcome the drawback of dragon fruit and also to add value to the drink in terms of nutrition. Mint leaves were added for their refreshing feel and the additional health and medicinal benefits they provide.

1.3 Objectives

1.3.1 General Objectives

The general objective of this study was to prepare and study the storage stability of Ready-To-Serve using dragon fruit, pineapple and mint.

1.3.2 Specific Objectives

The followings are the specific objectives of this work:

1. To analyze dragon fruit, pineapple and mint juices
2. To optimize the proportion of dragon fruit and pineapple juice
3. To optimize the best proportion of mint leaf extract in the RTS
4. To study the physicochemical properties of the prepared RTS
5. To study the microbiological changes occurring during the storage of RTS
6. To study the changes occurring during the storage of the RTS
7. To evaluate the cost of the prepared RTS

1.4 Significance of the study

This study can be beneficial to identify the possible utilization of dragon fruit, pineapple and mint as the raw materials in the preparation of highly nutritious and appetizing RTS. This work mainly focuses on the preparation of optimized dragon fruit-pineapple-mint RTS, its packaging and studying the storage stability of the RTS without added preservatives.

Dragon fruit and pineapple blended together in optimized proportion gives the best results in terms of both palate and health benefits. Furthermore, combined with mint leaves gives it an appealing aroma as well as refreshing feeling to the consumers. These fruits are still underutilized in Nepal despite of having high health benefits and also the favorable climate. This study could motivate the farmers to commercialize the production of dragon fruit, pineapple and mint such that juice processing industries could get their share of supply of the raw materials. This can help uplift the economy of the farmers involved by uplifting their living standards since dragon fruit and pineapple are high value fruits. This study might be useful to juice industries in Nepal for producing completely new product with high health benefits and refreshing taste as this modern world is super adamant on consuming highly nutritious diet without compromising on the taste. So we can definitely elevate the value of these fruits in the form of blended RTS drink. The result generated from this study might also work as an initiation for further studies related to blended dragon fruit and pineapple RTS.

1.5 Limitations of the study

1. The shelf life of the product was only studied for 45 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 specific variety of the fruits were selected for the study.
5. The optimization was carried out based on sensory parameters only.
6. Sensory panelists employed were semi-skilled.

Part II

Literature review

2.1 Introduction to juice blends

Fruits and vegetables are crucial parts of a healthy diet because they include large amounts of nutrients, particularly vitamins, carbohydrates, minerals, phytochemicals (bioactive components), antioxidants and fiber. Low intake of these commodities had accounted for more than 2.6 million death throughout the world. Regular fruit and vegetable eating lowers the risk of cancer, heart disease, stroke, diabetes premature ageing, stress, and other diseases and weariness, which is principally brought on by the combined effect of calcium, dietary fiber, ß-carotene, ascorbic acid, and oxygen radical scavengers (Lock *et al.*, 2005). However, consumption of fresh fruits and vegetables is matter of availability in particular season as most of them are seasonal and have very small shelf life. Because of their perishable nature, fruits and vegetables must be processed right away to prevent post-harvest losses (20–25%). Thus, processed products of fruits and vegetables are of great significance which can be preserved for long duration with minimum loss in consumptive value. Among different processed products, the consumption of fruit juices is on the rise in global diets (S. K. Singh and Sharma, 2017).

Fruit and vegetable beverages have become more and more popular over the past few years. This might be explained by changes in consumers' current lifestyles, taste preferences, and eating patterns. In comparison to synthetic beverages, fruit and vegetable drinks are superior due to their higher nutrient content, potential medicinal properties, and ability to supply additional calories (R. L. Bhardwaj and Pandey, 2011).

The presence of acidity, astringency, bitterness, and similar characteristics in certain fruits and vegetables poses a challenge when incorporating them into processed goods even though they provide significant nutritional value. Thus making a ready-to-drink beverage by blending multiple fruit and vegetable juices together can serve as a practical approach for utilizing these produced items (R. L. Bhardwaj and Pandey, 2011). The combination of fruit juices is believed to enhance the taste, increase nutritional benefits, and reduce production costs. Fruits that possess abundant nutrients but are often avoided due to their sourness or unpalatable taste can be combined with a variety of other fruits. This enhances their flavor while still providing us with their beneficial nutrients as well as it leads to development of new product. Because of the potential health risks associated with using food items that include synthetic food additives, one current trend in the food processing industry is to minimize or prevent their use. Thus, there is a growth in the use of natural flavors, colors, and preservatives, as well as the blending of various fruits (Sindumathi *et al.*, 2017).

2.2 Ready to Serve (RTS) beverage

Fruit juices and beverages are mainly processed food products that are conveniently used and liked by all age group consumers. They also assist in meeting your nutritional needs for vitamins and minerals in a healthy diet. There are many different products in market, such as sweetened carbonated soft drinks, clarified juice beverages, pulpy beverages, and soda water. Among these non-alcoholic beverages, the share of fruit juice based beverages is presently very small as compared to synthetic carbonated beverages. Consumers are now gradually shifting towards the consumption of natural fruit juice based beverages because of their quality, high nutritional content, medicinal importance, and good calorific value over synthetic beverages (R 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 (S. K. 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	≥10%
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 (2008)

2.2.1 Materials used in preparation of RTS beverage

2.2.1.1 Added sugar

Added sugar has several different definitions. Sugars added to foods during processing are referred to as "added sugars" according to the United States Department of Agriculture (USDA). By that standard, naturally occurring sugars found in meals like fruit's fructose and milk's lactose are not regarded as "added sugars". Usually referred to as table sugar, a disaccharide called sucrose is made up of linked units of glucose and fructose which are joined by a chemical link that is easily disrupted in the small intestine. Sucrose is present naturally in veggies and fruits. Sugars are desired for both their sweet flavor and their palatability. When sucrose is broken down or is exposed to an acidic environment (as occurs in several drinks that are ready to drink), it gets inverted to deliver 50% glucose and 50% fructose. Sugar is frequently added to the product due to manufacturing requirements and the desire to appeal to consumers. The maximum permitted amount of added sugar is standardized (Guthrie and Morton, 2000).

2.2.1.2 Citric acid

Citrus fruits contain anhydrous citric acid, a tricarboxylic acid with the molecular formula $C_6H_8O_7$. Due to its antioxidant characteristics, citric acid is utilized as an additive in medicinal formulations. It functions as a preservative and preserves the stability of the active components. It also functions as a pH regulator, an acidulant, and an anticoagulant by chelating blood calcium (Grewal and Kalra, 1995).

2.2.1.3 Colors and flavors

Food is given specific tastes and colors to replace colors lost during preparation and to enhance visual appeal. To improve flavor or affect the desired color, additives are used. Many spices, as well as artificial and natural flavors, improve food flavor. Similarly, colors improve the appearance of some items to satisfy consumer expectations. Due to the loss of volatile flavor components, flavor diminishes during storage (Byanna and Gowda, 2012).

2.3 Dragon Fruit

2.3.1 Introduction

Grown on a veining epiphytic cactus (Hylocereus sp.), pitaya, also known as dragon fruit, are native to tropical and sub-tropical forest regions of Mexico, Central America, and South America. The plants grow up tree trunks and are anchored by aerial roots. The fruits have red or pink thorn less skins, while the juicy flesh can range from white to magenta. Because of the bracts or scales on the fruit's skin, the fruit is known as pitaya, which translates to "the scaly fruit." The seeds are small and are consumed with the fruit. The fruit can weigh up to 900 grams, but the average weight is between 350 and 450 grams. Its peel accounts for more than 20 % of the fruit weight. The weight depends on pollination as well as the variety selection (Merten, 2003). Known as "Noble Woman" or "Queen of the Night," this plant has a long day and a magnificent blossom that blooms at night. The huge, creamy white flowers (25 cm in diameter) that bloom at night give it its beautiful significance (Pushpakumara *et al.*, 2006). The main benefit of this crop is that, once planted, it will continue to grow for over 20 years, and 800 dragon fruit plants may be grown on 1 hectare. It produces fruit in the second year after planting and attain in full production within five years. Commercial cultivation of it is practiced in the United States, Australia, Vietnam, Taiwan, Nicaragua, and Israel (Merten, 2003).

The rising trend in consumption of dragon fruit is a result of their medicinal advantages and beneficial effects on one's health (Sonawane, 2017). Due to its abundance of essential nutrients, this fruit holds great value in the global economy (Rifat *et al.*, 2019). The bioactive substances in Dragon fruit can be influenced by factors such as the variety of the fruit, the season in which it is grown, the prevailing weather conditions, the cultivation methods used, the availability of water, and the ways it is transported, handled, and stored (F. M. Hossain *et al.*, 2021). With its minimal water requirements and impressive tolerance for hot climates, dragon fruit is seen as a hopeful new crop for Mediterranean farmers (Trivellini *et al.*, 2020). As the red-fleshed Dragon fruits mature, they undergo a transformation and become red (Rahim *et al.*, 2009). Enriched with sugars and antioxidants, the fruit becomes both delightful and crunchy (Rao and Sasanka, 2015). Dragon fruit is an edible fruit with water-soluble fiber and contains high level of vitamin C and antioxidants like Betalains, Hydroxycinnamates and Flavonoids (Moshfeghi *et al.*, 2013). This item offers multiple advantages for your well-being. It can help you lose weight, improve digestion, lower bad cholesterol in your blood, and make your immune system stronger. The likelihood of developing cancer can be decreased by the use of hydroxycinnamates, while flavonoids exert their effects on brain cells and blood vessels to mitigate the possibility of heart disease. Furthermore, it defends against harmful microorganisms and supports the overall efficiency of the body (Verma *et al.*, 2017).

Dragon fruit is also popular in South East Asia (Patwary *et al.*, 2013). Dragon fruits become popular in Asian countries for its nutritional values, attractive feature and color. The cultivation of Dragon fruit has been on the rise in recent years due to its significance to both our well-being and the economy. It can be used to make functional materials to provide phytochemicals that have powerful antioxidant capacity. They are rich in fiber, vitamins, calcium, phosphorus, magnesium, phytochemicals and antioxidants (F. M. Hossain *et al.*, 2021). Due to its adaptability to different conditions, dragon fruit has a high potential to be grown in numerous countries. At present, there is not sufficient information accessible regarding the techniques for cultivating Dragon fruit (Karunakaran and Arivalagan, 2019). Cultivation of Dragon fruit has also been started in different places of Nepal due to suitable tropical climate and seasonal rainfall, light intensity and soil type (Atreya *et al.*, 2020).

2.3.2 Origin, history, distribution and production

During pre-Columbian times, H. undatus became prevalent in regions of the Americas and the Caribbean by dispersal thorough bird and peoples for its cultivation for fruits. The pitaya or dragon fruit is native to tropical and sub-tropical forests regions of Mexico, Central America, and Northern South America where it is distributed widely and occurs naturally (Britton and Rose, 1963). In the sixteenth century, this fruit was introduced into the Philippines by the Spanish and into Indochina by a French priest in the mid nineteenth century (earlier it was Vietnam, Laos and Cambodia) and later it was acclimatized and became a major fruit crop of region due to conducive climatic conditions. Later, it became an important fruit crop throughout South-East Asia and now it is cultivated widely in the tropics and subtropics (Nerd *et al.*, 2002; Nobel, 2002).

