A STUDY ON THE PROCESSING AND PRESERVATION OF MIXED FRUIT JUICE FROM PINEAPPLE, PAPAYA, BANANA AND ORANGE

A THESIS

BY

MD. MOSSARROF HOSSAIN

Student no: 1205045

Session: 2012-2013

Semester: January-June, 2013

MASTER OF SCIENCE
IN
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DEPARTMENT OF FOOD PROCESSING AND PRESERVATION

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR

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JUNE, 2013

DEDICATED TO
MY
BELOVED PARENTS

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The Author

ABSTRACT

Fruit juice is a drink made primarily by juice extractor. Mixed fruit juice is an easy way to add more fruits nutrient to any diet. Fruits are seasonal and perishable in nature and thus need to preserve by turning them into value added product like fruit juice. The experiment was conducted for processing and preservation of mixed fruit juice from pineapple, papaya, banana and orange. The fresh, fully ripe and sound fruits (pineapple, papaya, banana and orange collected from local market) were washed, peeled, removed seeds and finally transferred to the juice extractor for extracting juice. Sugar and sodium benzoate were added to the extracted and filtrated juice. The formulated juice was heated for 10 minutes, cooled and bottled for preservation. There were four treatments of mixed fruit juice marked as T_1 (Pineapple 50% + Papaya 20% + orange 20% +banana 10%), T_2 (Pineapple (20%) + Papaya 50% + orange 20% +banana 10%), T_3 (Pineapple 30% + Papaya 40% + orange 20%+banana 10%). Among the treatments, T_4 (Pineapple 40% + Papaya 30% + orange 20%+banana 10%), the treatment T_4 secured the highest scores for color, flavour, taste and overall acceptability and ranked as "Like very much" by a taste testing panel. The keeping quality, shelf life and consumer's acceptability of the products were investigated. Storage studies were carried out up to six months at refrigeration temperature and four months at room temperature. The mixed fruit juice stored at refrigerator rendered higher quality retention than those stored at room temperature. The mixed juice was analyzed for their total soluble solids (TSS), acidity, pH, vitamin C, reducing sugar, non-reducing sugar, total sugar, and mineral content. It was found that total soluble solids, acidity, total sugar, and bacterial load were increased slightly where as vitamin C and pH were decreased gradually during the storage periods. Fading of colour and off flavour was found at the end of storage periods. This research reveals that perishable fruits can be converted to attractive mixed juice and thus increase the preservation time for a reasonable time, which increase value of the product.

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CHAPTER I

INTRODUCTION

Fruits have been a part of human diet and food supplement over the years. They contain high quantity of water, carbohydrate, vitamins A, B_1, B_2, C, D and E and minerals such as Ca, Mg, Zn, Fe, K and organic compounds which are required in small amounts, to make the body function properly (Okwu and Emelike, 2006; Dosumu *et al*., 2009).Many fruits are used to make beverages, such as fruit juice (orange, apple, grape juice etc.) or alcoholic beverage such as wine, brandy or vinegar. Nutritionists have advised that eating at least five portions of fruits and vegetables a day can help people to maintain good health throughout their lives, protecting them from heart disease and cancer, Type 11 diabetes, kidney stones (Wenkam, 1990).

Fruits such as mangoes, bananas and pineapples play a significant role in human nutrition as they provide essential vitamins and minerals. Fruits also contain essential antioxidants which are responsible for scavenging free radicals responsible for various cancers. However, fruits and vegetables are highly perishable and need to be preserved to make them available for a long time (Brett, 1996).

Fruit juices are becoming an important part of the modern diet in many communities. They are nutritious beverages and can play a significant part in a healthy diet because they offer good taste and a variety of nutrients found naturally in fruits. Juices are available in their natural concentrations or in processed forms. Juice is prepared by mechanically squeezing fresh fruits or is extracted by water. Juices are fat-free, nutrient-dense beverages rich in vitamins, minerals and naturally occurring phytonutrients that contribute to good health. Fruit juices promote detoxification in the human body (Deanna & Jeffrey, 2007).

Papaya (*Carrica Papaya* L.*)* is a common fruit in all the tropical and sub-tropical countries. It is grown all over the country as a home yard fruit (Ahmed, 1984).Papaya (*Carica papaya* L.) belongs to the family Caricaceae grown in Australia, Hawaii, Philippines, SriLanka, South Africa, India, Bangladesh, Malaysia and other countries intropical America (Anuara *et al.,* 2008). Papaya is widely cultivated in the greater district of Pabna, Rajshahi, Jessore, Chittagong, Bandarban, Kushtia, Mymensingh, Dhaka, Rangpur, Faridpur, Rangamati etc. In Bangladesh, papaya occupies 20923 hector

(Ha) of land and total production is about 124764 metric tons with an average yield of 5963 Kg/ Ha (FAOSTAT, 2011). According to FAO and WHO (1972), the proximate composition of green papaya per 100 gm of edible portion as, moisture 92.1%, protein 1%, fat 0.1%, carbohydrate 6.2%, fibre 0.9%, ash 0.6%, calcium 38 mg, phosphate 20 mg, iron 0.3 mg, sodium 7 mg, potassium 215 mg, β- carotene 15 μg, thiamin 0.02 mg, riboflavin 0.03 mg, niacin 0.3 mg and ascorbic acid 40 mg/100g.

Pineapple (*Ananas comosus* L.) is one of the most appreciated tropical fruits around the world and is consumed as fresh fruits, juice and other forms. Pineapple was reported to contain polyphenolic compounds and also possess antioxidant activity (Hossain and Rahman, 2011). Pineapple, as a tropical fruit, is one of the most appreciated fruit due to its very attractive aroma and very nice flavour. Though pineapple is mainly processed into canned products, pineapple juice also a dominate part in the fruit juice sector, which ranks fourth in volume of fruit juice consumed (Grassin and Fauquembergue, 1996). The statistical data shows that about 39583 acreage of land is under pineapple cultivation in Bangladesh with an annual production of 234493 metric tons and the average yield of pineapple is 5.92 metric ton per acre (BBS, 2010). According to FAOSTAT (2011), in Bangladesh, pineapple occupies14988 hector (Ha) of land and total production is about 218582 metric tons with an average yield of 14583.8 Kg/ Ha.

The pineapple fruits are normally eaten fresh or as fresh pineapple juice. Pineapple fruits are an excellent source of vitamins and minerals and supply arrays of colour, flavour and texture to the pleasure of eating. One fresh, ripe, pineapple fruit can supply about 116.2% of the daily requirement for vitamin C (Shamsudin *et al.*, 2007).Juice extracted from pineapple can be processed into many forms such as concentrated juice, mixed juice and single strength juice. Single strength pineapple juice is categorized as juice that possess 12.5% total soluble solids, pH of 3.6, 0.54% of titratable acidity and 5.6% of suspended insoluble solid (Hongvalerat *et al.*, 2008).

Orange (*Citrus aurantium*) is a member of the family Rutaceae. It is an evergreen tree native of Asia and also cultivated in parts of Europe, America and West Indies. According to FAOSTAT (2011), in Bangladesh, oranges occupy5300 hector (Ha) of land and total production is about 21000 metric tons with an average yield of 3662.3 Kg/ Ha.

Orange juice are more nutrient dense than many commonly consumed 100 percent fruit juices, such as apple, grape, pineapple and prune (Rampersaud, 2007). It is a rich source

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Fruit juices are a ready and rich source of vitamins, fiber and mineral salts for human consumption (Ashurt, 1991). Fruit juices provide nutrients that are beneficial for human health and are in high and continually increasing demand (Ashurst, 2005). Fruit juices and drinks are nutritious which offer great taste and health benefit (Suaad and Eman, 2008). Fruit juices are product for direct consumption and are obtained by the extraction of cellular juice from fruit, this operation can be done by pressing or by diffusion (Mircea, 1995).

Fruit juice is a popular choice of beverage and the fruit juice market is one that has grown substantially over recent years. Recent research indicates that the market for fruit juice and juice drinks increased by 43% from 1999 to 2009 (Caswell, 2009). Its rise in popularity may be due to an increased in interest in health and nutrition among consumers and also due to the fact that the ever expanding fruit juice market now provides a range of different juice varieties catering for all tastes and need (Caswell, 2009).

Fruit juices are becoming more popular in comparison to synthetic beverages evidently because of their mixed flavor, colour, taste and nutritive value. Mixed fruit juices have a great demand in this sub-continent as well as many other foreign countries. Blending could lead to the production of delightful and delicious beverages with improved organoleptic quality and a high nutritive value (Hussain *et al.*, 2011). The present review mostly concerns summarization of published information to assess the composition and nutritive value of banana, orange, pineapple, papaya and related fruit juice and mixed fruit juices.

2.1Pineapple and its nutrient content

Brito *et al.* (2008) observed that the pineapple pulp had an ascorbic acid content of 21.4 mg/100 g and 15.7^0 Brix of soluble solids. The pineapple juice peroxidase presented optimum activity at 45-50 ^{0} C and pH 4.5. The enzyme remained stable at pH 4.0-9.0 and retained >80% of its activity after 24 hours of heat treatment at 50^0 C. The enzyme remained stable after 30 minutes of treatment at temperatures below 50^0 C, retaining more than 90% of its activity, and after 30 minutes at 70^0 C the residual activity was 15%.

Othman OC (2011) found that pineapple fruits Contain high moisture (68 – 89%), moderate titratable acidity (0.80 – 1.50%.),low crude fat content (0.12%), low crude fibre amounts (0.40%), low ash (0.20%), high reducing sugars (14.2 – 22.8%), high total sugars (15.2 – 30.0%), high total soluble solids (15.7 – 29.3%) and high ascorbic acid (7.9 –33.4%). Ascorbic acid, moisture, titratable acidity, sugars content and total soluble solids amounts varied within the season and with the days of storage-ripening.

Wen and Wrolstad (2002) reported that the phenolic composition of authentic pineapple juice concentrate was analyzed by HPLC. Nine major peaks accounting for 70% of total peak area were characterized and their concentrations measured in 54 commercial samples. Means and standard deviations were as follows (mg/100 ml single strength juice, normalized 12.8 ^{0}Brix), tyrosine 3.6(1.4); serotonin 1.8(0.8); di-methyl hydroxyl furanone 1.4(0.7); di -methyl hydroxyl furanone beta glucoside 6.2(3.0); tryptophan 2.2(0.9);S-sinapyl-L-cysteine1.1(0.6);N-gamma-L-glutamyl-S-sinapyl-L-cysteine 2.3(1.1); S-sinapyl glutathione 5.4(1.4); and ap-coumaric acid-like phenolic compound (calculated as P-coumaric acid) 0.5(0.4). This information will be useful for evaluation of authenticity and quality.

Askar (1998) investigated the importance and characteristics of tropical fruits. He discussed the properties of tropical fruits and their significance within the overall fruit and fruit juice industry. Aspects considered included: fruit production and consumption; the importance of quality management for successful production of tropical fruit products (Juices, nectars etc); importance of correct harvesting time; compositional aspects and nutritional and health benefits associated with tropical fruit.

Mohammed and Wickhom (1995) observed bio-chemical changes and sensory evaluation in pineapple during storage at refrigerated and non-refrigerated temperatures. They studies recently harvested pineapple. Deltada fruits stored at 10, 20 or 30°C and 65-80% RH for up to 12 days. During that storage time those were assessed for quality parameters (weight loss, shell and flesh color, firmness, decay, TSS, pH, acidity, sugars and, vitamin C contents, flesh translucency and taste score) at 4 days interval. The best results were obtained in the 10°C treatment in which all fruits were decay free after 12 days. This treatment resulted in 15.9 and 25.1% more marketable fruits than at 20°C and 30°C treatments, respectively. Significant correlations were found between taste test scores, decay free fruits and flesh translucency, but not between taste test scores and pH, sugars content, vitamin content or acidity.

Achinewhu and Hart (1994) studied the effects of processing and storage on the ascorbic acid (vitamin C) content of 4 pineapple varieties grown in the Rives State of Nigeria. They estimated ascorbic acid content of the juice of the varieties before and after storage of whole pineapple, and processing and storage of the juice for 2 months. They found that ascorbic acid of fresh juice ranged from 22.5 to 33.5 mg/100 g sample, while after storage of whole pineapple at 30to 32°C for 2 weeks. Ascorbic acid was reduced to between 59 and 65% of the fresh juice. They also found that processing the juice by pasteurization reduced ascorbic acid to between 28 and 46% while storage in plastic bottles for 2 months further reduced the ascorbic acid content to between 10 and 21%.

Botre *et al.* (1993) conducted an experiment to determine the effect of fruit weight on internal browning and quality in pineapple. They used fruits in 6 weight grades (700-899, 900-1009, 1100-1299, 1300-1499, 1500-1799 and 1800-2300 g) either at 25°C and 75% RH over 7 days or at 5°C and 90% RH over 1.5 days before assessed for the indices studied. They found that larger fruit (1500-1799 and 1800-2300 g) were more susceptible than smaller ones to internal browning and TSS content. They also found that ripe fruits held at 5°C had lower amounts of TSS.

Uddin and Islam (1985) studied the development of shelf-stable pineapple products by mechanical dehydration, sun drying and osmotic dehydration. Sugar syrup of different concentrations were used to study their effectiveness as an agent of osmotic dehydration and higher rate of dehydration observed with higher concentration of syrup. Studies on the influence of time and syrup fruit ratio showed that 6 hour contact time and 4:1 syrup fruit ratio would be optimum.

Anonymous (1960) reported the composition of pineapple, moisture content (75%); reducing sugar (3.06%); non-reducing sugar (6.88%); total sugar (19.94%); ascorbic acid (8.76 mg/100 g); ash (0.56%); acidity (0.64%); pH (2.57) and TSS (13%).

Hulme (1970) quoted the general composition ranges of pineapple extracted from numerous pineapple Research institute publication of Hawi covering at least 30 years of research, var; Smoth Cayenne, which was described, Brix (10.8-17.5) titrable acidity (0.6-

1.62) ash(0.3-0.42) water(81.2-86.2%) fibre (0.3-0.61) nitrogen(0.045-0.115) and esters(1.0-2.5) .