Production data for most new and expanding tropical fruit is rarely available. Available evidence from individual countries suggests that dragon fruit production is expanding rapidly in the past and current decade (Chen and Paull, 2019). It has proliferated from where it began to several other countries. Dragon fruit is being grown successfully in many countries. Australia, Cambodia, China, Colombia, Ecuador, Guatemala, Hawaii, Indonesia, Israel, Malaysia, New Zealand, Peru, Philippines, Taiwan, Thailand, Spain, Sri Lanka, and Vietnam and other countries. In Southeast Asia, especially in Thailand and Vietnam, dragon fruit is extensively cultivated as an export commodity and Vietnam is the largest producer of dragon fruit in the world today (Kakade *et al.*, 2022). Three major countries, Vietnam, China and Indonesia, provide over 93% of the world's dragon fruit production. Vietnam alone provides more than half (51.1%) of the world production. It has almost 55,419 ha devoted to dragon fruit farming with a volume of production more than 1 million metric tons (MT) valued at US$ 895.70 million (2018). The average productivity is 22-35 metric tonnes/hector/yr (Sharma *et al.*, 2021). It is the fifth most famous tropical crop fruit form Asia behind lychee, longan, banana and mango (Chen and Paull, 2019).

In the year 2000 AD, an American engineer introduced the white dragon fruit to Nepal from Vietnam. Its commercial farming started only after 2014 AD in Kabhre district. With the satisfactory results of these commercial farms, quite a number of farmers from various corners of the country have started plantations of white fleshed as well as red fleshed cultivars on trial basis in small scale or even on commercial scale (a total of about 8-10 ha) by a few enthusiastic persons including ex Minister Mr. Lokendra Bist in Dang district. This way, now dragon fruit is becoming an exciting new fruit crop for warmer region of the country (Atreya *et al.*, 2020).

The present market rate of this fruit in Nepal is around Rs 350 per kg. However the cost of dragon fruit was around Rs 800-1000 per kg in the initial phase of its introduction. This fruit can cultivate in region of less rainfall at altitude of 1500 meters above mean sea level. There is availability of thousands of fallow and marginal land in Terai, Bhitri madhes, valley and lower range of mountain which are suitable for cultivation. The chemical fertilizer demand of Dragon fruit is less so we can grow it organically using our local manures like FYM, compost, vermicompost which results into economical and eco-friendly production (Rijal, 2019).

2.3.3 Taxonomy and botanical description

The genus Hylocereus (Britton & Rose) is a small genus that contains 5 tropical American species (Kakade *et al.*, 2022). The nomenclature of dragon fruit is as follows:

Table 2.2 Nomenclature of dragon fruit

Kingdom	Plantae (Plants)
Sub kingdom	Trachebionta (vascular plants)
Division	Magnoliopshyta (flowering plants)
Class	Magnoliopsida (dicotyledons)
Order	Caryophyllales
Family	Cactaceae (cactus family)
Genus	*Hylocerus*
Species	*Hylocerus undatus*

Source: (Britton and Rose, 1963)

2.3.4 Chemical composition of dragon fruit and its uses

The extreme rise in popularity of dragon fruit can be attributed to its rich supply of vitamins and antioxidants (Gunasena *et al.*, 2006). This fruit is versatile and it can be enjoyed fresh or incorporated into recipes. Dragon fruit is an excellent option for salads due to its vibrant exterior, deep red interior and edible black seeds in white flesh. Dragon fruit pulp can be used to create delicious items such as juice, jam, candy and wine. Fruit peel contains high level of pectin and various methods are reported to yield 7.5% of pectin from fruit peel (Thirugnanasambandham *et al.*, 2014). It is suitable for adding color to food and manufacturing food colorants (Kakade *et al.*, 2022). Pitaya seeds contains 50 % of essential fatty acids, namely, linoleic acid and linolenic acid which are difficult to synthesize in-vivo (Ariffin *et al.*, 2009).

The chemical and nutritional composition of dragon fruit varies depending on the species, origin and harvesting time.

Table 2.3 Chemical composition of 100 g edible portion of Dragon fruit

Component	Amount (in g)
Moisture	87
Protein	1.1
Fat	0.4
Fiber	3.0
Carbohydrate	11.0
Iron	1.9
Vitamin C	20.5 mg
Calcium	8.5 mg
Phosphorus	22.5 mg

Source: (Thokchom *et al.*, 2019) with slight modifications

2.3.5 Varieties of dragon fruit

Dragon fruit is the name given to the fruit grown under two genera (Hylocerus and Selenicereus) belonging to the tribe Hylocereeae which are epiphytic, hemi-epiphytic and climbing cacti. There are five primary varieties of Hylocereus species, which are mostly distinguished by the features of their fruits. *Hylocereus undatus* is distinguished by its white pulped fruits and pink skin; similarly, *Hylocereus polyrhizus* has red pulped fruits and pink skin; *Hylocereus costaricencis* has violet-red pulp and pink skin; *Hylocereus guatemalensis* has red pulp and reddish-orange skin; and *Hylocereus megalanthus* has white pulp and yellow skin (Arivalagan *et al.*, 2021). The *H. Megalanthus* was previously categorized in genus Selenicereus and later by updating its taxonomy, (Bauer, 2003) gave the *Selenicereus* *megalanthus* species the name *H. megalanthus* and moved it into the Hylocereus genus. *Hylocereus polyrhizus* has also been renamed to *Hylocereus monacanthus.*

Hylocereus undatus, which is said to have originated in the tropical rainforests of Central and Northern South America, is one type of climbing cactus that has been eaten as food. The fruit is around 15-22 cm long, weighs about 300-800 g, and is rectangular with large and long scales that are red and green at the tips; it has white flesh with many black seeds, fleshy texture and good taste. It already appreciates the international recognition for the plant's big, fragrant, night-blooming blossoms and as an ornamental. Its fruit is now gaining popularity all over the world, particularly in Israel, Vietnam, and Australia (Britton and Rose, 1963).

Hylocereus polyrhizus and *Hylocereus megalanthus* are two other varieties of climbing cacti that are cultivated for their tasty fruit. While *H. megalanthus,* often known as the pitaya Amarillo or yellow pitaya, has yellow skin and clear to white flesh with edible black seeds while H. polyrhizus has red skin and red flesh that is speckled with edible black seeds and fruit is 10-15 cm in length and weighs about 130-350 grams and is oblong and covered with scales that vary in size (Britton and Rose, 1963).

2.3.6 Health benefits of dragon fruit

Dragon fruit is beneficial to human health due to its vital components such as vitamins, minerals, and complex carbohydrates, dietary fibers, and antioxidants. According to research, dragon fruit encouraged the formation of beneficial gut bacteria and Betacyanin, a red or purple pigment with anti-oxidative effects (Liaotrakoon, 2013). It is low in calories, zero cholesterol and full of antioxidants (Patel and Ishnava, 2019). Dragon fruit is rich in polysaccharides and mixed oligosaccharides which act as stimulating factors for growth of Lactobacilli and Bifidobacterium. These gastrointestinal microflora are called probiotics and suppress the growth of gastrointestinal pathogens. So dragon fruit can also be used as a natural probiotic (Sonawane, 2017; Wichienchot *et al.*, 2010; Xu *et al.*, 2016).

Dragon fruit promotes the healing of wounds and cuts. Moreover, the fruit improves appetite, eyesight and memory of human being. It has old-age retarding properties, cancer preventing effects, positive effects on metabolism, digestion, immune system, clear vision, oxidative stress, diabetes, cardiovascular diseases, reduce cholesterol level, prevent asthma, arthritis and also reduces aortic stiffness (Lim *et al.*, 2012; MR, 2010; Nurmahani *et al.*, 2012; Rao and Sasanka, 2015; Yusof *et al.*, 2012; Zhuang *et al.*, 2012).

Table 2.4 Functions of some of the main components contained in dragon fruit

Components	Amount	Functions
Flavonoids	Red fleshed 46.29 ± 2.47 mg RE/100 g FW & white fleshed 26.71 ± 4.46 mg RE/100 g FW	Flavonoids acts on brain cells and blood vessels to reduce the risk of heart diseases. It minimizes heart diseases and maintains blood pressure.
Betalains	42.71 ± 2.48 mg/100 g fresh pulp	Betalains can combat oxidative stress and may have the ability to suppress cancer cells. It has the ability to aid in weight loss, improves digestion, reduce LDL cholesterol in the blood and strengthen the immune system.
Hydroxycinnamates	Minor amounts of hydroxycinnamic acid	Hydroxycinnamates helps to prevent cancer.
Carotenoids (Beta-carotene)	1.4 mg/100 g	Reduced risk of cancer and cardio-vascular diseases.
Lycopene	3.4 mg/100 g	Lycopene inhibits the cell growth of various human cancer cell lines.
Linoleic acid and linolenic acid	Seeds rich with 50% of essential fatty acids	The seeds of dragon fruits are high in polyunsaturated fats (omega-3 and omega-6 fatty acids) that reduce triglycerides and lowers the risk of cardio vascular disorders.

Vitamin C	White fleshed 31.11±3.85 mg/100g FW and Red fleshed 20.00±1.33 mg/100g FW	Regular consumption of dragon fruit that contains high amount of vitamin C would help in fighting against cough and asthma; increases the wound healing properties and quickly heals the cuts areas; moreover, enhances the immune system and also stimulate the activity of other antioxidants in the body.
Phosphorus (P) and Calcium (Ca)	P 22.5 mg/100 and Ca 8.5 mg/100	Dragon fruit contains high levels of phosphorus and calcium; which helps to reinforce bones and plays an important role in tissue formation and forms healthy teeth.
Iron	1.9 mg/100	Red dragon fruit have so much iron which increases haemoglobin and erythrocyte levels in pregnant woman.

Source: (F. M. Hossain *et al.*, 2021)

The peel of dragon fruit has a significant potential for usage as a natural color (Rebecca *et al.*, 2009). Fresh and dried dragon fruit skins are both high in pectin and betalains, making them a natural culinary thickening and coloring agent. One natural food additive generated from the fruit's waste section (peel) known as 'Dragon Fruit Coloring Powder' (DFCP) is used as 'albedo'. As a result, it has no effect on the inherent benefits of dragon fruit. Dragon fruit's 'albedo' is traditionally used to color rice, milk, yoghurt, juice, and pastry (Moshfeghi *et al.*, 2013). It has therapeutic properties such as lowering blood pressure and diabetes (Kumar *et al.*, 2018).

2.4 Pineapple

2.4.1 Introduction

Pineapple (*Ananas comosus*) belonging to the family *Bromeliaceae* is one of the most important commercial fruit crops in the world grown in both the tropics and subtropics. It is renowned as the "Queen of fruits" because of its exceptional flavor and taste (Baruwa, 2013). This fruit, found in tropical regions, is rich in juice, has a distinctive tropical flavor and offers numerous health benefits. Fully matured fruit contains natural sugars, an enzyme called bromelain that breaks down proteins, citric and malic acids, and vitamins A and B (Joy, 2010). It also contains calcium, potassium, fiber, and vitamin C. Packed with vitamins and minerals, this fruit is a great option for a healthy diet. Eating it as a part of balanced diet can contribute to your overall health. After bananas and citrus fruits, pineapple ranks as the third most significant tropical fruit globally. Asia (Thailand, Philippines, Indonesia, India and China), South Central America (Costa Rica and Brazil) and Africa (Nigeria and South Africa) are the main producers (Rajendran Hemalatha and Anbuselvi, 2013; Rohrbach *et al.*, 2003).

The fruit is mostly produced in these nations for the processing and fresh fruit markets. Through the growth of regional industry and greater earnings for the farmers who produce it, it can raise national income. This fruit is incredibly well-liked and well-known worldwide. From a crown cutting of the fruit, pineapples can be grown; they may flower in 20–24 months and bear fruit in the subsequent six months. The plant has the potential to reach heights between 75 and 150 cm and can extend its reach to 90-120 cm. It has compact structure with thick stems and spiky, narrow leaves. The plant grows into a cone shaped, juicy fruit with a crown on top. It is predominantly preserved in cans and consumed by individuals around the world (Morton, 1987; Van Tran, 2006). Furthermore, it is processed into juices, concentrates, vinegar, alcohol, citric acid, calcium citrate and jams. Pineapple slices have also been preserved after freezing (Larrauri *et al.*, 1997). Moreover, the proteolytic enzyme bromelain, which is found in pineapple stems, is finding extensive use in food and pharmaceutical applications (Hebbar *et al.*, 2008).

The pineapple stems and leaves are an excellent source of fiber that may be treated to create paper and textile with exceptional pliability, smoothness, and thickness. Plant pieces are utilized to make hay and silage, which are fed to cattle. Animal feed is also made from centrifuged particles from juice production and processing wastes. Alcoholic drinks can also be produced from the fruit core's juice or nutrient-rich pulp (Lobo and Paull, 2017).

2.4.2 Origin, history and distribution

Pineapple is a native American plant first seen by people from the Old World when Columbus and some of his sailors landed on the island of Guadeloupe on November 4, 1493, on his second voyage to the New World. At the time that America was discovered the pineapple appears to have been well distributed throughout most of tropical America with the possible exception of the West Coast of South America. In many places evidences were found by the early explorers of its cultivation by the Indians. These pineapples included several distinct varieties, all of which were seedless or nearly so, although wild pineapple species growing in some parts of the American tropics were extremely seedy. Before the end of the 16th century it had also become established in China, Java and the Philippines. We believe that some of this transportation to other areas was incidental to the use of the pineapple as an item of food for the crews on these long sea voyages in the sailing vessels of that period (Collins, 1949).

Today, the world's tropical regions are the primary locations for pineapple cultivation. It is planted on more than 2.1 million acres with fruit in over 82 countries. According to Food and Agriculture Organization of the United Nations (FAO) data, pineapple stands at the eleventh position in cultivation, with just over 27,402,956 tons produced in 2017. Costa Rica is the world’s highest pineapple producing nation followed by Brazil and Philippines (Mohsin *et al.*, 2020).

In Nepal, three varieties of pineapple can be found; Giant kew, Queen and local. Five districts; Chitwan, Dhading, Nuwakot, Jhapa and Morang has been regarded as the pocket area for pineapple in Nepal. Pocket areas are divided by the government on the basis of agro ecological condition, potentiality and availability of fruits in Nepal (Devkota, 2016). In Nepal, pineapple is distributed in around 974 hector land with annual production of 13300 metric tonnes. Around 58% of pineapple is produced under Terai region. Most of the pineapple area and production occurs under central development region. Highest productivity occurs in Eastern Development Region (21.6 MT/ha) whereas average productivity of country in 2015/16 is 13.7 MT/ha. Highest pineapple area and production occurs in Jhapa, Sindhuli and Kaski districts (Pandey *et al.*, 2017).

2.4.3 Taxonomic and botanical classification

Pineapple is a perennial monocot belonging to the family Bromeliaceae which comprises of 56 genera, 2921 species with three sub-families. It can be cultivated in moist to extremely dry conditions and at altitudes ranging from sea level to above 1400 m above sea level. The cultivated pineapple has a short club-like stem and relatively narrow trough-shaped leaves arranged in a spiral around the stem. The plant produces a large multiple fruit from a terminal inflorescence. The adult pineapple plant is approximately 1-2 m tall and wide. The rosette of leaves is densely spirally arranged on the stem forming a heart shape in cross section. The stem is 25–50 cm long, and the diameter of the top (5–8 cm) is much wider than the bottom (2–5 cm). As with other monocots, the stem of pineapple contains closely spaced nodes and internode. The genus *Ananas* is distinguished from other genera by their special morphologies. The inflorescence is fused into a syncarp and has a unique dense rosette of scape-wide leaves, and the fruit sizes of this genus are usually medium to large (Cheng *et al.*, 2018).

Ananas comosus var. comosus is the most important of few commercial plants that perform constative CAM (Crassulacean acid metabolism), by which mature leaf always assimilates carbon dioxide through the CAM pathway. CAM is a carbon fixation pathway that evolved in some plants as an adaptation to arid conditions. This is a feature that certain plants including cacti have evolved to great adaptation because it allows the plant to conserve moisture during photosynthesis (Cheng *et al.*, 2018).

Taxonomically, this fruit plant is usually ordered as below:

Table 2.5 Taxonomic classification of pineapple

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Bromeliales
Family	*Bromeliaceae*
Genus	*Ananas*
Species	*Comosus*

Source: M. F. Hossain *et al.* (2015)

2.4.4 Chemical composition of pineapple and its uses

Table 2.6 Chemical composition of pineapple

Composition	Amount
Dietary fiber	1.40 g
Carbohydrate	13.7 g
Protein	0.54 g
Magnesium	12 mg
Calcium	16 mg
Potassium	150 mg
Vitamin A	130 I.U
Vitamin C	24 mg

Source: M. F. Hossain *et al.* (2015) with slight modifications

Pineapple fruits have low crude fiber content, high sugar content, high soluble solid content, high ascorbic acid and high moisture content. Therefore, pineapples can be utilized as an additional fruit with nutrients for maintaining good health (Rajendran Hemalatha and Anbuselvi, 2013). Typically, fresh pineapple fruits or fresh pineapple juice are consumed. The greatest fruits to eat fresh are field-ripe ones; all that needs to be done is remove the seeds the eyes, core, rind, and crown. Pineapples are used in a wide range of culinary items, such as desserts, fruit salads, jams, yoghurt, ice cream, candies, and meat meals. They can also be eaten fresh, tinned, or juiced (Debnath *et al.*, 2012). In Panama, tiny pineapples are harvested from the vine, leaving a few inches of stem attached for the handle. Larger fruits' meat is cut up in various ways and consumed raw, baked in pies, cakes, puddings, as a garnish on ham, or processed into sauces or preserves. It can also be eaten as dessert, in salads, compotes and other dishes. Pineapple is used by Malayan people in curries and other meat preparations. The fermented pulp is turned into a well-liked sweetmeat in the Philippines. Pineapples tend to develop off flavors when frozen, thus freezing does not work well for them. Worldwide, pineapple in cans is consumed. Young, tender shoots are consumed in salads in Africa. You can consume the inflorescences and terminal bud, sometimes known as "cabbage," uncooked or cooked. In Guatemala, young shoots known as "hijos de pina" are sold in vegetable markets (Joy, 2010).

2.4.5 Varieties of pineapple

Pineapple has been classified numerous times on the basis of different parameters since its introduction. But the classification on the basis of isozyme analysis is the most widely accepted one. This divides pineapple into five group of cultivars; Cayenne, Queen, Spanish, Abacaxi and Maipure (Joy and Anjana, 2015).

‘Smooth Cayenne’ is the standard for processing because of its cylindrical shape, shallow eyes, yellow flesh color, mild acid taste and high yields. Local selections are mostly known by their areas of origin, such as ‘Sarawak’ in Malaysia, ‘Champaka’ in India and widely grown in Hawaii. The group is susceptible to mealy bug wilt and nematodes. ‘Giant Kew’ grown in west Bengal, Goa and Meghalaya states of India belong to this group. ‘Kew’ cultivated in the north eastern states and in the southern region of India also is a cayenne member (Nakasone and Paull, 1998).

Queen group generally produces smaller plants and fruit with spiny, shorter leaves than the 'Cayenne' group. 'Queen' is grown in South Africa, Australia and India for the fresh fruit market. 'Z-Queen' or 'James Queen' is reported to be a mutant of 'Natal Queen' and is a natural tetraploid. Mauritius of the Queen group is cultivated broadly in Kerala, the southern state of India. It is used as a fresh fruit, for processing and in export (Nakasone and Paull, 1998).

Abacaxi group is grown mostly in Latin America and in the Caribbean region. It was also called as the Pernambuco group. The fruit is not considered suitable for canning or for fresh fruit export, but the juicy, sweet flavor of the fruit is favored in the local markets (Nakasone and Paull, 1998).

The plants of Spanish group are generally small to medium, spiny-leaved, vigorous and resistant to mealy bug wilt, but susceptible to gummosis caused by the larvae of the Batrachedra moth. It is acceptable for the fresh fruit market but not favored for canning, due to deep eyes and poor flesh color. 'Red Spanish' or 'Espanola roja' is the major cultivar in the Caribbean region. 'Singapore Spanish', or 'Singapore Canning' and 'Nanas Merah', are the principal canning pineapple in West Malaysia because of their adaptability to peat soil. The flesh has a bright yellow color. The fruit has light yellow flesh with adequate sugar and resistance to gummosis, is fairly tolerant to mealy bug wilt, and has good slip production and good shipping qualities (Nakasone and Paull, 1998).

The Maipure group is cultivated in Central and South America as fresh fruit for the local markets. Their clones may be of interest to breeders in the western hemisphere as they constitute a gene pool of adapted forms almost unused in breeding programmers (Nakasone and Paull, 1998).

The 'Smooth Cayenne' cultivar dominates commercial production for canning and is also one of the major fresh fruit varieties. However, 'Smooth Cayenne' has objectionably high acidity during the winter months, so newer hybrids such as 73-114 (MD-2, MG-3), which have comparable yield and a better sugar to acid balance during the winter months, have rapidly expanded in importance as fresh fruit varieties and now dominate international trade. Other varieties of some importance commercially include 'Queen' and 'Spanish', both of which are primarily consumed fresh (Joy and Anjana, 2015).

2.4.6 Health benefits of pineapple

Pineapple can be used as supplementary nutritional fruit for good personal health. Pineapple fruits are an excellent source of vitamins and minerals. One healthy ripe pineapple fruit can supply about 16.2% of daily requirement for vitamin C (Rajendran Hemalatha and Anbuselvi, 2013). Vitamin C is the body's primary water soluble antioxidant, against free radicals that attack and damage normal cells. A powerful antioxidant, vitamin C supports the formation of collagen in bones, blood vessels, cartilage and muscle, as well as the absorption of iron. Vitamin C also retards the development of urinary tract infections during pregnancy and reduces the risk of certain cancers, including colon, esophagus and stomach (Debnath *et al.*, 2012).

Malic acid makes up 13 percent of pineapple juice's acidic content. Malic acid is also beneficial for health. It boosts immunity; promotes smooth, firm skin; helps maintain oral health; and reduces the risk of toxic metal poisoning. Pineapple is also a good source of vitamin B1, vitamin B6, copper and dietary fiber (M. F. Hossain *et al.*, 2015).

Pineapple is a digestive aid and a natural anti-inflammatory fruit. Fresh pineapples are rich in bromelain used for tenderizing meat. Pineapple contains a proteolytic enzyme bromelain, which digests food by breaking down protein. Only modest quantities of bromelain are in the edible parts of the fruit, all commercially available bromelain is derived from the stem. Bromelain supplements are particularly popular among athletes for treating all sorts of physical aches and injuries. Bromelain has demonstrated significant anti-inflammatory effects, reducing swelling in inflammatory conditions such as acute sinusitis, sore throat, arthritis and gout and speeding recovery from injuries and surgery. Pineapple enzymes have been used with success to treat rheumatoid arthritis and to speed tissue repair as a result of injuries, diabetic ulcers and general surgery. Pineapple reduces blood clotting and helps remove plaque from arterial walls. Pineapple enzymes may improve circulation in those with narrowed arteries, such as angina sufferers (Debnath *et al.*, 2012).

Drinking pineapple juice can help hydrate the body and restore the immune system. It helps to build healthy bones. Pineapples are rich in manganese, a trace mineral that is needed for body to build bone and connective tissues. One cup of pineapple provides 73% of the daily recommended amount of manganese. The benefits of pineapple can affect the growth of bones in young people and the strengthening of bones in older people. Pineapple juice's high manganese content means it is a good choice for boosting fertility through sperm quality (Debnath *et al.*, 2012). Pineapples are used to help cure bronquitis and throat infections. Pineapple is an excellent cerebral toner; it combats loss of memory, sadness and melancholy. For any kind of morning sickness, motion sickness or nausea, drinking pineapple juice is advised. It works effectively in getting rid of nausea and vomiting sensation (Rajendran Hemalatha and Anbuselvi, 2013).