Remarkable works on analysis of pineapple composition was done throughout the world. The composition of pineapple according to FAO (1972) reported that moisture content (87%); Kcal (47); Ca (17 mg); Fe (0.3 mg); vitamin A (18 mg); vitamin C (22 mg/100g); ash (0.41%); fibre (0.5%) and fat (0.3%).They observed the considerably reduction of ascorbic acid of fresh juice with processing and storage and also observed that both the pasteurized and unpasteurized orange juice were acidic and the pineapple products were less acidic. They showed that the total solids, ash and the selected minerals were present in appreciable amount in the fruit products and were not significantly affected by processing and storage. They showed that pasteurized pineapple juice and pieces contained glucose, fructose and sucrose in appreciable amounts while pasteurized orange juice contained only glucose and fructose with traces of maltose but no sucrose.

Ahmed (1995) conducted an experiment to develop certain processing techniques to preserve the pineapple juice. He suggested that the juice can be preserved by can or bottle and may happily be consumed as drink for its delicious taste and characteristic flavors. His research was mainly conducted to preserve pineapple juice by bottling, reuse bottle, little or no syrup or additive and processing at water bath temperature. The juice was preserved by various heat treatments with or without KMS (preservative) in different types of containers. He found that the products developed by combined heat treatment and chemical preservative retained significantly better colour than those developed by heat treatment alone. However, all the products were found equally acceptable in so far as taste and general acceptability are concerned.

Rangana and Bajaj (1966) reported that SO2 is widely used throughout the world principally in treating food of plant origin. It is used in the preservation of fruit juices, pulps, beverages and concentrate. Concentration used may vary from 350 to 2000 ppm soluble salts (e.g. K.M.S.) usually used in treating fruits and vegetable products. The activity is higher at pH below 4.

According to USDA Nutrient Database, Nutritional value of fresh pineapple is presented below:

Table 2.1: Nutritional value per 100 g (3.5 oz) pineapple

Energy	202 kJ(48 kcal)	Folate (vit. B_9)	15 μg (4%)
Carbohydrates	12.63 g	Vitamin C	36.2 mg (44%)
Sugars	9.26 g	Calcium	13 mg (1%)
Dietary fiber	1.4 g	Iron	0.28 mg (2%)
Fat	0.12g	Magnesium	12 mg (3%)
Protein	0.54 g	Manganese	0.9 mg (43%)
Thiamine (vit. B_1)	0.079 mg (7%)	Phosphorus	8 mg (1%)
Riboflavin (vit. B_2)	0.031 mg (3%)	Potassium	115 mg (2%)
Niacin (vit. B_3)	0.489 mg (3%)	Zinc	0.10 mg (1%
Pantothenic acid (B_5)	0.205 mg (4%)	Vitamin B_6	0.110 mg (8%)

Source: USDA Nutrient Database

McCance and Widdowsons (1999) reported the composition of pineapple in three stages such as pineapple raw (flesh only), canned in juice and canned in syrup. These compositions per 100 gm are tabulated below:

Pineapple	Water (%)	Total sugar	Ash (%)μgm	Vit-C (mg/100ml)	Carotene (mg/100ml)	Protein (%)
Raw pineapple	86.5	10.1	245.0	12	18	0.2
Canned in juice	86.8	12.2	111.58	11	12	0.4
Canned in syrup	86.2	16.5	108.22	13	11	0.3

2.2 Papaya and its nutrient content

Papaya is an important fruit in Bangladesh but research works on prospect of processing green and ripe papaya for commercial products are insufficient. Some available research findings in this connection have been reviewed and presented as follows:

Akin *et al.* (2008) investigated the production of ethanol from *Carica papaya* (pawpaw) using dried active baker's yeast strain (*Saccharomyces cerevisiae*). The fermented papaya fruit waste produced ethanol at 2.82-6.60% (v/v). Proximate analyses of the dry fruit showed that pawpaw waste contained 90.82 g/100 g carbohydrate, 2.60 g/100 g lipid, 1.63 g/100 g crude protein and 4.95 g/100 g ash.

Papaya contains 108 mg ascorbic acid per 100g of fresh fruit, which is higher than oranges (67 mg/100 g). Papaya fruit is highly appreciated world-wide for its flavour, nutritional qualities, digestive properties and serotonin content (Fernandes *et al.*, 2006). Papaya is a good source of serotonin (0.99 mg/100 mg), which has been associated with enabling the gut to mediate reflex activity and also decreasing the risk of thrombosis (Santiago-Silva *et al.*, 2011).

Carmen *et al.* (1978) studied on the papaya and described that papaya is one of the largest tropical fruits; it has pulpy flesh. Its flavour and aroma are controversial; they are not as abundant and exotic as other tropical fruits but are quite characteristic. Papaya is usually eaten alone or in the mixtures of tropical fruits, peeled and cut in segments, cubes or balls. In mixtures it is useful for its texture and well defined organoleptic properties. Fruit pleases or the puree can be dehydrated by different methods. Both types of products, however, are rarely found in commerce, as most of their flavour and aroma is either changed or lost. In order to process papaya it must be at the proper ripening stage. The fruits usually collected green mature and it ripens under storage. Because of its size and fragile skin, transport and handling are critical. The fruit is affected by climate, is injured by chilling and during ripening physical and chemical changes occur. Papaya pulp is one of the best sources, among fruits, of vitamins, especially vitamin A and C. During fruit maturation and processing changes occurs in these compounds.

Rodriguez and Iguina-de-George (1975) investigated the evaluation of papaya nectar prepared from unpeeled papaya puree. It was found that the mature green papayas were ripened at room temperature, seeds were removed and unpeeled sections were mashed and pulped. The pulp with 17.5% sugar added was heated at 87.8°C and frozen at -23.3°C. Water to dilute it to 33.3% pulp content, sucrose and citric acid were added. The resulting nectar was pasteurized, canned and stored at 29.4°C for 12 months. The chemical composition of the nectar did not change significantly during storage although there were slight changes in the percentage of reducing sugars. Taste evaluation by a panel showed that the overall quality by of the sample remained acceptable throughout the storage period.

Chan and Kowrk (1975) reported that the total sugars, reducing and non-reducing sugar in papaya to be 5.8% and 0.3% respectively. Whereas Wilson (1980) analyzed the chemical composition of papaya and stated that the reducing and non-reducing and total sugar was 4.0%, 2.9% and 6.8% respectively

Firmin (1997) investigated the physical and chemical changes during maturation of the local and solo variety of papaya. It was found no change was observed in total acidity, though there was a slight increase in the pH of both types. Starch content decreased while total sugar, reducing sugar and ascorbic acid increased. A sensory panel preferred solo, confirming that it is suitable for the local and international market.

A compositional analysis found that per 100 g edible portion of papaya fruit there were 86.6g of water, 12.1 g of carbohydrates, 0.7 g of fiber, 0.5 g of ash, 0.5 g of protein, 0.3 g of fat, 450mg of vitamin A, 74 mg of vitamin C, 0.5 mg of niacin, 0.04 mg of riboflavin, 0.03 mg of thiamin, and 204 mg of potassium as the predominant mineral ion. The sugar profile contained 48.3% sucrose, 29.8% glucose, and 21.9% fructose (Chan and Kwok, 1975). Total soluble solids content in papaya flesh ranged from 5 to 19% (Paull *et al.*, 1997). It was relatively low in acid content. The titratable acidity of papaya, calculated as citric acid, is approximately 0.1% only, and the pH ranges between 5 and 5.5. Citric, malic, ascorbic, and a-ketoglutaric acids account for 85% of the titratable acidity (Chan *et al.*, 1975).

Aziz *et al.* (1975) harvested the fruits at edible maturity and observed the effects of thinning on size, yield and quality of papayas. Result showed length and total soluble solids (TSS) of papaya increased with increasing of level of fruit thinning. The 75% fruit thinning level produced the lowest and heavier fruits, with the highest TSS (10.80%).

Kuthe and Spoer (1974) studied on the 15-20 varieties of papaya fruits annually each containing 0.6% protein and 9% carbohydrate; yield per hector was about 28 tons. About 300 gm dried and powdered papain may be produced annually by each tree.

According to USDA Nutrient Database, Nutritional value of fresh papaya is presented below:

Table 2.2: Nutritional value per 100 g (3.5 oz) papaya

Energy	179 kJ (43 kcal)	Iron	0.25 mg (2%)
Carbohydrates	10.82 g	Magnesium	21 mg (6%)
Sugars	7.82 g	Manganese	0.04 mg (2%)
Dietary fiber	1.7 g	Phosphorus	10 mg (1%)
Fat	0.26 g	Potassium	182 mg (4%)
Protein	0.47 g	Sodium	8 mg (1%)
Vitamin A	equiv. 47 μg (6%)	Zinc	0.08 mg (1%)
beta-carotene	274 μg (3%)	Lycopene	1828 μg
lutein&zeaxanthin	89 μg	Vitamin B_6	0.038 mg (3%)
Thiamine (vit. B_1)	0.023 mg (2%)	Folate (vit. B_9)	38 μg (10%)
Riboflavin (vit. B_2)	0.027 mg (2%)	Vitamin C	62 mg (75%)
Niacin (vit. B_3)	0.357 mg (2%)	Vitamin E	0.3 mg (2%)
Pantothenic acid (B_5)	0.191 mg (4%)	Vitamin K	2.6 μg (2%)
Calcium	20 mg (2%)		

Source: USDA Nutrient Database

2.3 Orange and its nutrient content

Arthey and Ashurst (1996) reported thatthe sweet orange (*Citrus sinensis* L) are very tasty and nutritious, containing 5-10% sugar, 1-2% citric acid, along with vitamin C and beneficial fibre and pulp. Most sweet orange have an orange coloured rind when they are ripe as well as an orange interior and juice. Oranges are grown in enormous quantities and are readily available as an inexpensive fruit at anytime of the year.

Dhuique-Mayer *et al.* (2005) reported that orange fruit are the most popular ones for consumer throughout the world due to their pleasant flovours and nutritional value. The fruits are both consumed fresh and industrially processed orange peels contain abundant fragment substances which are extensively applied for processing into essential oils which are used commercially for flavoring foods, beverages, perfumes, cosmetics.

Akinyele *et al.* (1990) conducted an experiment to observe nutrient losses during and after processing of pineapple and orange. They analyzed ascorbic acid, pH, total titrable acidity, total solids, ash and contents of calcium, magnesium, sodium and potassium of various products of pineapple and oranges. They estimated sugars in the samples quantitatively and qualitatively and stored the samples of pasteurized pineapple pieces and pasteurized orange juice at room temperature for 3 months followed by chemical analyses.

The sweet orange (*Citrus sinensis* L.) are very tasty and nutritious, containing 5-10% sugar, 1-2% citric acid, along with vitamin C and beneficial fibre and pulp. Most sweet orange have an orange coloured rind when they are ripe as well as an orange interior and juice. Oranges are grown in enormous quantities and are readily available as an inexpensive fruit at anytime of the year (Arthey and Ashurst, 1996). Orange fruit are the most popular ones for consumer throughout the world due to their pleasant flovours and nutritional value (Dhuique-Mayer *et al.,* 2005). This fruits are both consumed fresh and industrially processed. Orange peels contain abundant fragment substances which are extensively applied for processing into essential oils which are used commercially for flavoring foods, beverages, perfumes, cosmetics (Dhuique-Meyer *et al.*, 2005).

Lim *et al.* (2006) conducted that, orange could be considered to have high vitamin C content compared to other fruits such as banana, dragon fruit and star fruit with vitamin C content of 4.9mg/100g, 8.0mg/100g and5.2mg/100g respectively. During storage the vitamin C content of the orange reduced significantly ($P<0.05$) in both waxed and unwaxed samples (42.46 to36.68mg/100g).

Protein is the most abundant material in citrus juice cloud; it contributes approximately 52.4% of the cloud of commercial orange juice (Klavons and others, 1991). In the protein constituent, 53% is insoluble protein, 30% is complexed with low molecular weight cloud components, and 17% is covalently bonded with components such as hemicellulose. Commercial orange juice cloud contains approximately 4.5% pectin, and in the pectin constituent, 60% is associated with insoluble protein, 25% is calcium pectate, and 15% is protopectin (Klavons and others, 1994).

According to USDA Nutrient Database, Nutritional value of fresh orange is presented below:

Table 2.3 Nutritional value per 100 g (3.5 oz) Orange

Energy	197 kJ (47 kcal)	Vitamin C	53.2mg (64%)
Carbohydrates	11.75 g	Vitamin E	0.18 mg (1%)
Sugars	9.35 g	Calcium	40 mg (4%)
Dietary fibre	2.4 g	Iron	0.1 mg (1%)
Fat	0.12 g	Magnesium	10 mg (3%)
Protein	0.94 g	Manganese	0.025mg (1%)
Water	86.75 g	Phosphorus	14 mg (2%)
Vitamin A	equiv.11µg (1%)	Potassium	181 mg (4%)
Thiamine (vit. B_1)	0.087 mg (8%)	Zinc	0.07 mg (1%)
Riboflavin (vit. B_2)	0.04 mg (3%)	Folate (vit. B_9)	0.06 mg (5%)
Niacin (vit. B_3)	282 mg (2%)	Choline	8.4 mg (2%)

Source: USDA Nutrient Database

2.4 Banana and its nutrient content

Figuerola *et al.* (2005) reported that banana fibre has a better quality than other fibre sources due to its high total and soluble fibre content, water and oil holding capacities, and colonic fermentability, as well as a lower phytic acid and caloric value content. A high dietary fibre content of banana peel (about 50 g/ 100 g) is indicative of a good source of dietary fibre (HappiEmaga *et al.*, 2007).

Happi Emaga *et al.* (2007) found that the maturation of banana fruits has shown to impact the dietary fibre compositions of banana peels. Cellulose, lignin, and hemicelluloses contents of banana peels, the components of the insoluble dietary fibre fractions, varied from 7 to 12 g/100 g, 6.4 to 9.6 g/100 g and 6.4 to 8.4 g/100 g, respectively, whereas pectin contents, a component of the soluble dietary fibre ranged from 13.0 to 21.7 g/100 g. The concentrations of hydrogen cyanide, an extremely poisonous substance, and oxalate contents in banana peels were found to be 1.33 mg/g and 0.51 mg/g, respectively, falling within the safety limits. These results indicated that banana peels were safe and valuable functional ingredients for human consumption (Anhwange, 2008)

Banana has high calorific and nutritional value carbohydrate content in the banana pulp as high as 36.4 % (Bajpai *et al.*, 1985). It also contains nearly other essential nutrients including minerals and vitamins. Nutritionists recommended that every person should have at least 85 gm of fruits in the daily diet (Chandra, 1981).