Pineapple is known to be very effective in curing constipation and irregular bowel movement. This is because it is rich in fiber, which makes bowel movements regular and easy. Pineapple is effective in getting rid of intestinal worms and also keeps the intestines and kidneys clean. It helps prevent gum disease and also prevents the formation of plaque, thus keeping the teeth healthy. The flesh of very young (toxic) fruits is deliberately ingested to achieve and as a drastic treatment for venereal diseases. Pineapple creates low blood pressure, cure inflammation disease, used for weight loss, control the death rate and prevent diabetes & radical damage. It cures the damaged teeth and makes them strong and healthy (Joy, 2010).

Atherosclerosis and immune disease can be also cured due to its high antioxidant content. It protects the cells of body against cancer, heart attack, nausea and helps in attaining long natural hair. It can also be used to solve acne, wrinkles, and age related problems. *Ananas comosus* leaves have anti- hyperglycemic and analgesic properties. That can be used as a cheaper and alternative source of medicine for reducing high blood sugar level of diabetic patients (Faisal *et al.*, 2014). The root and fruit are either eaten or applied topically as an anti-inflammatory and as a proteolytic agent. Phytochemical screening showed presence of alkaloids, flavonoids, saponins and tannins in the pineapple leave extract, which components can be responsible for the observed blood glucose lowering and analgesic effects (Faisal *et al.*, 2014).

One of the best known properties of pineapple is as a diuretic. This helps to eliminate toxins through the urine, helping patients with ailments of kidneys, bladder and prostate. Due to the fiber content of the pulp, pineapple prevents constipation and regularizes the intestinal flora. Furthermore, there is evidence of appetite reducer, heart protection and aid for fever, sore throat and mouth aches and inflammation. Lightly boiled ground pineapple can be used to clean infected wounds because it eliminates dead tissues, not affecting live tissue, acts as disinfectant (M. F. Hossain *et al.*, 2015).

2.5 Mint

2.5.1 Introduction

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).

Mentha spicata L. (Spearmint) is a perennial herb and is grown commercially worldwide. About 20 species of the genus Mentha belonging to the family Lamiaceae (Labiateae) are cultivated worldwide including well known plants such as Mentha arvensis, indigenous to East Asia, M. piperita, indigenous to the Mediterranean coastal areas and M. spicata, indigenous to Europe. 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).

2.5.2 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).

Table 2.7 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)

2.5.3 Chemical composition of mint

Table 2.8 Chemical composition of fresh mint leaves

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

Source: (Zheljazkov *et al.*, 2010) with slight modifications

2.5.4 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, Tucker and Naczi (2007), 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.

2.5.5 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).

2.5.6 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.

2.6 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).

2.7 Preservation of juice

Fresh juices are visually appealing and tasty, but they have a limited lifespan. It is crucial to maintain the freshness of juice by refraining from allowing it to sit for an extended period of time after it has been freshly squeezed in order to preserve its natural taste and aroma. Various methods are employed to maintain freshness, with each one offering its own benefits (Fitz and Kuipers, 2003). The methods generally used are:

a. Pasteurization

b. Preservation with chemicals

c. Addition of sugar

d. Freezing

e. Drying

2.7.1 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 (pH > 4.5, for example milk) 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).

2.8 Relation of juice acidity and thermal treatment

The concentration of hydrogen ions in a food is a governing factor in many chemical, biochemical, and microbiological reactions, and is represented by the term pH. The concentration of hydrogen ions is measured in moles, and pH is the negative log of the ion concentration. The pH of food is a deterministic element in microbial growth and activity. As a result, pH is crucial in calculating proper heating requirements. Foods are divided into three pH groups: (1) foods with a high acidity (pH 3.7); (2) foods with a low acidity (pH 4.5); and (3) foods with a low acidity (pH 3.7, pH 4.5) (Ağçam *et al.*, 2018).

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. 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 (Ağçam *et al.*, 2018).

2.9 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).

2.10 Sensory attributes of juice

For fruits and vegetables juices, the characteristics imparting the most important quality factors may be described by several different attributes, such as color (appearance), aroma and taste (flavor), texture, and nutritional value. Four characteristics, in the order shown above, are typically used by consumers to evaluate a product. Visual cues come first, then fragrance, taste, and tactile cues, in that order. While flavor comprises both scent and retro nasal (mouth) taste, aroma refers to the ortho nasal (sniff) smell of a fruit or vegetable product. Squeeze, handling stiffness, and sensory characteristics when chewing are all included in the very broad category of texture. Since items often get softer with ripening and storage, the perception of textural quality alters as chewing progresses. The nutritional value of a product is a crucial quality element that cannot be seen, tasted, or felt. Although nutritional value is a hidden attribute, this quality factor is becoming increasingly valued by consumers, scientists, and the medical profession as phytonutrients, functional foods, and antioxidants become more appreciate (Barrett *et al.*, 2010).

2.10.1 Color and appearance

The first impression of a food is usually visual and a major part of our willingness to accept a food depends upon its color and appearance. The human eye can detect thousands of different color and color perception is a phenomenon unique to the individuals. The color of food may vary considerably from place to place and season to season depending upon numerous factors. Color can serve as a useful criterion for quality. Special instrument have been designed to measure the clarity, turbidity or cloudiness of a liquid and various types of colorimeters or spectrophotometers can be used for color measurement. 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 (Kader, 2007).

2.10.2 Mouthfeel

Food's texture influences flavor and mouth feel in addition to look, taste, and fragrance. Food acceptability and taste are influenced by a variety of textural characteristics, including chewiness, gumminess, viscosity, elasticity, and others. Depending on its concentration, sugar has distinct effects on texture. It gives soft drinks more body and mouth feel when diluted. Both subjective and objective or instrumental methods can be used to gauge the texture of food. Human sense is the decisive factor in subjective approaches. Additionally, a variety of tools, including viscometers, tenerometers, texturometers, etc., are made for measuring texture (Barrett *et al.*, 2010).

2.10.3 Flavor

Flavor is a sensory phenomenon depending upon taste, odor or aroma and mouth feel. Flavor includes both taste and smell, is largely subjective and therefore hard to measure and thus leads to differences of opinion between judges of quality. Appearance of food is important, but it is the flavor that ultimately determines the quality and acceptability of food. Flavors have insignificant nutritive value but they exert a great influence on food acceptance as even hungry and nutritionally deprived populations reject the food that has not the flavor of their choice (Beaulieu and Baldwin, 2002).

2.11 Hedonic scale rating

Hedonic response quickly developed in the 20th century alongside the growth of the food processing industry. It contains a selection of techniques required for getting precise readings on how individuals react to food, which in turn affects how customers view things.

According to the Institute of Food Technologists, sensory evaluation is a scientific method for generating, measuring, analyzing, and interpreting reactions to products as experienced through the senses of sight, hearing, touch, smell, and taste. Using the senses of taste, smell, and touch, sensory analysis examines a product for its appearance, flavor, aroma, and other quality attributes (Falade and Omojola, 2010).

Customers decide whether to accept or reject food depending on the sensory analysis needed for verification of the new product's responsiveness. It is the study of weighing and judging food attributes utilizing human senses (Falade and Omojola, 2010). The hedonic scale is employed to determine how agreeable one or more things are. This scale is a categorical scale, with an odd number (five to nine) of categories ranging from dislike extremely to like extremely. There is a neutral choice included that is neither liked nor disliked. Customers rate the item on a scale based on their comments.

Part III

Materials and methods

3.1 Raw materials

3.1.1 Dragon fruit (*Hylocerus undatus*)

Dragon fruits of white variety 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 Pineapple (*Ananas comosus*)

Pineapples (variety: Giant kew) were also purchased from local market of Dharan. After being taken to the campus on the same day, they were sorted, graded, washed thoroughly to remove foreign materials, peeled, cut into small pieces, grinded and then passed through three folds of muslin cloth to extract pineapple juice.

3.1.3 Mint (Mentha spicata)

Mint leaves (pudina) were collected from a neighbor. 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 agent.

3.1.4 Sugar

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

3.1.5 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.

3.1.6 Chemicals

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.

3.1.7 Equipment

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.

3.1.8 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 dragon fruit, pineapple and mint juice at two different conditions.

3.2.1 Preparation of dragon fruit, pineapple and mint juice

The work was based on preparation and study of storage stability of RTS using dragon fruit, pineapple and mint at different conditions.

3.2.1.1 Selection of raw materials

Fresh and healthy dragon fruit, pineapple and mint leaves were selected.

3.2.1.2 Washing raw materials

Washing was done in the stainless steel vessel full of water. The dragon fruit and pineapple 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

Dragon fruit and pineapple were peeled manually using hands and knife respectively. The eyes, crown and core were removed from pineapple. About 40 % of the total weight of dragon fruit was discarded as peel while it was about 35 % for pineapple as peel, crown and core. For the extraction of dragon fruit and pineapple juice, electric mixer grinder was used. Dragon fruit and pineapple were 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.

Dragon fruit or Pineapple

↓

Washing

↓

Peeling and cutting

↓

Grinding

↓

Squeezing and filtration

↓

Dragon fruit or pineapple juice

Source: Timsina (2022) with modifications

Fig 3.1 Preparation of dragon fruit and pineapple 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.

Mint leaves

↓

Washing with clean water

↓

Cutting into small pieces

↓

Grinding with water at 1:1 ratio

↓

Squeezing and filtration

↓

Mint juice

Source: Timsina (2022) with modifications

Fig 3.2 Preparation of leaf mint extract

3.2.2 Experimental design

Design Expert 13 was used to create the recipe. Mixture design was used to formulate the recipe. The final product (RTS of dragon fruit, pineapple and mint) was prepared in two steps. First, the best proportion of dragon fruit and pineapple were selected followed by optimization of mint as a flavoring agent. Thus, the independent variable for RTS preparation were dragon fruit and pineapple concentration for the first design. Likewise, for the second design independent variables were mint juice and the optimized dragon fruit and pineapple RTS. The experimental designs are shown in the table 3.1 and table 3.2 respectively.

Table 3.1 Mixture design of mixed RTS of dragon fruit and pineapple

Sample	Proportion (Dragon fruit: Pineapple)
A	50:50
B	62.5:37.5
C	66.67:33.33
D	75:25
E	83.33:16.67
F	87.5:12.5
G	100:0

Table 3.2 Mixture design of mixed RTS of mint, dragon fruit and pineapple

Sample	Proportion (Dragon fruit and pineapple RTS: 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

3.2.3 Selection of best proportions of dragon fruit and pineapple in the RTS

The RTS samples were prepared according to various proportions of dragon fruit and pineapple juices by keeping the TSS of the mixed juice fixed at 15°Bx (by addition of sugar) and acidity at 0.3% (by addition of citric acid). The best RTS drink was selected on the basis of sensory evaluation (color, appearance, aroma, taste and overall acceptance). The different proportions of juices are shown in the table 3.1.

3.2.4 Optimization of mint extract as a flavoring agent in the best sample of dragon fruit and pineapple RTS

Mint juice was added as a refreshing flavoring agent. Different proportions of mint juice were added to the best RTS sample prepared by mixing dragon fruit and pineapple juice. The best flavored RTS drink was selected on the basis of sensory evaluation (color, appearance, aroma, taste and overall acceptance). The different proportions of mint juice are shown in the table 3.2.

The flowchart of dragon fruit and pineapple RTS blended with mint is given in fig 3.1.

Dragon fruit juice ↓ Pineapple juice ↓

↓

Mixing in different proportions

↓

Adjustment of TSS to 15°Bx by adding syrup, acidity to 0.3% by adding citric acid

↓

Ready to Serve (RTS) of dragon fruit and pineapple

↓

Blending with mint juice in different proportions

↓

Pasteurization of RTS at 80°C for 30 s, cooling, aseptically filling in pre-sterilized PET bottles and capping

↓

Storage at three different conditions; control (-4°C), refrigeration (7°C), room temperature (30±5°C)

Source: Timsina (2022) with modifications

Fig 3.3 Flowchart of dragon fruit and pineapple RTS blended with mint

3.2.5 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 three different storage conditions: frozen temperature (-4ºC), and room temperature (30±5 ºC) and refrigeration temperature (7ºC).

3.2.6 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 frozen temperature (-4°C) as control, refrigeration temperature (7°C) and room temperature (30±5°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.

3.2.7 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.

3.2.7.1 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).

3.2.7.2 Determination of titrable acidity

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

3.2.7.3 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).

3.2.7.4 Determination of vitamin C

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

3.2.7.5 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.

3.2.7.6 Determination of non-reducing sugar

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

3.2.7.7 Determination of protein

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

3.2.7.8 Determination of ash content

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

3.2.7.9 Determination of crude fiber content

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

3.2.7.10 Determination of antioxidant activity

The antioxidant activity dragon fruit, pineapple 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:

Scavenging effect (%) = [1-(A sample-A control)] ×100%

3.2.8 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.

3.2.9 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).

3.2.10 Statistical analysis

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 dragon fruit based RTS beverage blended with pineapple and study on its storage stability. Dragon fruit juice, pineapple juice 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 seven different formulations of dragon fruit and pineapple RTS were subjected to sensory evaluation. The best sample from sensory evaluation was then blended with mint juice at different proportions. The selected best sample was studied for storage stability by storing at three different temperatures; room temperature, refrigeration temperature and freezing temperature for 45 days.

4.1 Analysis of raw materials

4.1.1 Chemical composition of fruit

The chemical composition of dragon fruit and pineapple were determined and presented in the table 4.1.

Table 4.1 Chemical composition of dragon fruit and pineapple used for RTS beverage making*

Parameters	Dragon fruit	Pineapple
Moisture content (%)	89.19±0.5	88.54±0.5
Protein (%)	1.08±0.00	0.38±0.08
Ash (%)	0.4±0.01	0.23±0.01
Crude fiber (%)	0.68.±0.12	0.78±0.02
Antioxidant activity (%)	62±0.5	50±0.5

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

According to Ghorband *et al.* (2023), moisture content of dragon fruit showed 86.32 % , protein 1.1 %, fiber 1.24 %, ash content 0.45 % and 62.37% antioxidant activity. Thus the variation in composition can be due to seasonal variation, different maturity stages, origin and various other factors.

Similarly, pineapple showed 85.77 % moisture content, 0.53 % protein, 0.27% ash and 1.4 % crude fiber according to D. Islam *et al.* (2017) while antioxidant activity was found to be 65% according to Abd Hashib *et al.* (2019). Variation in the composition might be due to various factors like maturity stages and origin.

4.1.2 Chemical composition of juice

The chemical composition of dragon fruit and pineapple juice were determined and presented in the table 4.2.

Table 4.2 Chemical composition of extracted dragon fruit and pineapple juice used for RTS beverage making*

Parameters	Dragon fruit juice	Pineapple juice
TSS (ºBx)	11.5±0.0	17±0.0
pH	5.15±0.15	3.31±0.1
Reducing sugar (mg/100 g as dextrose)	4.49±0.37	4.01±0.23
Non reducing sugar (mg/100 g as dextrose)	3.65±0.31	4.32±0.42
Total sugar (mg/100 g as dextrose)	8.33±0.46	9.5±0.3
Vitamin C (mg ascorbic acid/100 g)	7.31±0.14	10±0.5
Acidity (% as citric acid)	0.18±0.01	0.98±0.02

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

According to Arivalagan *et al.* (2021), dragon fruit showed pH in the range 4.8-5.4, TSS 9.1-10.9 ºBx reducing sugar 3.39-4.98%, total sugar 5.13-7.6%, acidity 0.1-0.23% and vitamin C 7.4-14.1 %. Similar findings were obtained during this study.

Similarly, the chemical composition of pineapple was found to be 3.59 pH, 14.7 ºBx TSS, 15.74 mg ascorbic acid/100 ml and 0.74% titrable acidity as per reported by Odeyemi and Ojeleye (2021). While presence of 5.41 mg/100 g dextrose reducing sugar, 4.5 mg/100g non- reducing sugar and 9.91 mg/100 g total sugar was mentioned by Vishwokarma (2019). Similarity in findings of the studies can be observed.

Table 4.3 Chemical composition of mint*

Parameters	Mint
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.

4.2 Optimization of proportion of dragon fruit juice and pineapple juice in RTS

Seven different RTS containing different proportions of dragon fruit and pineapple juices were prepared and subjected for sensory evaluation. The results of the sensory analysis are shown in the fig 4.1-4.

4.2.1 Color

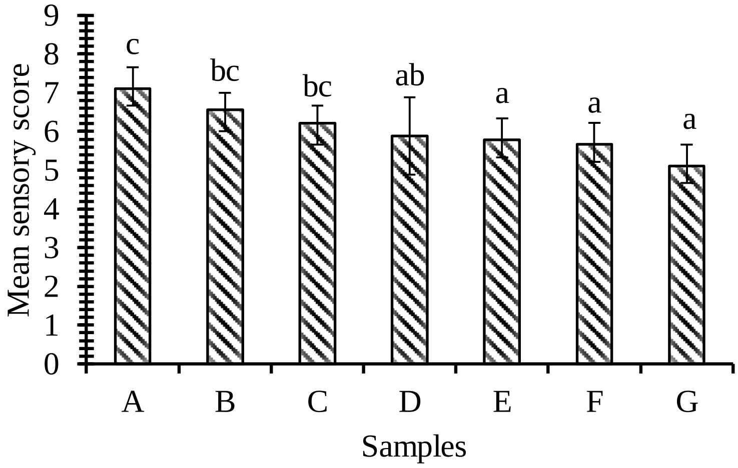

Fig 4.1 Mean color scores of different proportions of dragon fruit-pineapple RTS*

RTS A: RTS prepared by using 50% dragon fruit juice and 50% pineapple juice

RTS B: RTS prepared by using 62.5% dragon fruit juice and 37.5 % pineapple juice

RTS C: RTS prepared by using 66.67% dragon fruit juice and 33.33% pineapple juice

RTS D: RTS prepared by using 75% dragon fruit juice and 25% pineapple juice

RTS E: RTS prepared by using 83.33% dragon fruit juice and 33.33% pineapple juice

RTS F: RTS prepared by using 87.5% dragon fruit juice and 12.5% pineapple juice

RTS G: RTS prepared by using 100 % dragon fruit juice

Seven samples A, B, C, D, E, F and G with different proportions of dragon fruit and pineapple juice on RTS were subjected to sensory evaluation using two way ANOVA (no blocking) at 5% level of significance using GenStat Release 12.1. The mean sensory score of those samples with respect to color were observed and found to be 6.556, 6.444, 6.444, 6, 5.556, 5.556 and 5.556 respectively. The obtained means are represented as bar diagram in Fig 4.1. Different letters on the top of bar diagram showed that there is significant difference between the samples with respect to color while same letters at the top indicates that the samples are not significantly different at 5% level of significance. Statistical analysis showed that there was significant effect (p >0.05) of dragon fruit and pineapple variation on RTS at 5% level of significance. LSD indicated that the mean scores between the samples were significantly different (p>0.05). The mean color score of samples E, F and G (5.556) were not significantly different to each other and had the lowest value of all RTS samples. The sample A (6.556) got highest mean score which means the dragon fruit and pineapple juice made in proportion of 50:50 was found to be superior to other samples.

4.2.2 Aroma

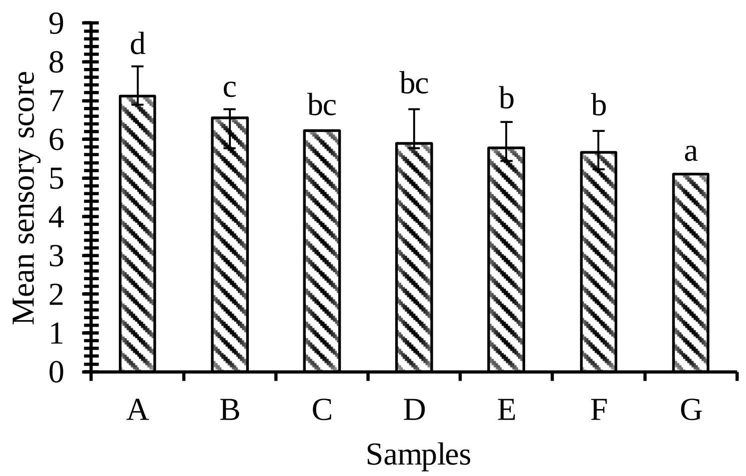

Fig. 4.2 Mean aroma scores of different proportions of dragon fruit-pineapple RTS

From the sensory evaluation for aroma of the seven different samples A, B, C, D, E, F and G, the mean sensory scores were found to be 6.778, 6.222, 6, 5.889, 5.667, 5.556 and 5 respectively. Statistical analysis showed that there was significant effect (p >0.05) of dragon fruit and pineapple variation on RTS at 5% level of significance with respect to aroma. LSD indicated that the mean scores between the samples C, D, E, F and B, C, D and E, F were not significantly different (p>0.05) but the mean aroma score of sample G (5) was lowest of all RTS samples. The sample A (6.778) got highest mean score which means the dragon fruit and pineapple juice made in proportion of 50:50 was found to be superior to other samples. This might be due to different proportions of dragon fruit and pineapple juice in the formulation of RTS.

4.2.3 Taste

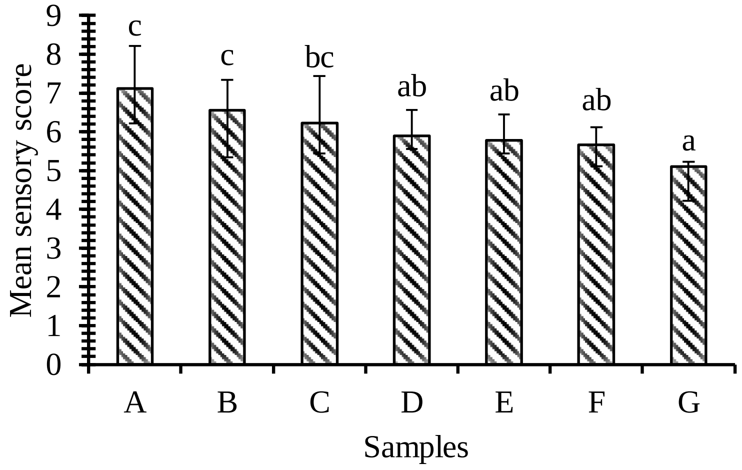

Fig: 4.3 Mean taste scores of different proportions of dragon fruit-pineapple RTS

Seven samples A, B, C, D, E, F and G of different proportions of dragon fruit and pineapple on RTS were subjected to sensory evaluation and the mean sensory scores were observed for their taste. The mean sensory scores observed were found to be 7.111, 6.778, 6.222, 5.667, 5.667, 5.444 and 5.111 respectively. Statistical analysis showed that there was significant effect ($p<0.05$) of dragon fruit and pineapple variation on RTS at 5% level of significance. LSD indicated that the mean taste scores between samples A, B, C and D, E, F, G were not significantly different ($p<0.05$) but the taste score of sample G (5.111) was the lowest of all the samples. The highest mean sensory score was observed for the sample A (7.111).