Sharaf *et al.* (1979) studied the chemical composition of banana. They found that the composition (%dry basis) and nutrients in banana pulp were moisture 75.7%, crude fiber 3.44%, starch 14.60%, total sugar 76.12%, pectin 2.30%, and ash 3.60% and the following nutrients(mg/100 gm dry basis): P 88.38 mg/100 gm, Ca 70.19 mg/100 gm, Fe 0.47 mg/100 gm, vitamin C 40.00 mg/100 gm, vitamin B_1 0.88 mg/100 gm, vitamin B_2 0.93 mg/100 gm respectively. They also reported that among the amino acid, methionine was absent in pulp.

Ketiku (1976) and Vilong (1981) reported that the importance of banana fruit is due to its caloric and nutritive value and of its versatile use to the consumers. It contains appreciable amount of vitamin B and certain amount of vitamin A and C, and also minerals such as K, P, Ca, Fe.

Sarathathevy and Ganesharanee (1993) reported that the flours of banana have on average, crude protein 2.2%, crude fat 1.3%, ash 3.7, neutral-detergent fiber 8.9 , acid detergent fiber 3.2, cellulose 3.1, lignin 1.0 and hemicelluloses 5.0% (dry basis). Carbohydrates are soluble sugars 2.8, starch 70.0 and non-starch polysaccharides 12.0%.Potassium is the main mineral in the banana flour, about 275 with phosphorus about 130 and magnesium about 95mg/100g23 (dry basis). Fresh green banana have vitamin C about 90 mg/100 g on a dry basis.

Palmer (1971) reported constant pectinesterase activity in ripening banana. He suggested that phenolic compounds were inhibiting the enzyme. This assertion was verified when he determined pectin esterase activity in the presence of polyvinylpyrolidone (a chemical commonly used to bind phenolics). Polygalacturonase may be involved in softening of banana pulp, but little information is available. Banana pulp contains 2-3% cellulose and this decreases only slightly during ripening. In the green fruit, the hemicelluloses make up 8-10% of the fresh banana pulp, decreasing to about 1% in the ripe fruit. Despite this decrease, the hemicellulose content of ripe banana pulp is higher than that of most fruits and vegetables, whereas cellulose and lignin contents are lower.

The chemical composition of plantain varies with the variety, maturity, degree of ripeness and where it is grown (soil type). The water content in the green plant is about 61% and increases on ripening to about 68%. The increase in water is presumably due to the breakdown of carbohydrates during respiration. Green plantain contains starch which is in the range of 21 to 26%. The starch in the unripe plantain is mainly amylose and amylopectin and this is replaced by sucrose, fructose, and glucose during the ripening stage due to the hydrolysis of the starch (Marriott *et al.*, 1983). The carbohydrate content reduces to between 5 and 10% when ripe. The sugar content is between 0.9 to 2.0% in the green fruit but becomes more prominent in the ripe state. The titrable acidity of plantain is about twice that of sweet potato (Aurand *et al.*, 1987).

Plantains therefore have a high carbohydrate content (31 g/100 g) and low fat content (0.4 g/100 g). They are good sources of vitamins and minerals (Adeniji *et al.*, 2006), particularly iron (24 mg/kg), potassium (9.5 mg/ kg), calcium (715 mg/kg), vitamin A, ascorbic acid, thiamin, riboflavin and niacin.

As a starchy staple food, plantain supply about 1 g protein/100 g edible portion (USDA, 2009). As a healthy adult requires about 0.75 g protein kg-1 day-1 (Burton and Willis, 1976), plantain alone cannot meet adult protein needs. The fat content of plantains and bananas is very low, less than 0.5%, and so fats do not contribute much to the energy content. Although the total lipid content remains essentially unchanged during ripening, the composition of fatty acids, especially within the phospholipids fraction has been observed to change, with a decrease in their saturation (Ogazi, 1996).

The 2005 Dietary Guidelines Advisory Committee recommended increasing the dietary intake of vitamins A, C, and E, Ca, Mg, K, and fiber (USDA, 2004). Most of these nutrient requirements can be met by increasing the consumption of fruits and vegetables to 5–13 servings/day (USDA, 2004). Bananas contribute about 2.7% of the total K and fiber consumed by the average adult (USDA, 2004).

It is reported (http://answers.yahoo.com) that a serving size of 3.5 oz (99.23g) of banana supplies the following: Total fat 0.7g ,1% saturated Fat 0.1g , monounsaturated fat 0.4g polyunsaturated fat 0.2g, Sodium 1.4 mg, Potassium 486.9 mg/100g, total carbohydrate 31.1g, dietary fibre 3.5g, Sugars 16.6g,Protein 1.5g, Vitamin A 2%,Vitamin C 20%, Calcium 1%, Iron 2%. Nutritionally, bananas are considered to be good for the treatment of gastric ulcers and diarrhoea because they contain vitamin A, bananas act as a digestive aid. Due to their high content of vitamin B_6, bananas help in the reduction of stress and anxiety and the high content of carbohydrates makes bananas a very good source of energy for sports practicing people (INFO COMM, 2005).

Plantain (*Musa paradisiacea*) belongs to the family of banana and is popularly called cooking banana, since it is seldom eaten raw. It is widely grown in the southern states of Nigeria where there is adequate rainfall distribution (Oyenuga, 1972). The fruit is an excellent source of nutrient when eaten as food. It contains carbohydrate (32%), protein (1%), fat (0.02%), water (60%), some vitamins and mineral elements (Kure *et al.,* 1998). It is recommended to produce plantain flour from green fruits, since it has high starch content of about 35% on wet weight basis (Simmond, 1976).

Banana fruits are wholesome and fairly well balanced source of nutrient containing various mineral salts, vitamins and high amount of carbohydrates with a little oil and protein (Simmonds, 1966; Ketiku, 1973; Ahenkora *et al*., 1997). Banana fruits are eaten raw as desert fruits. Nutritional information is used increasingly by public agencies and agricultural industries to promote fresh produce. Consumers are looking for variety in their diets, and are aware of the health benefits of fresh fruits and vegetables. Of special interest are food sources rich in anti-oxidant vitamins (vitamins C, A, and E), calcium (Ca), magnesium (Mg), and potassium (K), (Marisa, 2006). Bananas are considered nutritive with high content of vitamins A and C but poor in vitamins B. Generally, bananas contain a considerable amount of mineral elements and could therefore serve as a good source of mineral supplement in human/animal diets.

According to Louis *et al.,* (2009) yellow plantain variety gave higher yield of starch than the white variety. The two varieties differed in the purity of starch extract; white plantain starch contained: ash (1.09%), protein (0.640%) and fat (0.276%) while yellow plantain starch contained: ash (0.95%), protein (0.325%) and fat (0.403%). The amylose content of yellow plantain starch (24.36% (apparent), 26.13% (total)) was similar to that of white plantain starch (24.24% (apparent), 26.01% (total)).

The average composition of raw banana per each 100g is as follows; water, 74.2g; energy, 92 Kcal; fat, 0.48g; protein, 1.03g; carbohydrate, 23.43g; fiber, 2.4g; potassium, 396mg; phosphorus, 20mg; iron, 0.31mg; sodium, 1mg; magnesium, 29mg; calcium, 6mg; zinc, 0.16mg; selenium, 1.1mg; vitamin C, 9.1mg; vitamin A, 81IU; vitamin B1, 0.0045mg; vitamin B2, 0.10mg; vitamin E, 0.27mg; niacin, 0.54mg (UADA, 2009).

In a research conducted by Adegboyega (2006) on the proximate chemical composition of the carbohydrate constituents and the amino acid make-up of green and ripe plantain, the quantity of total sugars considerably increased during ripening from 3.0 to 31.6% in the peel and from 1.3 to 17.3% in the pulp while starch concentration decreased from 50 to 35% and from 83 to 66% in the skin and the pulp, respectively. The skin was richer in cellulose (10%) and hemicellulose (13%) than the pulp which had 1.4% cellulose and 1.3% hemicellulose. The pulp protein was abundantly rich in arginine, aspartic acid and glutamic acid. Methionine was present in the lowest amount with tryptophan and cystine conspicuously being absent.

Plantain (*Musa spp.*) is an important dietary source of carbohydrate in the humid tropical zones of Africa, Asia and South America (Robinson, 1996). Plantain is rich in vitamins A, C and B group as well as minerals such as calcium and iron (Marriott and Lancaster, 1983; Robinson, 1996). Plantain provides between 9% and 35% of the total calories in the diets of more than 14 million people in sub-Sahara Africa. The banana is shown to contribute to the recommended daily requirements of K, Mg, Cu and B (Hardisson *et al.,* 2001).

According to USDA Nutrient Database, Nutritional value of fresh banana is presented below:

Table 2.4 Nutritional value per 100 g (3.5 oz) banana

Energy	371 kJ (89 kcal)	Manganese	0.27 mg (13%)
Carbohydrate	22.84 g	Phosphorus	22 mg (3%)
Sugar	12.23 g	Potassium	358 mg (8%)
Dietary fiber	2.6 g	Sodium	1 mg (0%)
Fat	0.33 g	Zinc	0.15 mg (2%)
Protein	1.09 g	Fluoride	2.2 μg
Thiamine (vit. B_1)	0.031 mg (3%)	Vitamin B_6	0.4 mg (31%)
Riboflavin (vit. B_2)	0.073 mg (6%)	Folatevit.(B_9)	20 μg (5%)
Niacin (vit. B_3)	0.665 mg (4%)	Choline	9.8 mg (2%)
Pantothenic acid (B_5)	0.334 mg (7%)	Iron	0.26 mg (2%)
Vitamin C	8.7 mg (10%)	Magnesium	27 mg (8%)

Source: USDA Nutrient Database

It is reported that plantain composes of mainly water, 60% and 27-31% carbohydrate with only 2-3%, protein about 1% fat (Oyenuga, 1968; Simmonds, 1970; Ketiku, 1976). The protein is deficient in methionine and tryptophan (Omole*et al.*, 1978). Comparatively, plantain is rich in phosphorus, calcium and iron with 34, 10 and 0.55mg/100g of pulp respectively (Oyenuga, 1968). Other minerals found in plantains includes, potassium, magnesium, manganese, copper, iodine, zinc, cobalt. Plantain is also a very good source of pro-vitamin A, vitamins B_6 and vitamin C (0.285, 0.65, 17.0mg/100g of pulp respectively). A significant of amount of thiamin, riboflavin, nicotinic, acid and folic acids are also reported to be found in this fruit (Marriott *et al.*, 1983). Plantain is rated as an important source of energy and provides about 104cal/100g of the pulp (Oyenuga, 1968). Of special interest are food sources rich in anti-oxidant vitamins (vitamins C, A, and E), calcium (Ca), magnesium (Mg), and potassium (K), (Marisa, 2006).

2.5 Mixed fruit juice and its nutrient content

Hussain *et al.* (2011) investigated the effect of sodium benzoate (0.1%) on apricot –apple blend juice stored at refrigerated (4^0C) temperature. The samples were; apple juice (T_1), apricot juice (T_2),75% apple and 25% apricot juice (T_3), 50% apple and 50% apricot (T_4), 25% apple and 75% apricot juice (T_5). From (T_6) to (T_{10}) above concentrations were repeated with 0.1% sodium benzoate as preservative. The samples were analyzed for parameters like ascorbic acid, acidity and pH, total soluble solids, reducing and non reducing sugars, overall acceptability and microbial study after the intervals of 15 days for the period of three months. The results showed minimum ascorbic acid content decreased in T_8 (44.61%), while maximum in T_5 (78.26%) during storage. Acidity was increased in all treatments, maximum acidity increase was recorded for T_1 (46.87%) and minimum for T_8 (31.25%). The maximum pH value decreased recorded for T_3 (25.02%) and minimum for T_7 (7.92%) during storage. The total soluble solids (TSS) (°brix) increased in juice sample, maximum in T_8 (6.97%) and minimum in T_1 (4.76%). Reducing sugar increased during storage of juices samples; the maximum value regarding reducing sugars was recorded for T_2 (8.82%) and minimum for T_6 (8.02). Non-reducing sugars decreased observed during storage, maximum value observed for T_5 (33.92%) and minimum for T_7 (17.00%). Results regarding overall acceptability showed that maximum mean score observed for T_8 and T_{10} in maintaining the sensory characteristics compared to others during storage. Minimum microbial load were observed in T_8 and maximum in T_1 to T_5(uncountable). Among all the treatments T_8 and T_{10} were most effective in maintaining the sensory and nutritional quality during storage.

Awsi Jan and Dorcus Masih (2012) studied the development of pineapple (Ananascomosus), carrot (Drocuscarota), and orange (*Citrus sinensis*) blended beverage which was stored for 21 days in pet bottles (400 ml capacity) at refrigerated temperature. They observed there were marginal changes in pH, total soluble solids, acidity, vitamin C and beta-carotene. The beta carotene content of juice was found (1583µg) to be increased with increasing the proportion of carrot juice. Estimation of vitamin C content of sample (19.50mg) showed high improvement in nutritional value of pineapple juice incorporated with carrot and orange juice. May be due to the excessive fermentation and presence of lactic acid reducing micro- organism the acidity increased (0.97-1.83) and pH of the juice decreased progressively during the storage period . The mean overall acceptability scores of more than 8 for juice samples T_2 (Pineapple:carrot:orange= 60:10:30) indicates that up to 30% orange juice incorporation indicated the commercial scope for manufacturing good and nutritious pineapple juice blended with carrot and orange juice, which will also be helpful in providing dietary requirement of beta carotene to the consumer. Heat pasteurization (90^{0}C for 25 sec) was more effective for inactivating the microbial flora. The product was recommended for children, youth and elderly persons to be used within 21 days.

Kausar *et al.* (2012) reported that addition of muskmelon to cucumber juice increases the nutritional value of the drink and also provides various health benefits to consumers. They prepared cucumber and muskmelon functional drinks by blending different ratios of cucumber and muskmelon (100:0, 90:10, 80:20, 70:30, and 60:40) and evaluated physico-chemical parameters and sensory characteristics of blended drinks for four months at 15 days of storage interval. It was observed that TSS mean values increased (15.49-16.09%) during storage. Increase in acidity (0.41-0.51%) and decrease in pH (4.89-4.82) was also observed. Reducing sugars increased from 1.9 to 2.48 percent while non-reducing sugars decreased from 9.36 to 8.70 percent. Regarding sensory attributes, maximum scores (7.51) for overall acceptability was observed in cucumber and muskmelon ratio of 90:10 followed by ratio of 100:0 (7.33) Drink prepared at 90:10 was also found as the most acceptable in maintaining the physico-chemical and organoleptic characteristics as compared to other treatments.