4.3.4 Overall acceptance

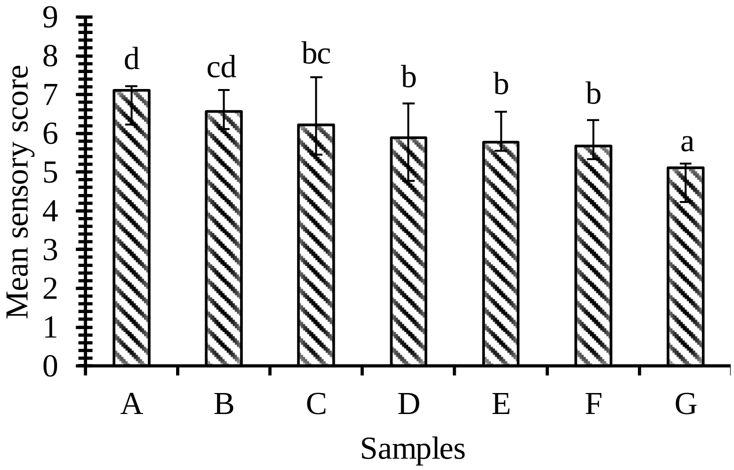

Fig: 4.4 Comparison of mean overall acceptance scores of different proportions of dragon fruit-pineapple RTS

Like all other parameters, juice variation has significant effect on overall acceptance at 5% level of acceptance. The mean overall scores for the RTS samples A, B, C, D, E, F and G were found to be 7.111, 6.556, 6.222, 5.889, 5.778, 5.667 and 5.111 respectively. At 5% level of significance, the samples B, C and D, E, F were not found significantly different from each other. The sample A however was found to be significantly different from all other samples and had the highest mean score. Therefore, based on overall acceptance, juice made by using proportion of dragon fruit and pineapple as 50:50 was found to be best among all the samples.

From the sensory evaluation and statistical analysis, it showed that the sample A had the highest mean sensory score. Therefore, the proportion of sample A (50:50) was selected for further optimization of proportion of dragon fruit and ash pineapple juice. According to Pavithra and Mini (2023) RTS beverage prepared by blending dragon fruit: pineapple in the ratio 50:50 had maximum mean score of flavor, taste and overall acceptability among different samples formed by independently blending dragon fruit and pineapple in nine different ratios. This test was conducted within the Department of Postharvest Technology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala amid the period 2019-2021.

4.3 Optimization of the best proportion of mint extract in the dragon fruit and pineapple RTS

The best RTS obtained from the optimization of dragon fruit and pineapple juice was further subjected for optimization with mint as a flavoring agent in different proportions. The effect of blending of mint juice on sensory quality of dragon fruit and pineapple RTS is shown in the fig 4.5-4.8.

4.3.1 Color

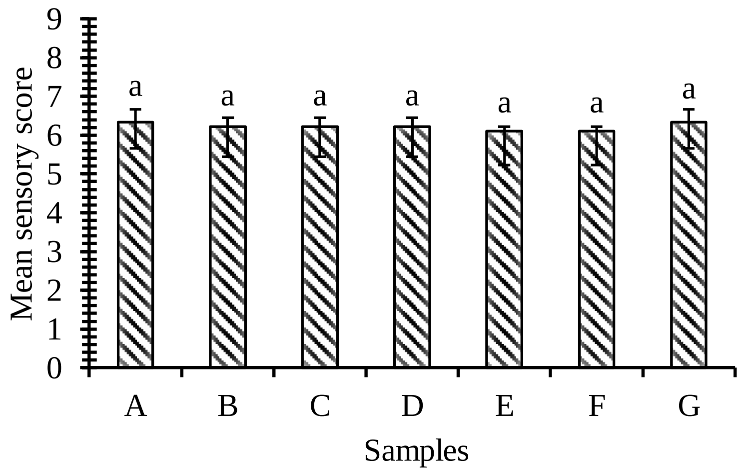

Fig. 4.5 Mean color scores of different dragon fruit, pineapple and mint RTS

RTS A: RTS prepared by using 94% dragon fruit, pineapple juice and 6% mint juice

RTS B: RTS prepared by using 95.5% dragon fruit, pineapple juice and 4.5% mint juice

RTS C: RTS prepared by using 96% dragon fruit juice, pineapple juice and 4% mint juice

RTS D: RTS prepared by using 97% dragon fruit, pineapple juice and 3% mint juice

RTS E: RTS prepared by using 98% dragon fruit, pineapple juice and 2% mint juice

RTS F: RTS prepared by using 98.5% dragon fruit, pineapple juice and 1.5% mint juice

RTS G: RTS prepared by using 100% dragon fruit and pineapple juice (control)

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. Statistical analysis showed that there was no significant effect ($p<0.05$) of variation of dragon fruit, pineapple and mint RTS of different proportions at 5% level of significance.

4.3.2 Aroma

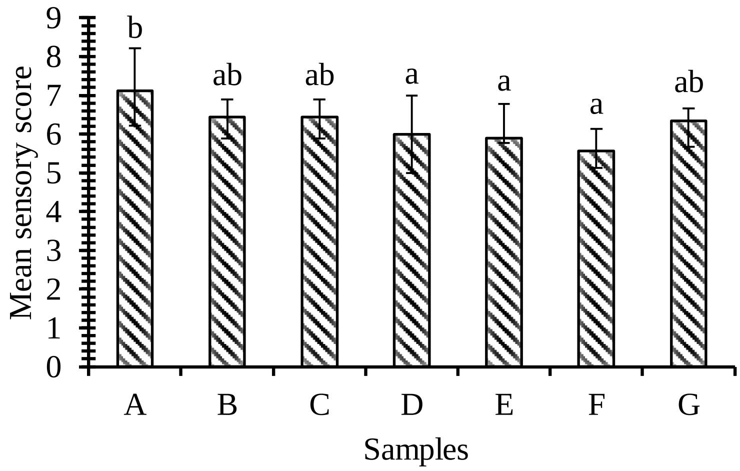

Fig. 4.6 Mean aroma scores for different dragon fruit, pineapple and mint RTS

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 dragon fruit, pineapple and mint RTS at 5% level of significance. Sample A (7.111) had the highest mean score among other RTS samples. LSD 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

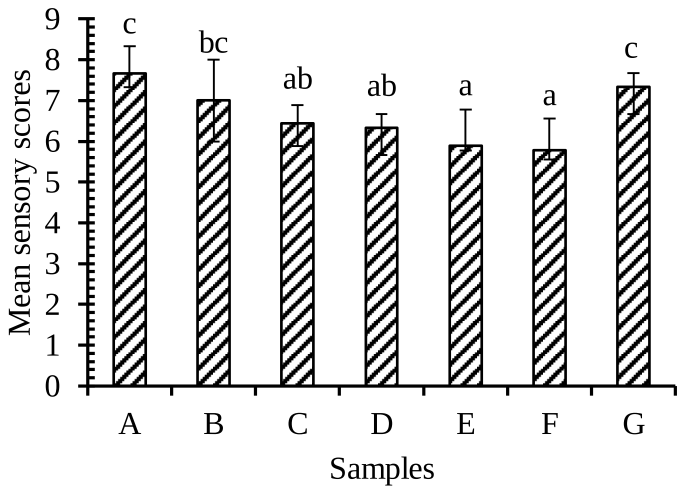

Fig. 4.7 Mean taste scores of different dragon fruit, pineapple and mint RTS samples

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 7.333 respectively. Statistical analysis showed that there was significant effect ($p<0.05$) of variation of dragon fruit, pineapple and mint RTS at 5% level of significance. Samples A, B, G and C, D, E, F, were not significantly different ($p<0.05$) to each other but sample A (7.667) had the highest mean score as indicated by LSD while sample F (5.778) had the lowest among all. Therefore based on taste, sample A was found best among all the RTS.

4.3.4 Overall acceptance

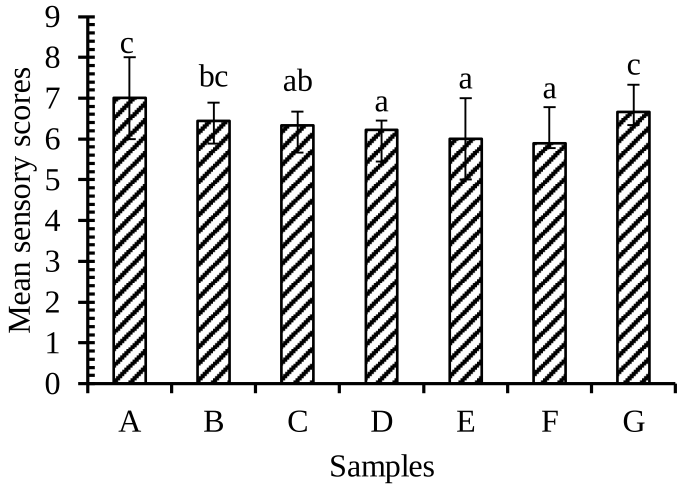

Fig. 4.8 Comparison of the mean score of overall acceptance between the samples

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 dragon fruit, pineapple 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 LSD, 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 dragon fruit and pineapple RTS blended with mint juice is presented in the table 4.4. The best sample (dragon fruit, pineapple and mint RTS) was compared with the control sample (dragon fruit and pineapple 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 *et al.* (2021) while acidity, pH, reducing sugar, non-reducing sugar, ascorbic acid content and total sugar were slightly lower than that reported by Mahar *et al.* (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 at three different temperatures; normal temperature, refrigeration temperature and frozen 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 below.

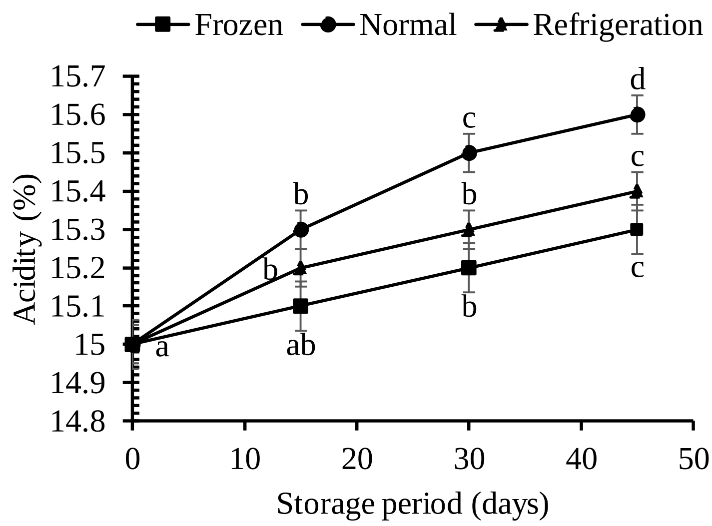

Fig 4.9 Effect of storage time and temperature on TSS of RTS

*Values are the means of three determinations. Vertical bars represent ± standard deviation. Means having similar alphabets are not significantly different by LSD at $p<0.05$.

A slight increase in the TSS of the beverage during 45 days of storage at three different temperatures were observed as shown in fig 4.9. TSS of the beverage were found to be 15, 15.3, 15.5, 15.6 ºBx for normal sample (30±5 ºC), 15, 15.2, 15.3, 15.4 ºBx for refrigeration sample (7 ºC) and 15, 15.1, 15.2, 15.3 ºBx for frozen sample (-4 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.

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 (R. L. Bhardwaj and Pandey, 2011). Similar results were observed in the preparation and storage of mixed fruit juice spiced beverage RTS (Deka and Sethi, 2001). 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 (S. K. Singh and Sharma, 2017). According to R. Bhardwaj and Nandal (2014), the longer blended Kinnow juice is stored, the higher the total soluble solids (TSS) becomes. The spike in sugar content is especially prominent when the juice is stored at room temperature, due to the rapid breakdown of acids into sugars.