Mishra *et al.* (2012) developed a suitable formulation for preparation of mixed amla–grape juice and analyzed for its chemical composition and different formulations of mixed juices prepared by different proportions of amla and grape juice. The prepared mixed juices were packed in glass bottle with cork cap and stored at room temperature for a period of 2 months. The prepared mixed juices were analyzed for its keeping quality and acceptability at intervals of 10 days. They found that mixed beverage having composition 50:50 juice, 0.4% acidity, 10% sugar (of amla juice), 15.24° Brix was found to be optimum among the other formulation.

Chowdhury *et al.* (2008) developed a suitable formulation for preparation of mixed wood apple–papaya juice and studied physico-chemical composition of wood apple and papaya pulp which reveals that the moisture content 68.45% and 87.50%, total soluble solid 18.52% and 10.50%, acidity 0.52% and 0.31%, total sugar 12.10% and 8.70%, reducing sugar 4.20% and 3.40%, non-reducing sugar 7.90% and 5.30%, vitamin-C, 8.52 mg/100g and 28.75mg/100g, ash 0.75% and 0.35%, pH 5.3 and 4.22, respectively. The different formulations of mixed juices were prepared by different proportions of mixed wood apple-papaya pulp, Gum (acacia) and CMC (carboxy methyl cellulose) were used as a thickening agent and prepared mixed juice packed in glass bottle with lug cap and stored at room temperature for a period of 6 months. They analyzed for its keeping quality and acceptability at intervals of one month. It was revealed that the sedimentation was minimized by using CMC and found to be more effective for minimizing the sedimentation than gum (acacia). The prepared mixed juices were evaluated by a taste-testing panel for sensory attributes. The taste-testing panel opined that formulation T_8 (13% mixed wood apple-papaya pulp+ 12.38% sugar + 0.25% citric acid + 0.5% CMC + 0.06% KMS) had the highest overall acceptability among other treatments.

Bhardwaj and Mukherjee (2010) studied different fruit juice blends prepared as (Kinnow juice: Anola juice: Ginger juice in 100: 0: 0, 95: 5:0, 92: 5: 3 ratio and Kinnow juice: Pomegranate juice: Ginger juice in 90: 10: 0, 87: 10: 3 ratio) for improving flavour, palatability, nutritive and medicinal value. The juice blends were preserved by pasteurization (75°C for 15 min) and by addition potassium meta-bi-sulphite (750 ppm). These blends were stored in 200 ml. colourless glass bottles at room temperature (28 ± 4°C) for six months and tested at two months interval for physico-chemical, sensory evaluation and microbial population. It was observed that the kinnow juice blend with pomegranate and ginger juice in ratio of 87:10:3 was most effective juice blend for minimum changes in TSS (12.00 to 14.13°Brix), acidity (0.720 to 0.510%), ascorbic acid (18.38 to 12.90 mg/100 ml juice), and limonin (0.103 to 0.250). Sensory evaluation score was also higher in the same treatment due to better consistency and flavour up to end of storage. The juice blend ratio of Kinnow juice: Aonla juice: Ginger juice (92:5:3) was best in view of non-enzymatic browning (0.081 to 0.104) and minimum population of bacteria (4.0×10^3log cfu/ml), mould (1.5×10^3 log cfu/ml) and yeast (2.1×10^3 log cfu/ml) at end of storage (six months). It contained fair amount of vitamin 'C' (38.95 mg/100 ml juice)at six month of storage. It was also observed that the addition of ginger juice in blends improves the quality and reduces microbial growth. Further, the juice was found acceptable after six months of storage at room temperature.

Rivas *et al.* (2005) showed the effect of different Pulsed Electric Fields (PEF) intensities (25 kV/cm and 280 ms, P_1; and 25 kV/cm and 330 ms, P_2) and conventional HTST treatment (98^0C, 21s, T) on quality characteristics (pH, ^{0}Brix, total acidity, turbidity, hydroxyl methyl furfural (HMF), color, microbial flora, pectin methyl esterase (PME) activity, and sensory analysis) of blended orange and carrot juice. HMF, L (luminosity) and C (saturation or chrome) color parameters did not vary with any of the treatments. Total acidity and turbidity were slightly higher after HTST treatment. Sensory characteristics of the PEF-treated juice were more similar to the untreated juice than the HTST-pasteurized juice. Nevertheless, heat pasteurization (98^0C, 21 s) was more efficient in inactivating microbial flora and PME and preventing the growth of microbial flora and reactivation of PME at 2 and 12^0C for 10 weeks. However, the shelf-life of the PEF-treated juice was established as 4 weeks at 2^0C.This appears to be a reasonable shelf-life for this type of foodstuff.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The study was conducted in the laboratory of the Department of Food Processing and Preservation under the Faculty of Engineering and some analysis were conducted in Agri-chemistry Laboratory of Hajee Mohammad Danesh Science and Technology University, Dinajpur, during the year of 2012-2013.

3.1 Materials

Ripe banana, pineapple, papaya, orange, sugar, plastic bottles etc were collected from local market of Dinajpur. Other ingredients like preservatives (sodium benzoate) were used from the laboratory stock.

3.2 Methods

3.2.1Preparation of pineapple juice

Fresh, fully ripe and sound pineapples were used for extraction of juice. After washing, they were peeled and cores were removed. The juice was extracted from pineapple by juice extractor. The juice was collected and the residue was discarded. The extracted juice was filtered by muslin cloth. The filtrated juice then heated for 5 minutes at 65^0C and cooled immediately. Then it was stored in deep freeze at a temperature of -20°C for future use. Preparation of juice in this way was chosen from Hussain *et al. (2011).*

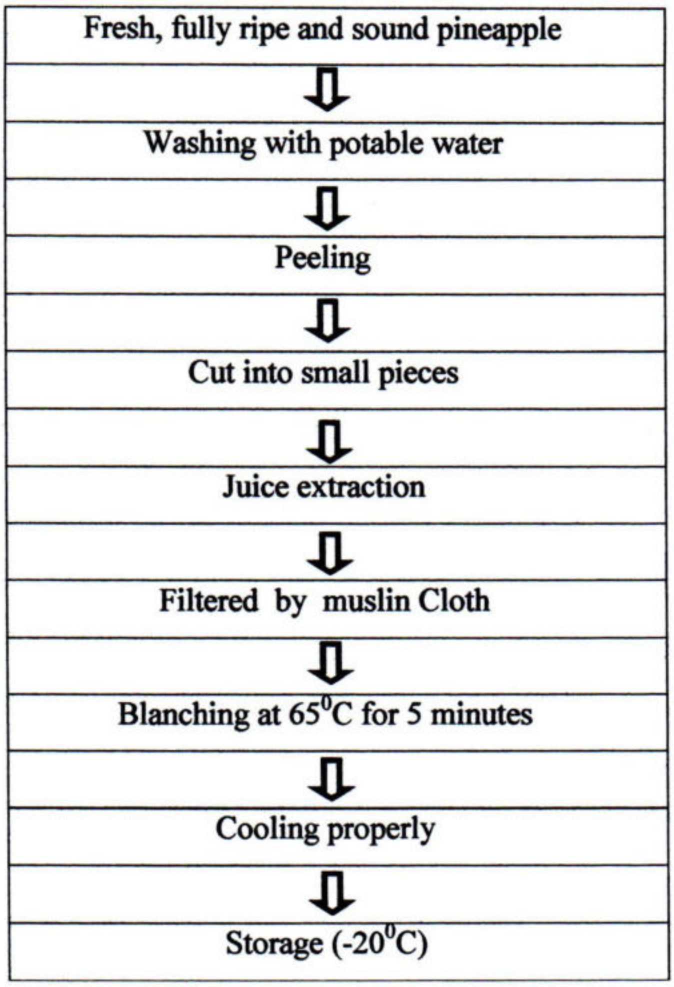

Figure 3.1: Schematic diagram for preparation of pineapple juice

3.2.2 Preparation of papaya juice

Fresh, fully mature and sound papayas were used for the extraction of juice. After washing with clean water they were peeled and cut into longitudinal section and removed the seeds. Juice was extracted from longitudinal section of papaya by juice extractor. The extracted juice was filtered by muslin cloth. The filtrated juice then heated for 5 minutes at 65^{0}C and cooled immediately. Then it was stored in a deep freeze at -20°C for future use.

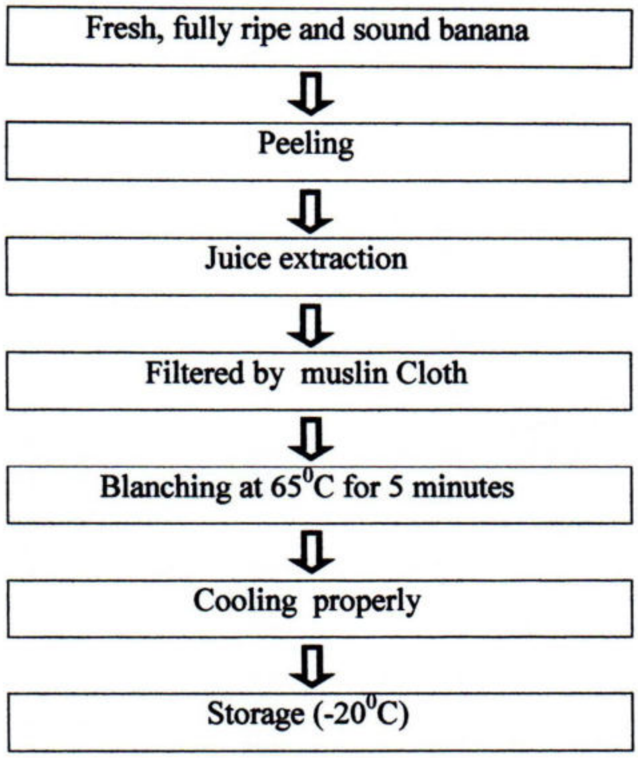

Figure 3.3: Schematic diagram for preparation of banana juice

3.2.4 Preparation of orange juice

Fresh, fully ripe and sound oranges were used for extraction of juice. After washing thoroughly with clean water, the orange were peeled by hand. The peeled orange then cut into halves. The piece of orange then transferred into juicer. The extracted juice was filtered by muslin cloth. The filtrated juice then heated for 5 minutes at 65^0C and cooled immediately. Then the juice was stored in a deep freeze at a temperature of -20°C for future use.

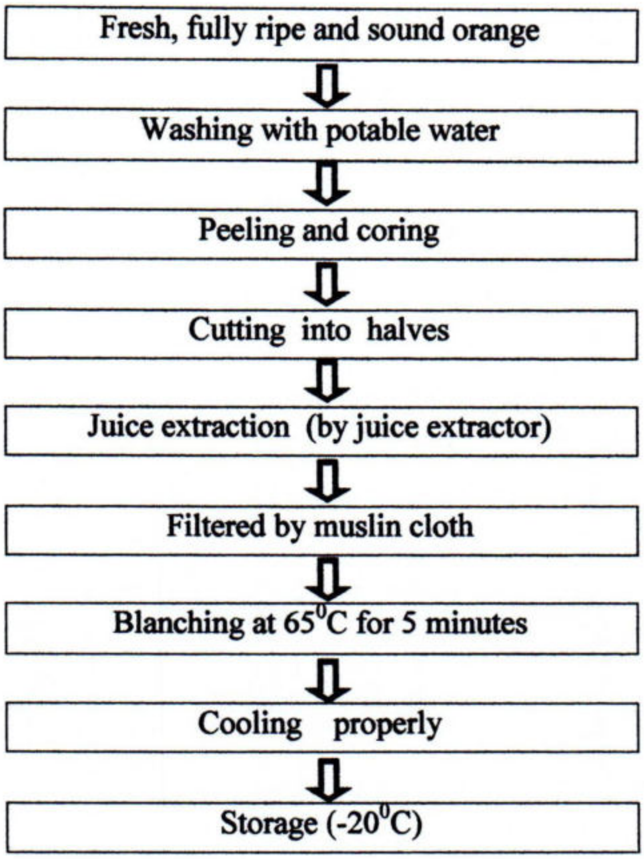

Figure 3.4: Schematic diagram for preparation of orange juice

3.3 Procedure for preparation of mixed fruit juice

3.3.1 Basic treatmentfor preparation of mixed fruit juice

Table 3.1 shows different treatments for mixed fruit juice preparation. The table shows that proportions of orange juice, banana juice, sugar and sodium benzoate remains same for all treatments, only the proportions of pineapple juice and papaya juice were changed for the treatments. The choice of juice blends ratio was selected from different published papers in national and international journals.

Table 3.1: Basic treatments for the preparation of mixed fruit juice

Ingredients	Treatments			
	T_1	T_2	T_3	T_4
Pineapple juice	50%	20%	30%	40%
Papaya juice	20%	50%	40%	30%
Orange juice	20%	20%	20%	20%
Banana juice	10%	10%	10%	10%
Sugar	10%	10%	10%	10%
Sodium benzoate	0.08%*	0.08%*	0.08%*	0.08%*

* Amount of sodium benzoate was chosen from Hussian *et al.* (2011)

3.3.2 Preparation of mixed fruit juice from papaya juice, pineapple juice, orange juice and banana juice

At first papaya juice, pineapple juice, orange juice and banana juice were taken and then properly weighed by a balance. All treatments commonly contained 10% banana juice, 20%orange juice,10% sugar and 0.08% sodium benzoate. All the ingredients were mixed thoroughly and heated at 65^0C for 5 minutes for proper mixing. The heated mixed juice then cooled. After proper cooling the mixed juice were filled into plastic bottles. This method was followed according to Hussain *et al.* (2011).

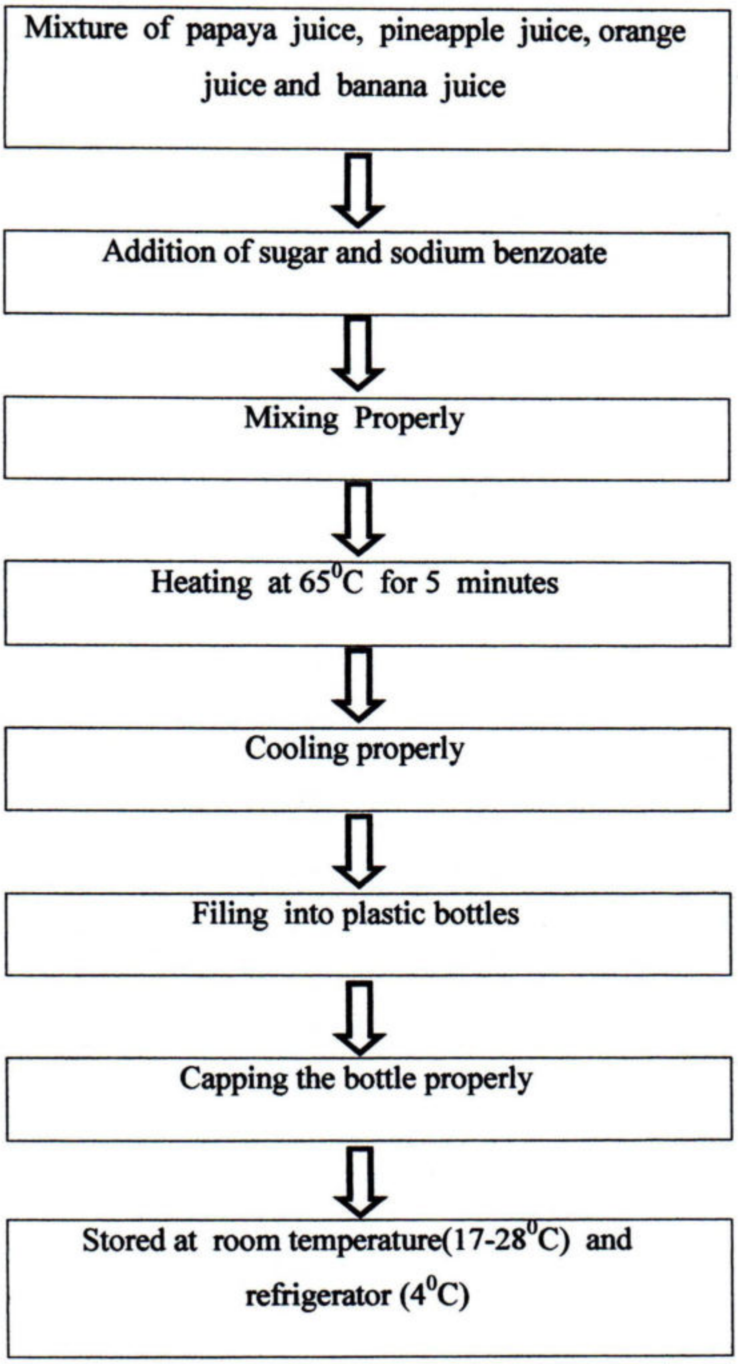

Figure 3.5: Schematic diagram for preparation of mixed fruit juice

3.4 Chemical analysis

The stored mixed fruit juice was analyzed for their mineral composition (Na, K, Ca, Mg, S, P), titratable acidity, pH, total soluble solids (TSS), reducing sugar & non-reducing sugar, total sugar and vitamin C. All the determinations were done in triplicate and the results were expressed as average value. All analysis were done at a regular interval of 30 days but mineral content was determined only the day of preparation of juice.

3.4.1 Total soluble solids (TSS)

Two drops of prepared juice was taken in a refractometer (Model no. HI 96801, HANNA INSTRUMENT,ROMANIA) plate and the total soluble solids of the juice were read directly from the refractometer.

3.4.2 Acidity

Acidity was determined following the methods of Jacob (1959) and Rangana (1977). Known volume of the fruit juice was measured in graduated cylinders and then they were transferred to beakers and sufficiently stirred. The juices were then cooled and poured back to the same graduated measuring cylinder and made up to the lost volume with distilled water.

Titration

10mljuice was taken in a 100ml conical flask. A few drops of 1% phenolphthalein solution (indicator) was added to the flask and titrated with 0.1N NaOH solution from a burette until a light pink colour appeared and persist for 15 seconds.

The titration was done for several times for accuracy. Percent titrable acidity was calculated using the following formula:

$$\% \text{ Titrable acidity} = \frac{T \times N \times V_1 \times E}{V_2 \times W \times 1000} \times 100$$

Where,

T= Titre

N= Normality

V_1=Volume made up

E= Equivalent weight of acid

V_2=Volume of sample taken for estimation

W= Weight of sample

3.4.3 Determination of pH

The pH means the negative logarithm of hydrogen ion concentration in a solution. The pH of the selected samples was determined by the conventional procedure by a pH meter.

Materials

A pH meter (Hanna instruments- ORPP), salinity-sodium tester (ISO-9001 certified company; Woonsocket, RI 02895), the supplied pH 4.0 buffer solution, distilled water and 50 ml beaker.

Using standard buffer solution of pH 4.0 for calibration the pH buffer solution was used to calibrate the pH meter.

Procedure

The electrode assembled to the pH meter was dipped into the standard buffer solution of pH 4.0 taken in a clean and dry beaker.

The fine asymmetry potential knob was adjusted to pH 4.0. The electrode assembled pH meter was dipped into the selected fruit juice, the pH was then readout washed twice with distilled water. Again it was dipped into another sample to determine the pH. The pH of all samples was determined by the procedure.

3.4.4 Vitamin-c content (ascorbic acid)

Ascorbic acid was determined following the method of Rangana (1977). The equations used for the estimation of vitamin-C were follows:

$$\text{mg of vitamin C per 100g sample} = \frac{T \times D \times V_1}{V_2 \times W} \times 100$$

Where,

T = Titre

D = Dye factor

V_1 = Volume made up

V_2 = Aliquot of extract taken for estimation

W = Weight of sample taken for estimation

1. 3% Meta phosphoric acid (HPO_3): Prepared by dissolving the sticks or pellets of HPO_3 in glass-distilled water.

2. Ascorbic acid standard: Weighted accurately 100 mg of L ascorbic acid and made up to 100 ml with 3% $HPO_3$and diluted 10 ml to 100 ml with 3% HPO_3 mg =0.3 mg of ascorbic acid.

3. Dye solution: Dissolve 50 mg of the sodium salt of 2, 6 dichlorophenol indophenol in approximately 150 ml of hot glass distilled water containing 42 mg of sodium bicarbonate. Cool and dilute with glass-distilled water to 200 ml. Store in a refrigerator and standardize every day. The dye 2, 6 Dichlorophenol-indophenols is blue in alkaline solution and reduced to light red colour by an ascorbic acid at pH range of 1-3.5

Standardization of Dye

5 ml of standard ascorbic acid solution were added to 5 ml of HPO_3. Filled a micro burette with the dye. Titrate with the dye solution to a pink colour, which should persist for 15 seconds. Determined the dye factor i.e. mg of ascorbic acid per ml of the dye, using the following formula:

$$\text{Dye factor} = \frac{0.5}{\text{Titre}}$$

Preparation of the samples

10 ml of the juice was taken and made up to 100 ml with 3% HPO_3 and then filtered. Now 10 ml of the aliquot was taken in a 150 ml conical flask. 1ml of 40% formaldehyde and 0.1N of HCI were added to it and kept for 10 minutes. This was titrated with standard dye to a pink colour (end point) when persisted for 15 seconds.

Calculation

$$\text{Mg of ascorbic acid per 100 ml} = \frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up ml}}{\text{Aliquot of extract} \times \text{weight of the sample}} \times 100$$

3.4.5 Reducing sugar

The reagents used for the estimation of reducing, non-reducing and total sugar were follows:

1. Fehling's solution (A)
2. Fehling's solution (B)
3. Methylene blue indicator
4. 45% Neutral lead acetate solution
5. 22% Potassium oxalate solution

Standardization of Fehling's solution

10 ml of both Fehling's solution A and Fehling's solution B were mixed together in a beaker. 10 ml of mixed solution was pipetted into a 250 ml conical flask and 25 ml distilled water was added to it. Standard sugar solution was taken in a burette. The conical flask containing mixed solution was heated on a hot plate. When the solution began to boil, three drops of methylene blue indicator solution was added to it. Mixed solution was titrated by standard sugar solution. The end point was indicated by decolorization of the indicator. Fehling's factor was calculated by using the following formula:

$$\text{Fehling's factor} = \frac{\text{Titre} \times 2.5}{1000}$$

Preparation of the sample

10 gm of filtered juice and 100 ml of distilled water were mixed in homogenizer and transferred to 250 ml volumetric flask. The mixture was neutralized with 0.1N NaOH and 2 ml of lead acetate solution was added and followed to stand for 10 minutes. 5 ml potassium oxalate solution was added and made to a volume of 250 ml. Then the mixture was filtered and made the dilution.

Titration for reducing sugar

10 ml of mixed Fehling's solution was taken in a conical flask and 25 ml of distilled water was added to it. Purified juice was taken in a burette. Conical flask containing mixed Fehling's solution was added to the flask when boiling started and titrated with solution taken in the burette at the same time. The end point was indicated by decolorization of indicator. Percent reducing sugar was calculated by using the following formula:

$$\% \text{ Reducing sugar} = \frac{I \times D \times 100}{T \times W \times 100}$$

Where,

I = mg of invert sugar required to reduce known volume of Fehling's solution

D = dilution factor

T = titration

W = weight of sample

3.4.6 Non-reducing sugar

50 ml purified solution was taken in conical flask 50 ml distilled water and 5 gm of citric acid were added to it. Then the conical flask was heated for 10 minutes for addition of sucrose and finally cooled. The sample was then neutralized by 0.1 N NaOH solution using phenolphthalein as indicator. The volume was made up to 100 ml with distilled water. The mixed Fehling's solution was titrated using similar procedure followed as that for reducing sugar. The percent invert sugar was then

calculated by the similar procedure as for reducing sugar from which the percent non-reducing sugar is calculated as follows:

% Non-reducing sugar =% Invert sugar-%Reducing sugar

3.4.7 Estimation of total sugar

Total sugar can be calculated as follows:

% Total sugar = %Reducing sugar +%Non-reducing sugar.

3.4.8 Determination of minerals content in mixed juice

The minerals content were determined by the method of Pearson (1976).

Sample preparation

1 ml sample with 15 ml of diacid mixture (2:1) (HNO3: $HClO_4$) was taken in a beaker and boiled until the solution become clear. Cooled and made the volume in 100 ml.

Estimation of calcium

5 ml solution mixed with 20-25 ml hot distilled water. Added 10 drops of each solution of Potassium pherocyanite, OH- amine, hydrochloride, triethanolamine and 5 ml of NaOH buffer (10%) and 5-6 drops calcon indicator. test sample was titrated against EDTA (0.01M) solution from a burette until pink color completely turned to pure blue color.

Calculation

1 ml 1 M Na2-EDTA= 40.08 mg Ca

Estimation of magnesium

5 ml solution mixed with 20-25 ml hot distilled water. Added 10 drops of each solution of Potassium pherocyanite, OH- amine hydrochloride, triethanolamine, Na tungstate and 5 ml ammonium buffer and 5-6 drops EBT (Erriochrome Black-T). Test sample was titrated against EDTA (0.01M) solution from a burette until pink color completely turned to pure blue color.

Calculation

1 ml 1 M Na_2-EDTA=2 4.305 mg ofMg

Estimation of sulphur

$MgSO_4.7H_2O$ solution (For 100 ppm sulphur)

Exactly 0.769 g $MgSO_4.7H_2O$ was taken in a 1000ml volumetric flask and added 200ml distilled water. Shaken well to dissolve. Then made the volume up to the mark with distilled water.Concentration of that solution is made 100 ppm of sulphur.

Series solution of sulphur

0 ml, 0.2 ml, 0.5 ml, 1.0 ml, 2.0ml, 4.0 ml and 6.0 ml of 100 ppm S solution was taken in several 100 ml volumetric flask and made volume upto 20 ml with distilled water. For unknown sample a blank solution was made which contained 20 ml distilled water.

Procedure

Each standard and unknown solution was taken in a 150 ml conical flask. In each conical flask 0.3 g Barium chloride, waited for 30 minutes and reading was taken in a spectrophotometer at 425 nm wavelength.

Estimation of phosphorus

KH_2PO_4 (50 ppm phosphorus)

Exactly 0.2195 g dried KH_2PO_4 was taken in a 1000ml volumetric flask and added 200ml distilled water. Then 4.86 ml conc. H_2SO_4 was added and shaken well to dissolve. Finally made the volume up to the mark with distilled water.

Stannous chloride solution

5 g Stannous chloride solution was taken in a 150 ml beaker and 10 ml HCl was added. Then heat was applied to dissolve the solution and finally made the volume 100 ml in a100 ml volumetric flask.

Sulphomolybdic acid solution

a. Exactly 25 g ammonium heptamolybdate was taken in 500 ml beaker and about 200 ml distilled water added. Warmed at 600 C until the solution become clear then cooled it.

b. 500 ml distilled water was taken in a 1000 ml beaker. 275 ml conc. H_2SO_4 was added slowly and cooled.

Sample a and sample b was added. Ammonium heptamolybdate solution was transferred in a 1000 ml volumetric flask. 200 ml H_2SO_4 solution was added slowlyand made volume upto the mark with distilled water. Now it is called Sulphomolybdic acid solution.

Series solution of phosphorus

2.5 ml, 5 ml, 10 ml, 15 ml, 20ml, 25 ml and 30 ml of 2 ppm P solution was taken in several 500 ml volumetric flask and made volume upto 20 ml to 500 ml with distilled water. For unknown sample a blank solution was made which contained 5 ml of 2 ppm solution.

Procedure

Each standard and unknown solution was taken in several 100 ml volumetric flask. 20 ml distilled water and 4 ml sulphomolybdic acid was added. 5-6 drops stannous chloride was added and made volume upto the mark with distilled water. Then absorbance reading is taken in a spectrophotometer at 660 nm wavelength within 3-4 minutes.

Estimation of potassium

Preparation of 100 ppm solution

Exactly 1.907g KCl was taken in a 1000ml volumetric flask and added 300-400 ml distilled water. Shaken well to dissolve completely.Then made the volume up to the mark with distilled water.Diluted 50 ml to 500 ml solution. Concentration of that solution is made 100 ppm of potassium.