4.5.2 Titrable acidity

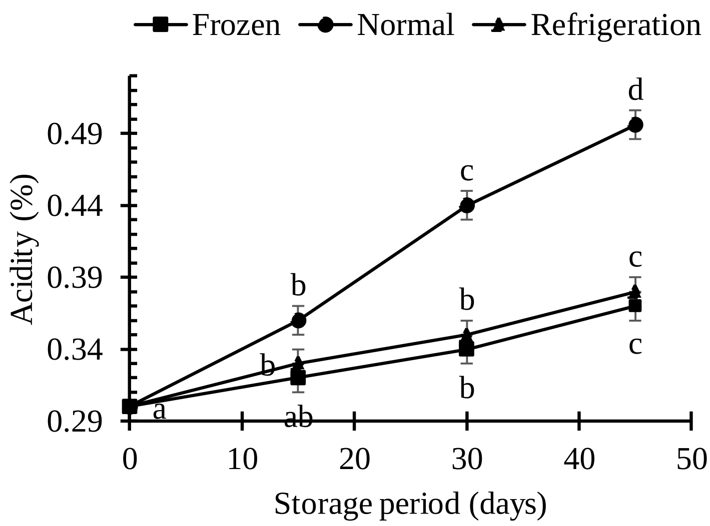

Fig 4.10 Effect of storage time and temperature on acidity of the RTS

*Values are the means of three determinations. Vertical bars represent ± standard deviation. Means having similar alphabets are not significantly different by LSD at $p<0.05$

The steady increase in the titrable acidity of the beverage during 45 days of storage at three different temperatures were observed as shown in fig 4.10. Acidity of the beverage were found to be 0.3, 0.36, 0.44, 0.496 for normal sample (30±5 °C), 0.3, 0.33, 0.35, 0.38 for refrigeration sample (7 °C) and 0.3, 0.32, 0.34, 0.37 for frozen sample (-4 °C) 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 acidity content of all the samples were significantly different to each other.

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.*, 2018; Mehmood *et al.*, 2008) . Similar results were reported for juice blend of bottle guard and basil leaves juice by Majumdar *et al.* (2011).

4.5.3 pH

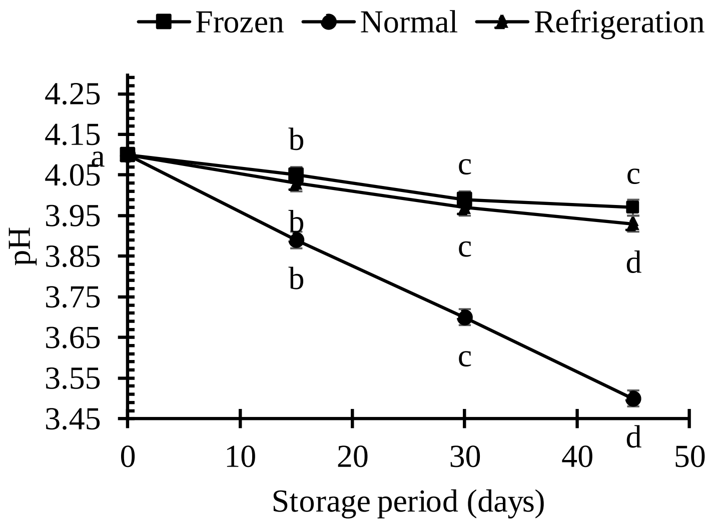

Fig 4.11 Effect of storage time and temperature on pH of the RTS

*Values are the means of three determinations. Vertical bars represent ± standard deviation. Means having similar alphabets are not significantly different by LSD at $p<0.05$

A slight decrease in the pH of the beverage during 45 days of storage at three different temperatures were observed as shown in fig 4.11. PH of the beverage were found to be 4.1, 3.89, 3.7, 3.5 for normal sample (30±5 ºC), 4.1, 4.03, 3.97, 3.93 for refrigeration sample (7 ºC) and 4.1, 4.05, 3.99, 3.97 for frozen sample (-4 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 pH of the beverage. Thus LSD indicated that the pH values were significantly different to each other.

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.* (2011).

4.5.4 Reducing sugar

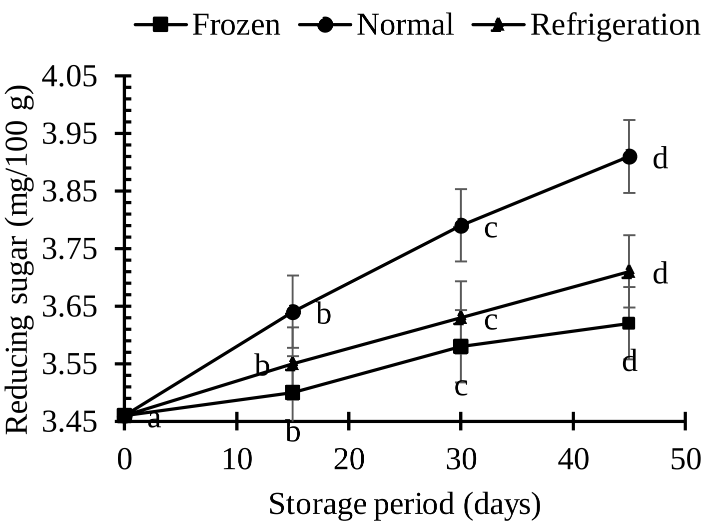

Fig 4.12 Effect of storage time and temperature on reducing sugar of the RTS

*Values are the means of three determinations. Vertical bars represent ± standard deviation. Means having similar alphabets are not significantly different by LSD at $p<0.05$

Gradual increase in the reducing of the beverage during 45 days of storage at three different temperatures were observed as shown in fig 4.12. Reducing sugar of the beverage were found to be 3.46, 3.64, 3.79, 3.91 for normal sample (30±5 ºC), 3.46, 3.55, 3.63, 3.71 for refrigeration sample (7 ºC) and 3.46, 3.5, 3.58, 3.62 for frozen sample (-4 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 reducing sugar content 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 (K. K. Singh and Mathur, 1983). M. Ahmed *et al.* (2008) reported a significant increase in reducing sugar during storage of citrus juice which may be due to acid hydrolysis of sucrose (non-reducing sugar) to glucose and fructose. Similar results were reported for a juice blend of bottle guard and basil leaves by (Majumdar *et al.*, 2011).

4.5.5 Total sugar

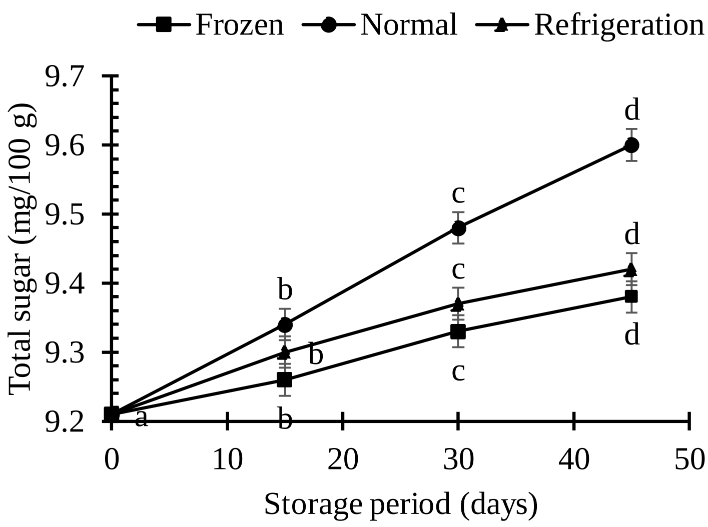

Fig 4.13 Effect of storage time and temperature on total sugar of the RTS

*Values are the means of three determinations. Vertical bars represent ± standard deviation. Means having similar alphabets are not significantly different by LSD at p<0.05

An increase in total sugar content of the beverage during 45 days of storage at three different temperatures were observed as shown in fig 4.13. Total sugar of the beverage were found to be 9.1, 9.34, 9.48, 9.6 for normal sample (30±5 ºC), 9.1, 9.3, 9.37, 9.42 for refrigeration sample (7 ºC) and 9.1, 9.26, 9.33, 9.38 for frozen sample (-4 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 total sugar of the beverage. Thus LSD indicated that the values were significantly different to each other.

Sugars are one of the most important constituents of fruit products, essential for and also act as a natural food preservative (R. L. 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 K. K. Singh and Mathur (1983). Similar findings by Kausar *et al.* (2012) suggests increase in sugar content with increased storage time of a cucumber-melon functional drink.

4.5.6 Non-reducing sugar

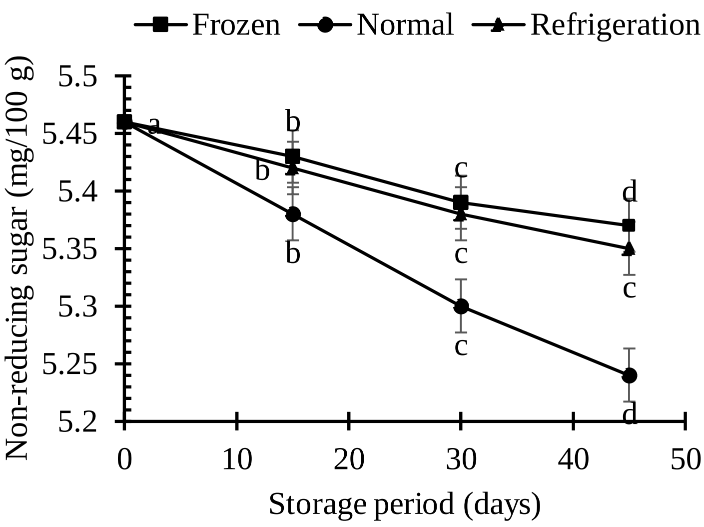

Fig 4.14 Effect of storage time and temperature on non-reducing content of the RTS

*Values are the means of three determinations. Vertical bars represent ± standard deviation. Means having similar alphabets are not significantly different by LSD at p<0.05

Decrease in non-reducing sugar of the beverage during 45 days of storage at three different temperatures were observed as shown in fig 4.14. Non-reducing sugar of the beverage were found to be 5.46, 5.38, 5.3, 5.24 for normal sample (30±5 ºC), 5.46, 5.42, 5.38, 5.35 for refrigeration sample (7 ºC) and 5.46, 5.43, 5.39, 5.37 for frozen sample (-4 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 non-reducing sugar content of the beverage. Thus LSD indicated that the values were significantly different to each other.

4.5.7 Ascorbic acid

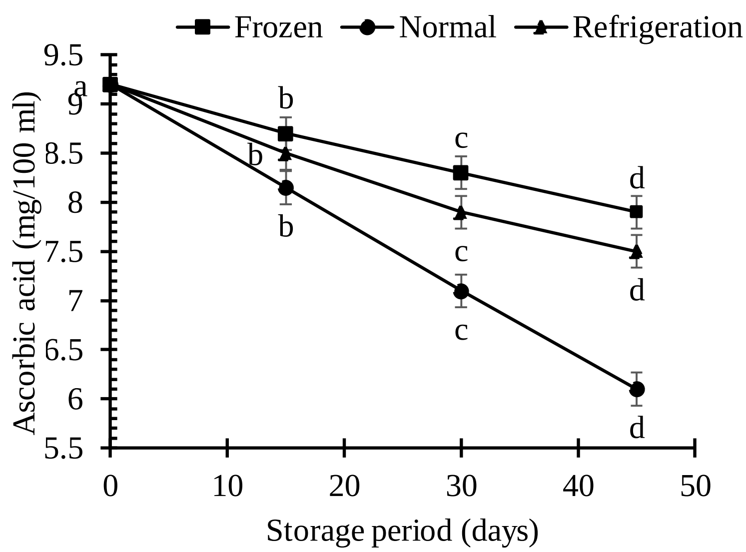

Fig 4.15 Effect of storage time and temperature on ascorbic acid content of the RTS

*Values are the means of three determinations. Vertical bars represent ± standard deviation. Means having similar alphabets are not significantly different by LSD at $p<0.05$

The gradual decrease in the ascorbic content of the beverage during 45 days of storage at three different temperatures were observed as shown in fig 4.15. Ascorbic acid content of the beverage were found to be 9.2, 8.15, 7.1, 6.1 for normal sample (30±5 ºC), 9.2, 8.5, 7.9, 7.5 for refrigeration sample (7 ºC) and 9.2, 8.7, 8.3, 7.9 for frozen sample (-4 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 ascorbic acid content of the beverage. Thus LSD indicated that the values were significantly different to each other. 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 (M. Ahmed *et al.*, 2008). Storage temperature is one of the measure contributing factors for ascorbic acid degradation during storage as it is highly thermal sensitive (S. K. Singh and

Sharma, 2017). According to Vikram *et al.* (2005), the degradation was rapid at higher temperatures. The ascorbic acid degradation rate in the orange–carrot juice stored at 2°C was less than in the juice stored at 10°C (Torregrosa *et al.*, 2006).