Series solution of K

5 ml, 10 ml, 15 ml, 20ml, 25 ml, 30 ml, 40 ml, 50 ml and 60 ml of 100 ppm K solution was taken in several 100 ml volumetric flask and made volume upto the mark with distilled water.

Finally in Flame Emission Photometer showed the reading dirrectly for unknown solution. Final result got in ppm.

Estimation of sodium

Preparation of 100 ppm solution

Exactly 2.54 g NaCl was taken in a 1000ml volumetric flask and added 300-400 ml distilled water. Shaken well to dissolve completely. Then made the volume up to the mark with distilled water. Diluted 50 ml to 500 ml solution. Concentration of that solution is made 100 ppm of potassium.

Series solution of Na

5 ml, 10 ml, 15 ml, 20ml, 25 ml, 30 ml, 40 ml, 50 ml and 60 ml of 100 ppm Na solution was taken in several 100 ml volumetric flask and made volume upto the mark with distilled water.Finally in Flame Emmision Photometer, on the switch for Na estimation. For unknown solution the reading is measured dirrectly and final result got in ppm.

3.5 Sensory evaluation

Sensory evaluations of all the samples of mixed fruit juice were done by taste testing panel. The taste testing panel was made up with 10 test panelists. They were asked to evaluate colour, flavour, taste and overall acceptability by a scoring rate on a 9 point hedonic scale, means 9= Like extremely, 8= Like very much, 7= Like moderately, 6= Like slightly, 5= Neither like nor dislike, 4= Dislike slightly, 3= Dislike moderately, 2= Dislike very much and 1= Dislike extremely. The preference differences were evaluated by statistical analysis of the data for variance and consequently Duncan's Multiple Range Test (DMRT). Procedures of the Statistical Analysis System (SAS, 1985) were used for statistical analysis.

3.6 Studies on storage stability of prepared mixed fruit juice

The mixed fruit juice samples were filled into plastic bottles. The samples were stored at two temperature such as room temperature (17-28°C) and refrigerated temperature (4^{0}C).

Table 3.2: Treatments applied in the storage studies of mixed fruit juice

Sample	**Storage temperature (^{0}C)**	**Period of storage (Days)**
Mixed fruit juice	Room temperature(17-28^{0}C)	120
	Refrigerated temperature(4^{0}C)	180

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

This chapter describes the results obtained from the experiments described in Chapter 3.The experiment was conducted to determine the effective means of processing and preservation of mixed fruit juice from pineapple, papaya, orange and banana. The prepared mixed juice were analyzed for Total soluble solids (TSS), acidity, pH, vitamin-C, reducing sugar, non-reducing sugar, total sugar and mineral contents sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), sulpher (S), phosphorus (P).The compositions of mixed fruit juice on the day of preparation are given on Table 4.1 and the changes of composition during storage periods are described right after.

Table 4.1: Chemical composition of mixed fruit juice

Components/Parameters		Treatments			
		T_1	T_2	T_3	T_4
TSS (%)		11	12.3	11.5	10.8
Acidity (%)		0.34	0.36	0.30	0.33
pH		3.84	3.91	3.85	3.92
Vitamin C (mg/100g)		14.36	13.21	14.25	14.62
Reducing sugar (%)		5.41	4.94	5.21	5.30
Non-reducing sugar (%)		12.16	11.95	12.05	12.40
Total sugar (%)		17.57	16.89	17.26	17.70
Mineral constituent (mg/100g)	Sodium(Na)	44.83	45	43.33	41
	Potassium(k)	44.16	44.16	45	43.16
	Calcium(Ca)	14.97	9.05	10.37	7.67
	Magnesium(Mg)	22.22	18.27	18.49	18.33
	Sulphur (S)	16.18	18.59	18.44	18.86
	Phosphorus(P)	13.39	13.46	13.41	13.41

Table 4.2: Effects of treatment and storage on TSS (%) of mixed fruit juice

Storage Condition	Treatment	Storage periods (Days)						
		00	30	60	90	120	150	180
Storage at refrigerated temperature (4°C)	T_1	11	11.02	11.05	11.09	11.14	11.20	11.25
	T_2	12.3	12.33	12.36	12.40	12.44	12.47	12.50
	T_3	11.5	11.53	11.57	11.60	11.64	11.68	11.72
	T_4	10.8	10.81	10.83	10.86	10.89	10.93	10.97
Storage at room temperature (17-28°C)	T_1	11	11.03	11.05	11.10	11.18	ND	ND
	T_2	12.3	12.35	12.43	12.50	12.57	ND	ND
	T_3	11.5	11.54	11.57	11.61	11.65	ND	ND
	T_4	10.8	10.83	10.85	10.88	10.93	ND	ND

ND: not determined

4.2 Acidity

Acidity was calculated on the basis of titratable acidity. Titratable acidity is directly proportional and is a measure of shelf life of the product and guard against the attack of micro-organisms. It also helps to ensure some chemical changes during preparation and storage. Acidity for all the treatments at various storage periods was observed (Table 4.3). The initial acidity was 0.34%, 0.36%, 0.30% and 0.33%in treatments T_1, T_2, T_3 and T_4, respectively. After six months of storage at refrigerated temperature, the acidity changed to 0.64%, 0.55%, 0.56% and0.48% in treatments T_1, T_2, T_3 andT_4, respectively. After four months of storage at room temperature the acidity changed to0.58%, 0.51%, 0.52 % and 0.45%in treatments T_1, T_2, T_3 and T_4, respectively. Dhaliwal and Hira (2004) reported that there were minor changes in acidity i.e. from 0.39 % to 0.42% in carrot-spinach and carrot-pineapple juices during storage. Similarly, Majumdar *et al.* (2010) reported that acidity increased from 0.25-0.36 % during storage of cucumber-basil juice. Hossain *et al.* (2011) found that acidity changed from 0.39% to 0.51% in apple and appricot blend juice during storage. Mishra *et al.* (2012) reported change in acidity in amla-grape juice from 0.4% to 0.49%. Krishnaveni *et al. (*2001*)* reported marginal changes in acidity in jack fruit RTS beverage (0.25-0.27%).The increased acidity might be due to increased storage periods and the inactivation of enzymes and other reactions responsible for decrease in acidity. Other reason might be due to degradation of carbohydrates present in mixed fruit juice by the action of microorganisms. Decrease in pH-values and increase in total titratable acidity during the storage period may be due to activity of some acid-producing bacteria such as *Alicyclobacillus acidoterrestris*. (Sheikha, 2004)

Table 4.3: Effects of treatment and storage on acidity (%) of mixed fruit juice

Storage Condition	Treatment	Storage periods (Days)						
		00	30	60	90	120	150	180
Storage at refrigerated temperature (4°C)	T_1	0.34	0.38	0.42	0.48	0.52	0.58	0.64
	T_2	0.36	0.39	0.43	0.45	0.48	0.51	0.55
	T_3	0.30	0.34	0.38	0.43	0.48	0.52	0.56
	T_4	0.33	0.35	0.38	0.40	0.43	0.45	0.48
Storage at room temperature (17-28° C)	T_1	0.34	0.38	0.48	0.52	0.58	ND	ND
	T_2	0.36	0.39	0.45	0.48	0.51	ND	ND
	T_3	0.30	0.34	0.43	0.48	0.52	ND	ND
	T_4	0.33	0.35	0.40	0.43	0.45	ND	ND

ND: not determined

4.3 pH

pH is inversely proportional to the acidity of any medium. The results of present study showed significant effect of storage period and treatments on pH of all fruit juice with the increase in acidity and decrease in pH. pH for all the treatments at various storage periods was observed (Table 4.4).After six months of storage at refrigerated temperature the pH were changed from 3.84 to 3.30, 3.91 to 3.40and 3.85 to 3.00 in treatments T_1, T_2, T_3 and T_4,respectively. On the other hand, after four months of storage at room temperature the pH were changed from3.84 to 3.40, 3.91 to 3.50, 3.85 to 3.30, and 3.92 to 3.60in treatments T_1, T_2, T_3 and T_4 respectively. These finding are almost similar to earlier study by Dhaliwal and Hira (2004) where pH of fruit juices decreased (from 3.9 to 3.6) with corresponding increase in acidity during storage. Majumdar *et al.* (2010) reported that pH values decreased from 4.0-3.93 in ash gourd-mint leaves juice during six months storage. Mishra *et al.* (2012) reported that pH values decreased from 4.02 to 3.41 in amla-grape blend juice during storage. Husssain *et al.* (2011) found that pH decreased from 4.30 to 2.90 in apples and appricot blend juice during storage. High acid and low pH may be due to production of acetic acid and lactic acid during storage. The decrease in pH during storage might be due to degradation of carbohydrates present in mixed fruit juice by the action of microorganisms.

Table 4.4: Effects of treatment and storage on pH of mixed fruit juice

Storage Condition	Treatment	Storage periods (Days)						
		00	30	60	90	120	150	180
Storage at refrigerated temperature (4^{o} C)	T_1	3.84	3.80	3.75	3.70	3.65	3.40	3.30
	T_2	3.91	3.87	3.83	3.80	3.76	3.50	3.40
	T_3	3.85	3.81	3.80	3.70	3.50	3.30	3.00
	T_4	3.92	3.88	3.83	3.77	3.80	3.60	3.50
Storage at room temperature (17-28^{o} C)	T_1	3.84	3.80	3.70	3.40	3.40	ND	ND
	T_2	3.91	3.87	3.80	3.60	3.50	ND	ND
	T_3	3.85	3.81	3.70	3.50	3.30	ND	ND
	T_4	3.92	3.88	3.77	3.60	3.60	ND	ND

ND: not determined

4.4 Vitamin C

Table 4.5 showed that vitamin C or ascorbic acid reduced to significant amount in mixed juices. The initial vitamin C of different treatments were 14.36 mg/100g, 13.21 mg/100g, 14.25 mg/100g and14.62 mg/100g for T_1, T_2, T_3 and T_4, respectively. After six months of storage at refrigerated temperature, vitamin C was changed to 8.50mg/100g,7.25 mg/100g, 8.35 mg/100g and8.65 mg/100g in treatments T_1, T_2, T_3 and T_4 respectively. After four months of storage at room temperature vitamin-c were changedto8.10 mg/100g, 7.90 mg/100g, 8.00 mg/100g and 8.30mg/100gin treatments T_1, T_2, T_3 and T_4, respectively. Hussain *et al.* (2011) found that ascorbic acid changed from 5.67% to 3.64% in apple and appricot blend juice during storage. Ascorbic acid changed from 18.38 to 12.90 mg/100 ml juice in kinnow-anola-ginger blend juice (Bhardwaj and Mukherjee, 2010). Similar results were also observed by Mishra *et al.* (2011); Puranik *et al.* (2011), Singh and Roy (1984) etc. The ascorbic acid content of the juice decreased during storage with the advancement of storage period, which was probably due to the fact that ascorbic acid being sensitive to oxygen, light and heat and easily oxidized in presence of oxygen by both enzymatic and non-enzymatic catalyst (Mapson, 1970).The loss in ascorbic acid content with the progress of storage period could be attributed to rapid conversion of L-ascorbic acid into dihydro-ascorbic acid in the presence of L-ascorbic acid oxidase (Bashir and Abu-Goukh, 2002). Concerning the effect of storage temperature on fruit juice, vitamin C content significantly decreased with increasing storage temperature (Thanaa *et al.*, 2011).

Table 4.5: Effects of treatment and storage on vitamin C (mg/100g) of mixed fruit juice

Storage Condition	Treatment	Storage periods (Days)						
		00	30	60	90	120	150	180
Storage at refrigerated temperature (4^oC)	T_1	14.36	13.31	12.61	11.34	10.58	9.78	8.50
	T_2	13.21	12.25	11.37	10.35	9.57	8.70	7.25
	T_3	14.25	13.27	12.50	11.61	10.55	9.40	8.35
	T_4	14.62	13.15	12.51	11.38	10.65	9.85	8.65
Storage at room temperature ($17-28^oC$)	T_1	14.36	13.15	11.80	11.15	8.10	ND	ND
	T_2	13.21	11.97	10.50	9.20	7.90	ND	ND
	T_3	14.25	13.10	11.40	9.90	8.00	ND	ND
	T_4	14.62	13.35	11.90	10.20	8.30	ND	ND

ND: not determined

4.5 Reducing sugar

The reducing sugar content of the formulated treatments slightly increased during storage periods (Table 4.6). The initial reducing sugar content of T_1, T_2, T_3 and T_4, was observed 5.41%, 4.94%, 5.21% and5.30% respectively. After six months of storage at refrigerated temperature (4^0C), reducing sugar was changed to 5.65%, 5.15%,5.48%and 5.57% in treatments T_1, T_2, T_3 and T_4,respectively.On the other hand, after four months of storage at room temperature, the reducing sugar was changed to 5.60%, 5.10%, 5.35% and5.47% in treatments T_1, T_2, T_3 and T_4, respectively. Similar results were found that reducing sugar changed from 2.71% to 8.82% in apple and appricot blend juice during storage

(Hussain *et al.* 2011). Ewaidah (1972) reported that the reducing sugar increased due to the hydrolysis of sucrose present in fruit beverage. It was observed that for all the treatments, the reducing sugar slightly increased due to hydrolysis of sugar or may be due to the inversion of sucrose under acidic environment.