4.5.8 Microbiological analysis

In microbiological analysis, TPC and YMPC were performed and the changes in microbial counts during storage is given in table 4.5.

Table 4.5 Microbial changes during storage

Parameters/Days	TPC (cfu/ml)	YMPC (cfu/ml)
Day 0	$1×10^3$	$1×10^3$
Day 15		
C	$2×10^3$	$1×10^3$
F	$3×10^3$	$1×10^3$
R	$4×10^3$	$2×10^3$
Day 30		
C	$6×10^3$	$4×10^3$
F	$7×10^3$	$5×10^3$
R	$1.3×10^4$	$9×10^3$
Day 45		
C	$8×10^3$	$5×10^3$
F	$9×10^3$	$6×10^3$
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 (T. Ahmed *et al.,* 2018). RTS stored in refrigeration condition and frozen condition were well under the microbial safety limits till the end of 45 days of storage while that stored in normal room temperature was found to cross the permissible limits after 15 days.

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. The total cost associated with the best product was calculated and the cost per 250ml of dragon fruit: pineapple with mint RTS beverage was NRs 73.428. The processing and packaging cost are excluded. The calculation is made price per liter in Appendix E.

The cost associated with production of this RTS is nearly 50 % higher than that of market as of today. With increased farming of dragon fruit, pineapple 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. Dragon fruit and pineapple RTS juice where 50% dragon fruit juice and 50% pineapple juice were blended together was found to be the best from sensory analysis.
2. The best among different variations of mint incorporated dragon fruit and pineapple RTS beverage was sample A (94:6) maintaining constant TSS and acidity of 15ºBx and 0.3%.
3. On statistical analysis of the best blend of RTS after storage for 45 days at three different storage temperatures; refrigeration temperature, room temperature and frozen temperature, the storage period was found to have significant effect ($p < 0.05$) on physiochemical changes and sensory parameters.
4. Juice stored at refrigeration temperature was found to be superior to the RTS stored at room temperature with respect to microbial load.
5. The juice can be stored at refrigeration and frozen 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. Dragon fruit juice and pineapple juice were obtained by following process. Dragon fruit and pineapple 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.

Accordingly, seven different samples were prepared as A (50:50), B (75:25), C (100:0), D (87.6:12.4), E (67.6:32.4) and F (83.2:16.8) by maintaining 15 °Bx TSS and 0.3% acidity. Sample A was found to be the best according to the sensory evaluation for dragon fruit: pineapple RTS. The selected sample was then used as control for further experiment. Thereafter, mint juice was incorporated in the RTS and seven further 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 under three different conditions: room temperature (30±5°C), in the fridge (7°C), and frozen (-4°C). Any chemical or bacterial changes on a 15 days basis was carried out. The data were analyzed by one way and two way ANOVA using GenStat at 5% level of significance. From statistical analysis, it was found that the storage period had significant effect ($p<0.05$) in 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: 2080/03/21

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

Appendix C

ANOVA (two way) result for sensory analysis of dragon fruit-pineapple RTS

Table C.1.1 ANOVA (no blocking) for color of dragon fruit-pineapple RTS

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr
Samples	5	3.0370	0.6074	1.01	0.422
Panelists	8	4.7037	0.5880	0.98	0.465
Residual	40	23.9630	0.5991		
Total	53	31.7037			

Table C.1.2 ANOVA (no blocking) for aroma of dragon fruit-pineapple RTS

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr
Samples	5	10.9815	2.1963	5.21	<0.001
Panelists	8	8.7037	1.0880	2.58	0.022
Residual	40	16.8519	0.4213		
Total	53	36.5370			

Table C.1.3 ANOVA (no blocking) for taste of dragon fruit-pineapple RTS

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr
Samples	5	20.0000	4.0000	6.32	<0.001
Panelists	8	4.6667	0.5833	0.92	0.510
Residual	40	25.3333	0.6333		
Total	53	50.0000			

Table C.1.4 ANOVA (no blocking) for overall acceptance of dragon fruit-pineapple RTS

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr
Samples	5	8.1481	1.6296	6.01	<0.001
Panelists	8	4.7037	0.5880	2.17	0.051
Residual	40	10.8519	0.2713		
Total	53	23.7037			

ANOVA (two way) result for sensory analysis of dragon fruit-pineapple-mint RTS

Table C.2.1 ANOVA (no blocking) for color of dragon fruit-pineapple-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 dragon fruit-pineapple 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 dragon fruit-pineapple 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	<0.001
Residual	48	17.3651	0.3618		
Total	62	62.0000			

Table C.2.4 ANOVA (no blocking) for overall acceptance of dragon fruit-pineapple 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			

Appendix D

ANOVA (one way) results for chemical changes during storage of dragon fruit-pineapple-mint RTS

Table D.1.1 ANOVA results (no blocking) for TSS of dragon fruit-pineapple-mint RTS beverage (control)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.150	0.050000	26.67	<0.001
Residual	8	0.015	0.001875		
Total	11	0.165			

Table D.1.2 ANOVA results (no blocking) for TSS of dragon fruit-pineapple-mint RTS beverage (normal)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.630	0.210000	112.00	<0.001
Residual	8	0.015	0.001875		
Total	11	0.645			

Table D.1.3 ANOVA results (no blocking) for TSS of dragon fruit-pineapple-mint RTS beverage (refrigeration)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.2625	0.087500	46.67	<0.001
Residual	8	0.0150	0.001875		
Total	11	0.2775			

Table D.2.1 ANOVA results (no blocking) for acidity of dragon fruit-pineapple-mint RTS beverage (control)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.0080250	0.0026750	26.75	<0.001
Residual	8	0.0008000	0.0001000		
Total	11	0.0088250			

Table D.2.2 ANOVA results (no blocking) for acidity of dragon fruit-pineapple-mint RTS beverage (normal)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.0672360	0.0224120	224.12	<0.001
Residual	8	0.0008000	0.0001000		
Total	11	0.0680360			

Table D.2.3 ANOVA results (no blocking) for acidity of dragon fruit-pineapple-mint RTS beverage (refrigeration)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.0102000	0.0034000	34.00	<0.001
Residual	8	0.0008000	0.0001000		
Total	11	0.0110000			

Table D.3.1 ANOVA results (no blocking) for pH of dragon fruit-pineapple-mint RTS beverage (control)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.0314250	0.0104750	104.75	<0.001
Residual	8	0.0008000	0.0001000		
Total	11	0.0322250			

Table D.3.2 ANOVA results (no blocking) for pH of dragon fruit-pineapple-mint RTS beverage (normal)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.0494250	0.0164750	164.75	<0.001
Residual	8	0.0008000	0.0001000		
Total	11	0.0502250			

Table D.3.3 ANOVA results (no blocking) for pH of dragon fruit-pineapple-mint RTS beverage (refrigeration)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.5942250	0.1980750	1980.75	<0.001
Residual	8	0.0008000	0.001000		
Total	11	0.5950250			

Table D.4.1 ANOVA results (no blocking) for reducing sugar of dragon fruit-pineapple-mint RTS beverage (control)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.0480000	0.0160000	160.00	<0.001
Residual	8	0.0008000	0.0001000		
Total	11	0.0480000			

Table D.4.2 ANOVA results (no blocking) for reducing sugar of dragon fruit-pineapple-mint RTS beverage (normal)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.3402000	0.1134000	1134.00	<0.001
Residual	8	0.0008000	0.0001000		
Total	11	0.3410000			

Table D.4.3 ANOVA results (no blocking) for reducing sugar of dragon fruit-pineapple-mint RTS beverage (refrigeration)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.1034250	0.0344750	344.75	<0.001
Residual	8	0.0008000	0.0001000		
Total	11	0.1042250			

Table D.5.1 ANOVA results (no blocking) for total sugar of dragon fruit-pineapple-mint RTS (control)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.0507000	0.0169000	169.00	<0.001
Residual	8	0.0008000	0.0001000		
Total	11	0.0515000			

Table D.5.2 ANOVA results (no blocking) for total sugar of dragon fruit-pineapple-mint RTS (normal)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.2576250	0.0858750	858.75	<0.001
Residual	8	0.0008000	0.0001000		
Total	11	0.2584250			

Table D.5.3 ANOVA results (no blocking) for total sugar of dragon fruit-pineapple-mint RTS (refrigeration)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.0747000	0.0249000	249.00	<0.001
Residual	8	0.0008000	0.0001000		
Total	11	0.0755000			

Table D.6.1 ANOVA results (no blocking) for non-reducing sugar of dragon fruit-pineapple-mint RTS (control)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.0146250	0.0048750	48.75	<0.001
Residual	8	0.0008000	0.0001000		
Total	11	0.0154250			

Table D.6.2 ANOVA results (no blocking) for non-reducing sugar of dragon fruit-pineapple-mint RTS (normal)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.0825000	0.0275000	275.00	<0.001
Residual	8	0.0008000	0.0001000		
Total	11	0.0833000			

Table D.6.3 ANOVA results (no blocking) for non-reducing sugar of dragon fruit-pineapple-mint RTS (refrigeration)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	0.0206250	0.0068750	68.75	<0.001
Residual	8	0.0008000	0.0001000		
Total	11	0.0214250			

Table D.7.1 ANOVA results (no blocking) for ascorbic acid content of dragon fruit-pineapple-mint RTS (control)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	2.78250	0.92750	92.75	<0.001
Residual	8	0.08000	0.01000		
Total	11	2.86250			

Table D.7.2 ANOVA results (no blocking) for ascorbic acid content of dragon fruit-pineapple-mint (normal)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	16.07063	5.35688	535.69	<0.001
Residual	8	0.08000	0.01000		
Total	11	16.15063			

Table D.7.3 ANOVA results (no blocking) for ascorbic acid content of dragon fruit-pineapple-mint (refrigeration)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Time (days)	3	4.94250	1.64750	164.75	<0.001
Residual	8	0.08000	0.01000		
Total	11	5.02250			

Appendix E

Particular	Quantity	Rates (Rs/kg)	Amount (Rs)
Dragon fruit	2 kg	350	700
Pineapple	2 pieces	150 per piece	300
Mint	250 g	200	50
Sugar	500 g	100	50
Citric acid	10 g	142	1.42
Sub total			1101.42
Overhead cost (20%)			220.284
Total cost			1321.704

Therefore, price of 4500 ml of dragon fruit: pineapple and mint RTS beverage cost Rs. 1321.704, thus the price of 250 ml of drink cost Rs.73.42 Processing and packaging costs are excluded.

Photo gallery

P.1 Raw materials for the preparation of RTS

P.2 Chemical analysis of raw materials