Table 4.6: Effects of treatment and storage on reducing sugar (%) of mixed fruit juice

Storage Condition	Treatment	Storage periods (Days)						
		00	30	60	90	120	150	180
Storage at refrigerated temperature (4^{o} C)	T_1	5.41	5.43	5.46	5.48	5.53	5.58	5.65
	T_2	4.94	4.94	4.95	5.02	5.05	5.08	5.15
	T_3	5.21	5.23	5.28	5.34	5.38	5.43	5.48
	T_4	5.30	5.33	5.37	5.43	5.48	5.52	5.57
Storage at room temperature (17-28^{o} C)	T_1	5.41	5.45	5.48	5.55	5.60	ND	ND
	T_2	4.94	4.96	4.99	5.04	5.10	ND	ND
	T_3	5.21	5.23	5.27	5.30	5.35	ND	ND
	T_4	5.30	5.33	5.37	5.42	5.47	ND	ND

ND: not determined

4.6 Non-reducing sugar

The non-reducing sugars decreased during storage. Table 4.7 shows that the initial non-reducing sugar of the formulated juice T_1, T_2, T_3 and T_4 were observed 12.15%, 11.95%, 12.03% and 12.38% respectively. After six months of storage at refrigerated temperature non-reducing sugar were changed to 11.93%, 11.83%, 11.87% and12.21% in treatments T_1, T_2, T_3 and $T_{4,}$ respectively. On the other hand ,after four months of storage at room temperature non-reducing sugar were changed to12.00%, 11.85%, 11.90 and12.25%in treatments T_1, T_2, T_3 and T_4 respectively .It was observed that for all the treatments, the non-reducing sugar decreased slightly. These findings are similar to those of Hussain *et al.* (2010) where non-reducing sugars decreased from 2.56 to 1.88 %t in apple-apricot blended juice. Hussain *et al.* (2011) reported decreasing in non-reducing sugars from 6.96-6.47 % in apple-apricot blended juice during storage. Majumdar *et al.* (2011) reported decreasing trend in non-reducing sugars from 8.27-7.59 % in bottle gourd-basil juice during storage of six months. The observations were also similar to Ewaidah (1972). Decreasing non-reducing sugar may be due to conversion of some reducing sugar to non-reducing sugar through the process of glucogenesis. Sucrose content of the fruit convert to glucose and fructose during the storage, results in the change in sucrose contents of juices. (Ruiz-Nieto *et al.*, 1997)

Table 4.7: Effects of treatment and storage on non-reducing sugar (%) of mixed fruit juice

Storage Condition	Treatment	Storage periods (Days)						
		00	30	60	90	120	150	180
Storage at refrigerated temperature (4^{o} C)	T_1	12.16	12.15	12.12	12.08	12.05	12.00	11.93
	T_2	11.95	11.95	11.94	11.91	11.87	11.85	11.83
	T_3	12.05	12.03	11.97	11.95	11.93	11.90	11.87
	T_4	12.40	12.38	12.35	12.32	12.28	12.25	12.21
Storage at room temperature ($17\text{-}28^{o}$ C)	T_1	12.16	12.14	12.12	12.07	12.00	ND	ND
	T_2	11.95	11.94	11.92	11.89	11.85	ND	ND
	T_3	12.05	12.03	11.97	11.93	11.90	ND	ND
	T_4	12.40	12.38	12.35	12.30	12.25	ND	ND

ND: not determined

4.7 Total sugar

A negligible change in total sugar content in the prepared juice through 4 months storage periods was observed at room temperature ($17\text{-}32^{0}$C) and six months storage at refrigerated temperature (Table 4.8). It was observed that the initial total sugar ranges found in all treatments were16.89-17.70%. After 6 months of storage at refrigerated temperature, the total sugar ranges were changed slightly and found 16.98% to 17.80% and after four months storage at room temperature the total sugar ranges were17.00% to 17.80%. Bhardwaj and Mukherjee (2010) found that total sugar increased from 7.44 to 11.11% during storage of kinnow-pomegrantae-ginger blend juice. The increased total sugar might be due to variation in percent different juice content and conversion of sugar.

Table 4.8: Effects of treatment and storage on total sugar (%) of prepared mixed fruit juice

Storage Condition	Treatment	Storage periods (Days)						
		00	30	60	90	120	150	180
Storage at refrigerated temperature(4^0C)	T_1	17.57	17.58	17.58	17.60	17.61	17.62	17.63
	T_2	16.89	16.89	16.90	16.92	16.94	16.95	16.98
	T_3	17.26	17.27	17.29	17.29	17.31	17.32	17.35
	T_4	17.70	17.71	17.72	17.75	17.76	17.77	17.80
Storage at room temperature(17-28°C)	T_1	17.57	17.60	17.62	17.65	17.70	ND	ND
	T_2	16.89	16.91	16.93	16.95	17.00	ND	ND
	T_3	17.26	17.28	17.30	17.33	17.35	ND	ND
	T_4	17.70	17.72	17.75	17.77	17.80	ND	ND

ND: not determined

4.8 Storage studies

Four treatments of mixed juice were stored at ambient temperature (17-28°C) and refrigerated temperature (4°C).The colour, flavour, TSS, acidity, pH, sugar content and visual fungal growth in the mixed juice were observed during the storage period of four months at room temperature (Table 4.9) and storage period of six months at refrigerated temperature (Table 4.10). In refrigerated temperature, there was no change in colour and flavour up to four months (120 days) of storage, then slightly changed. There was no fungal growth occurred up to four months of storage. However growth of fungus initiated right after. But here were no change in color, flavour and no fungal growth occurred up to five months (150 days) in treatment T_4. In room temperature storage, there were no change in colour and flavor and no fungal growth occurred up to three months (90 days) of storage. After three months of storage colour and flavour were changed and fungal growth occurred. But no change in colour and flavour and no fungal growth occurred up to 3.5 months (105days) in treatment T_4.

Table 4.9: Shelf life of prepared mixed fruit juice stored at room temperature

Storage period(days)	Treatment	Colour	Flovour	Visual fungal growth	Overall acceptability
00	T_1	Light yellow	Natural flavour of pineapple, papaya, banana and orange	No growth	Good
	T_2	Dark yellow			
	T_3	Dark yellow			
	T_4	Yellow			
30	T_1	No change	No off flavour	No growth	Good
	T_2	No change	No off flavour	No growth	Good
	T_3	No change	No off flavour	No growth	Good
	T_4	No change	No off flavour	No growth	Good
60	T_1	No change	No off flavour	No growth	Good
	T_2	No change	No off flavour	No growth	Good
	T_3	No change	No off flavour	No growth	Good
	T_4	No change	No off flavour	No growth	Good
90	T_1	No change	No off flavour	No growth	Good
	T_2	No change	No off flavour	No growth	Good
	T_3	No change	No off flavour	No growth	Good
	T_4	No change	No off flavour	No growth	Good
105	T_1	Change slightly	Slightly off flavour	Growth slightly	Slightly spoiled
	T_2	Change slightly	Slightly off flavour	Excessive growth	spoiled
	T_3	Change slightly	Slightly off flavour	Growth slightly	Not so good
	T_4	Change slightly	No off flavour	No growth	Good
120	T_1	Change significantly	Excessive off flavour	Excessive growth	spoiled
	T_2	Change significantly	Excessive off flavour	Excessive growth	spoiled
	T_3	Change significantly	Excessive off flavour	Excessive growth	spoiled
	T_4	Change slightly	Slightly off flavour	Growth slightly	Slightly spoiled

Figure 4.1: Mixed fruit juice sample at the day of preparation

Figure 4.2: Mixed fruit juice sample after two months of storage at refrigerated temperature

Figure 4.3: Mixed fruit juice sample after four months of storage at refrigerated temperature

Figure 4.4: Mixed fruit juice sample after six months of storage at refrigerated temperature

Figure 4.5: Mixed fruit juice sample after two months of storage at room temperature

Figure 4.6: Mixed fruit juice sample after four months of storage at room temperature

Flavour

In case of flavour preference, among the treatment ANOVA analysis (Appendix 4.1 and Appendix 4.2) showed that there was significant ($p<0.05$) difference in flavour acceptability among the fruit juice. From Appendix4.3, it is seen that sample T_4 secured highest score 8.1 for flavour and was ranked as "Like very much" and followed by the treatment T_2 and T_3 securing score 7.0 and 6.1 respectively. The treatment T_1 secured score 5.7 and rank as "Like slightly".

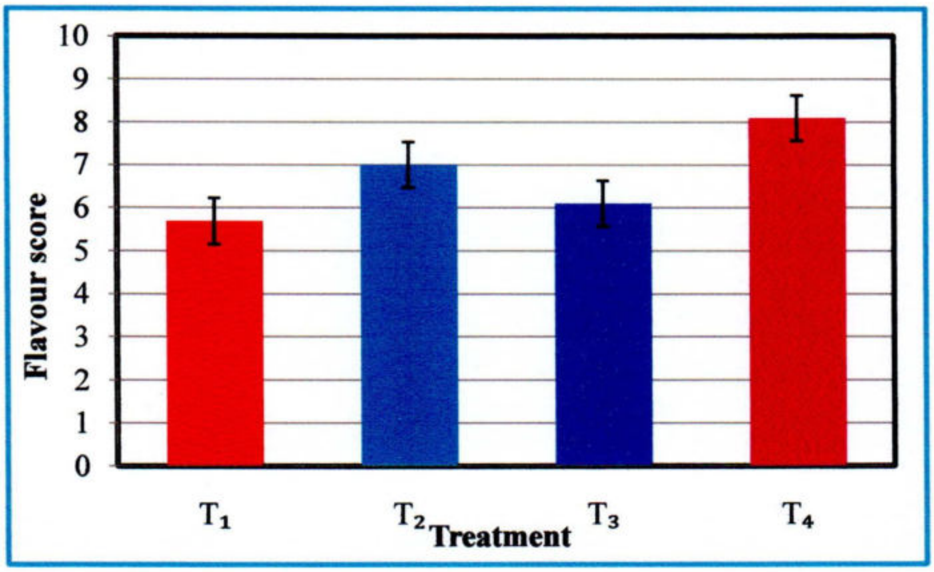

Figure 4.8: Acceptability of flavour preference of prepared mixed fruit juice based on mean score

Taste

In case of taste preference, among the sample (Appendix 5.1 and Appendix 5.2) showed that there was significant ($p<0.05$) difference in taste as shown in Appendix 5.3. Treatment T_4 secured the highest score 8.4 for taste and ranked "Like very much". Treatment T_1 secure score 6.1 and posed lowest score.

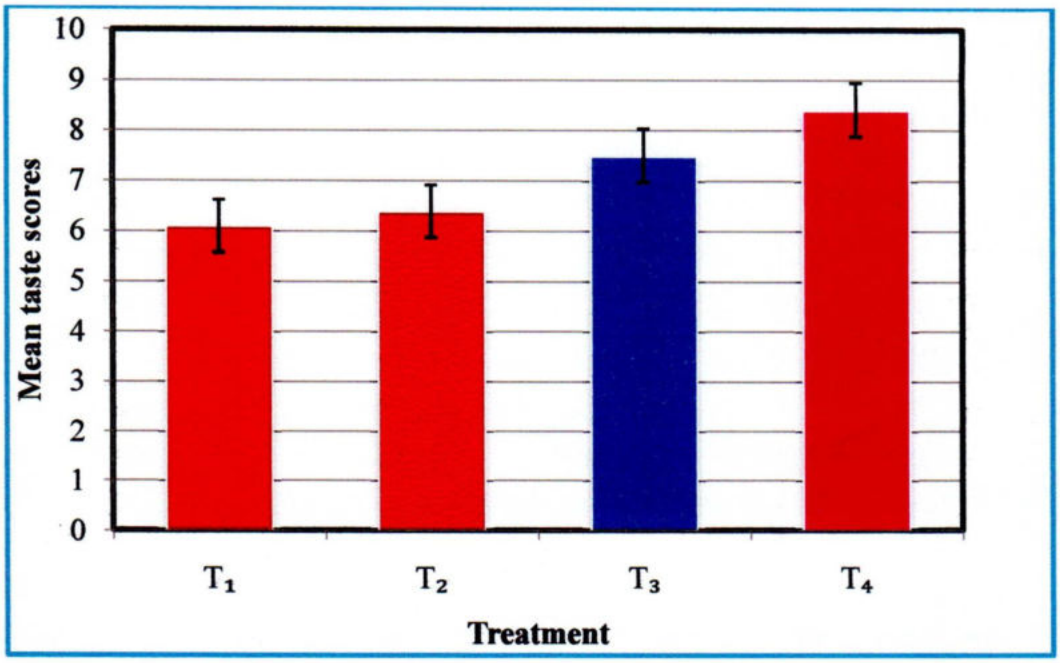

Figure4.9: Acceptability of taste preference of prepared mixed fruit juice based on mean score

Overall acceptability

It was apparent from the results of the ANOVA (Appendix 6.1 and Appendix 6.2), that there was significant ($p<0.05$) difference in overall acceptability of the treatment tested as the calculated F (31.988) greater than the tabulated F value (2.960). This indicates that so far as overall acceptability is concern the treatments were not equally acceptable. It can be seen from Appendix 6.3, that the treatment T_4 is the most acceptable product receiving 8.1 out of 9.0 compared to the other treatment and ranked as "Like very much". Treatment T_2 securing 7.1 ranked as "like moderately ". However T_3 and T_1securing 6.1 and 5.6 respectively ranked "Like slightly ".

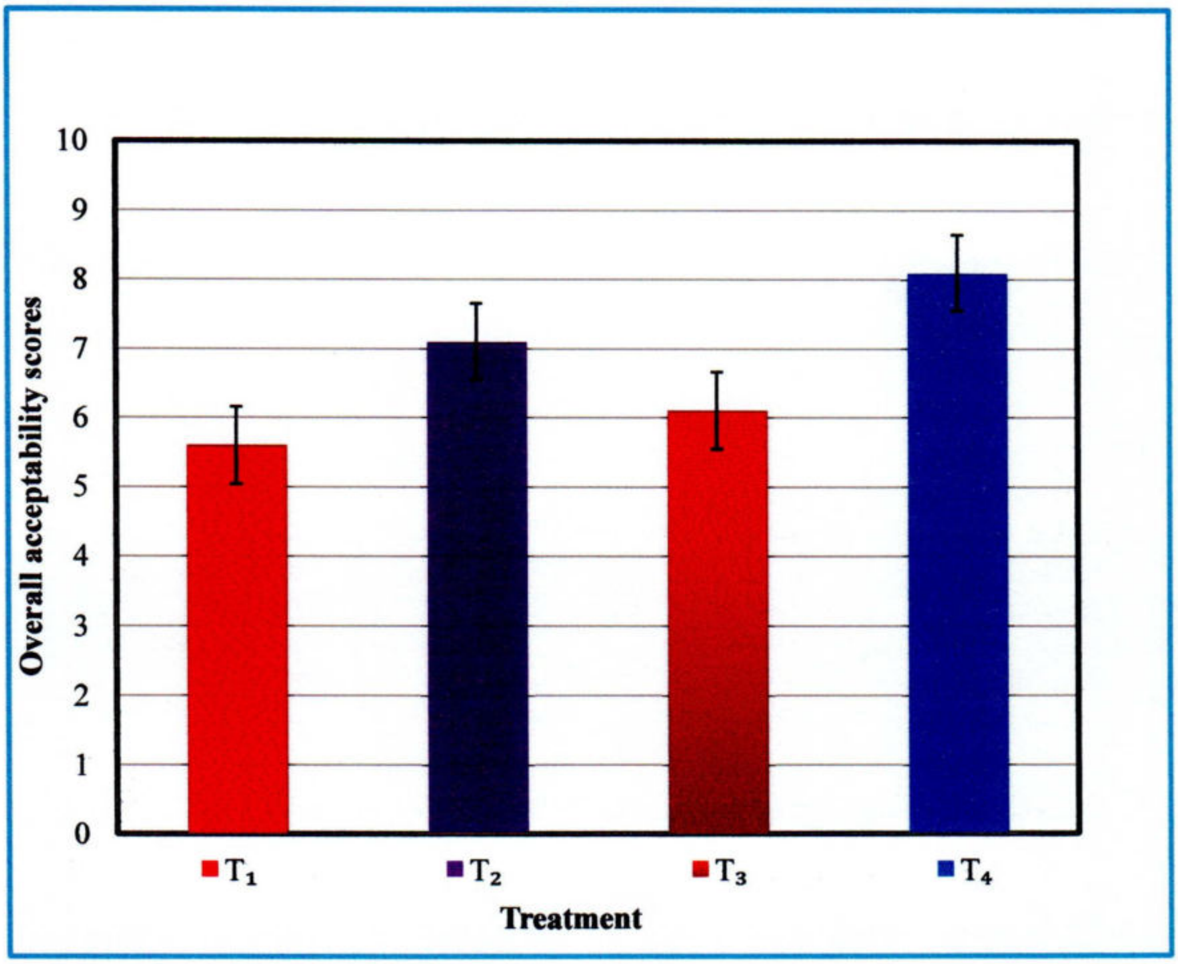

Figure 4.10: Acceptability of overall acceptability preference of prepared mixed fruit juice based on mean score

Mixed fruit juice T_4 containing 40% pineapple juice, 30% papaya juice, 20% orange juice and 10% banana juice secured the highest score for colour, flavour, taste and overall acceptability among all the treatment and was closely followed by fruit juice T_2 having 20% pineapple juice, 50% % papaya juice , 20% orange juice and 10% banana juice . So, treatment T_4 mixed fruit juice may be regarded as the best mixed juice among the four treatments.

CHAPTER V

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSIONS

The experiment was carried out in the laboratory of the Department of Food Processing and Preservation and some analysis were conducted in Agri-chemistry laboratory of Hajee Mohammad Danesh Science and Technology University, Dinajpur to explore the acceptable formulation of mixed fruit juice incorporating pineapple, papaya, orange, and banana fruits. The pineapple, papaya, orange and banana juice were prepared and used for production of mixed fruit juice using different proportions along with other ingredients in the treatments. Four treatments namely, T_1, T_2, T_3 and T_4 were considered for study. Four treatments of the mixed juice T_1, T_2, T_3 and T_4 were prepared with 20% orange juice, 10% banana juice, 10% sugar and 0.08% sodium benzoate varied with pineapple and papaya juice. Pineapple and papaya juice was varied as 50% and 20%, 20% and 50%, 30% and 40%, 40% and 30% respectively for T_1, T_2, T_3 and T_4, treatments. The shelf life and consumer`s acceptability of the prepared mixed juice were studied. The juice was stored at room temperature (17-28^0 C) and refrigerated temperature (4^0 C) for four months and six months respectively.

The mixed fruit juice was prepared as per standard procedures and was analyzed for chemical composition. The chemical analysis (TSS, acidity, pH, vitamin C, reducing sugar, non-reducing sugar, total sugar) of the prepared mixed fruit juice were done at an interval 0, 30,60,90,120,150 and 180 days but mineral content such as sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S) and phosphorus (P) was determined on the day of preparation only. On the day of preparation the **Treatment T_1** contained TSS 11%, acidity 0.34% , pH 3.84 ,vitamin C 14.36 mg/100g, reducing sugar 5.41%, non reducing sugar 12.16%, total sugar 17.57%, sodium 44.83 mg/100g, potassium 44.16 mg/100g, calcium 14.97 mg/100g, magnesium 22.22 mg/100g, sulphur16.18 mg/100g and Phosphorus 13.39 mg/100g. **Treatment T_2** contained TSS 12.3%, acidity 0.36% , pH 3.91 ,vitamin C 13.21 mg/100g, reducing sugar 4.94%,non reducing sugar 11.95% ,total sugar 16.89%, sodium 45 mg/100g, potassium 44.16 mg/100g, calcium 9.05mg/100g, magnesium 18.27mg/100g,sulpher 18.59 mg/100g and phosphorus 13.46 mg/100g **Treatment T_3** contained TSS 11.5% ,acidity 0.30% , pH 3.85 ,vitamin -C 14.25 mg/100g, reducing sugar 5.21%, non reducing sugar 12.05%, total sugar17.26%, sodium 43.33 mg/100g, potassium 45 mg/100g, calcium 10.37 mg/100g, magnesium 18.49 mg/100g, sulpher 18.44 mg/100g and phosphorus 13.41 mg/100g. **Treatment T_4** contained TSS 10.8%, acidity 0.33%, pH 3.92, vitamin C 14.62 mg/100g, reducing sugar 5.30%, non reducing sugar 12.40%, total sugar 17.70%, sodium 41 mg/100g, potassium 43.16 mg/100g, calcium 7.67 mg/100g, magnesium18.33 mg/100g, sulphur 18.86 mg/100g and phosphorus 13.41 mg/100g.

The statistical analysis of the score response by the taste-testing panelists on the sensory attributes of juice revealed that colour, flavour, taste and overall acceptability of the differently treated juice were significantly ($p<0.05$) different. It was found that colour, flavour, taste and overall acceptability of mixed fruit juice of treatment T_4 (pineapple 40%, papaya 30%, orange 20%, banana 10%) was more acceptable than other treatments. It indicates that higher proportions of pineapple juice than papaya juice but not far difference ratio between them rendered the most acceptable mixed fruit juice.

All of the treatments of the juice possessed the mixed natural colour, flavour and taste of pineapple, papaya, orange and banana fruits and fading of colour and off flavour was observed at the end of storage period. It was observed that acidity, reducing sugar and total sugar was always higher but non- reducing sugar, pH and vitamin-C was lower at room temperature than in refrigerator temperature. The study demonstrated that the mixed juice was effective to maintain the edible quality during three months of storage at room temperature (17-28^{0}C) and five months of storage at refrigerated temperature (4^{0}C). The experimental finding concludes that, it is possible to prepare mixed juice from cheap and available fruits in Bangladesh those cannot be preserved for long time. The mixed fruit juice with a simple preservatives sodium benzoate (0.08%) and packed in plastic bottles can be easily preserved for three months in room temperature and for up to five months in refrigerated temperature without any spoilage and loss of taste, colour and flavour.

The fruit juice preparation is a simple technique for preservation and suitable for cottage and small scale enterprises. Inadequate and improper processing and preservation facilities for many important fruits like pineapple, papaya and banana are responsible for increasing post harvest losses of these commodities. Proper utilization and value addition of these important fruits through preparation of mixed fruit juice may help encourage development of cottage and small scale industries in the country.

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APPENDICES

APPENDICES

Appendix 3.1 Rating score for colour of mixed fruit juice from pineapple, papaya, orange and banana

Panelist No.	Treatment No.				Total
	T_1	T_2	T_3	T_4	
1	6	7	5	8	26
2	4	6	5	7	22
3	6	7	6	8	27
4	5	6	5	7	23
5	6	7	5	8	26
6	5	6	5	7	23
7	6	7	5	8	26
8	6	8	7	9	30
9	6	7	5	7	25
10	5	7	6	8	26
Total	55	68	54	77	254
Mean	5.5	6.8	5.4	7.7	

Hedonic scale used: 9 = Like extremely; 8 = Like very much; 7 = Like moderately; 6 = Like slightly; 5 = Neither like nor dislike; 4 = Dislike slightly; 3 = Dislike moderately; 2 = Dislike very much; 1 = Dislike extremely.

Appendix 3.2 Analysis of variance (ANOVA) for colour

Source	Degree of freedom	Sum of squares	Mean squares	F- value	
				Calculated	Tabulated
Judges	9	12.1	1.344	8.067	2.250
Products	3	36.5	12.167	73	2.960
Error	27	4.5	0.167		
Total	39	53.1			

Appendix 3.3 Duncan's Multiple Range Test (DMRT) for colour LSD value =0.375, P<0.05

Treatment code	Original order of means	Treatment Code	Ranked order of means
T_1	5.5^c	T_4	7.7^a
T_2	6.8^b	T_2	6.8^b
T_3	5.4^c	T_1	5.5^c
T_4	7.7^a	T_3	5.4^c

Appendix 4.1 Rating score for flavour of mixed fruit juice from pineapple, papaya, orange and banana

Panelist No.	Treatment No.				Total
	T_1	T_2	T_3	T_4	
1	7	8	6	9	30
2	6	7	5	8	26
3	5	7	6	7	25
4	5	6	7	8	26
5	7	8	6	9	30
6	6	6	5	7	24
7	5	6	7	8	26
8	6	8	7	9	30
9	6	8	7	9	30
10	4	6	5	7	22
Total	57	70	61	81	269
Mean	5.7	7	6.1	8.1	

Hedonic scale used: 9 = Like extremely; 8 = Like very much; 7 = Like moderately; 6 = Like slightly; 5 = Neither like nor dislike; 4 = Dislike slightly; 3 = Dislike moderately; 2 = Dislike very much; 1 = Dislike extremely.

Appendix 4.2 Analysis of variance (ANOVA) for flavour

Source	Degree of freedom	Sum of squares	Mean squares	F- value	
				Calculated	Tabulated
Judges	9	19.225	2.136	5.402	2.250
Products	3	34.075	11.358	28.728	2.960
Error	27	10.675	0.395		
Total	39	63.975			

Appendix 4.3 Duncan's Multiple Range Test (DMRT) for flavour LSD value =0.577, P<0.05

Treatment Code	Original order of means	Treatment Code	Ranked order of means
T_1	5.7^c	T_4	8.1^a
T_2	7^b	T_2	7^b
T_3	6.1^c	T_3	6.1^c
T_4	8.1^a	T_1	5.7^c

Appendix 5.1 Rating score for taste of mixed fruit juice from pineapple, papaya, orange and banana

Panelist No.	Treatment No.				Total
	T_1	T_2	T_3	T_4	
1	6	7	8	9	30
2	8	6	7	8	29
3	7	6	8	9	30
4	6	7	8	8	29
5	6	7	7	8	28
6	7	6	8	9	30
7	5	6	7	8	26
8	4	5	6	7	22
9	6	7	8	9	30
10	6	7	8	9	30
Total	61	64	75	84	284
Mean	6.1	6.4	7.5	8.4	

Hedonic scale used: 9 = Like extremely; 8 = Like very much; 7 = Like moderately; 6 = Like slightly; 5 = Neither like nor dislike; 4 = Dislike slightly; 3 = Dislike moderately; 2 = Dislike very much; 1 = Dislike extremely.

Appendix 5.2 Analysis of variance (ANOVA) for taste

Source	Degree of freedom	Sum of squares	Mean squares	F- value	
				Calculated	Tabulated
Judges	9	15.1	1.678	4.978	2.250
Products	3	33.4	11.133	33.033	2.960
Error	27	9.1	0.337		
Total	39	57.6			

Appendix 5.3 Duncan's Multiple Range Test (DMRT) for taste LSD value =0.532, P<0.05

Treatment Code	Original order of means	Treatment Code	Ranked order of means
T_1	6.1^c	T_4	8.4^a
T_2	6.4^c	T_3	7.5^b
T_3	7.5^b	T_2	6.4^c
T_4	8.4^a	T_1	6.1^c

Appendix 6.1 Rating score for overall acceptability of mixed fruit juice from pineapple, papaya, orange and banana

Panelist No.	Treatment No.				Total
	T_1	T_2	T_3	T_4	
1	5	7	6	8	26
2	4	6	5	7	22
3	6	8	7	9	30
4	6	8	5	8	27
5	5	6	5	7	23
6	7	6	5	8	26
7	5	7	6	8	26
8	6	8	7	9	30
9	6	7	8	8	29
10	6	8	7	9	30
Total	56	71	61	81	269
Mean	5.6	7.1	6.1	8.1	

Hedonic scale used: 9 = Like extremely; 8 = Like very much; 7 = Like moderately; 6 = Like slightly; 5 = Neither like nor dislike; 4 = Dislike slightly; 3 = Dislike moderately; 2 = Dislike very much; 1 = Dislike extremely.

Appendix 6.2 Analysis of variance (ANOVA) for overall acceptability

Source	Degree of freedom	Sum of squares	Mean squares	F- value	
				Calculated	Tabulated
Judges	9	18.725	2.081	5.414	2.250
Products	3	36.875	12.292	31.988	2.960
Error	27	10.375	0.384		
Total	39	65.975			

Appendix 6.3 Duncan's Multiple Range Test (DMRT) for overall acceptability LSD value =0.569, P<0.05

Treatment Code	Original order of means	Treatment Code	Ranked order of means
T_1	5.6^c	T_4	8.1^a
T_2	7.1^b	T_2	7.1^b
T_3	6.1^c	T_3	6.1^c
T_4	8.1^a	T_1	5.6^c

Appendix 7

TASTE TESTING OF MIXED FRUIT JUICE

Name of Tester: ………………………….. Date: ………………………

Please taste these samples and check how much you like or dislike each one on four sensory attributes such as Colour, Flavour, Taste and Overall Acceptability. Use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample. Please give a reason for this attitude. Remember you are the only one who can tell what you like. An honest expression of your personal feeling will help me.

HEDONIC	COLOUR				FLAVOUR				TASTE				OVERALL ACCEPTABILITY			
	Treatment				Treatment				Treatment				Treatment			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Like extremely																
Like very much																
Like moderately																
Like Slightly																
Neither like nor dislike																
Dislike slightly																
Dislike moderately																
Dislike very much																
Dislike extremely																

Extra comments on each sample if any:

N.B. Overall Evaluation: Hedonic scale used: 9 = like extremely; 8 = like very much; 7 = like moderately; 6 = like slightly; 5 = neither like nor dislike; 4 = dislike slightly; 3 = dislike moderately; 2 = dislike very much; 1 = dislike extremely.

Signature of Judge