STUDIES ON PHYSICO-CHEMICAL AND MICROBIOLOGICAL QUALITIES OF SOME SELECTED BRAND OF MANGO FRUIT JUICE OF BANGLADESH

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DECLARATION

This is to declare that the research work embodying the results reported in this thesis entitled "STUDIES ON PHYSICO-CHEMICAL AND MICROBIOLOGICAL QUALITIES OF SOME SELECTED BRAND OF MANGO FRUIT JUICE OF BANGLADESH" submitted by Md. Ruhul Amin, has been carried out under the joint supervision and able guidance of Professor Dr. Naiyyum Choudhury, Coordinator, Biotechnology and Microbiology program, and Associate Professor Dr. M. Mahboob Hossain, Microbiology program, BRAC University in partial fulfillment of MS in Biotechnology, at BRAC University, Dhaka. It is further declared that the research work presented here is original and has not been submitted anywhere else for any degree or diploma.

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DEDICATED TO

MY BELOVED PARENTS

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ABSTRACT

Among many local fruits, mango is very delicious and nutritious for our health. Many people want to take test of mango around the year. Consumption of mango juice continues to increase in Bangladesh because of its taste, nutrition value and meet the needs of busier lifestyles. Mango juices have always been considered as delicious, nutritious, healthful popular drink, but processed mango juice may not always be safe due to chemical and microbiological hazard. Determination of physico-chemical and microbiological qualities of some packed mango juice of Bangladesh will help consumer to know present scenario or condition.

Six samples were collected from different manufacturing companies which were commercially packed juices available in the markets. In this study, carbohydrate profiles of juices were determined using HPLC, crude protein content of the samples were determined using the Kjeldahl method and other parameters were determined using standard AOAC methods (Horwitz, 2003). Standard culture techniques were followed to assess total viable count (TVC), fecal coliform and *Escherichia coli*. In this study, the pH of the fruit juices varied from 3.55 to 3.80. The highest quantity of monosaccharide (58.88%) was recorded in ACME mango juices while the lowest in Homemade and Mangolee juices (5.648% and 9.867% respectively). Maximum content of acidity 0.24% (as citric acid) was recorded and minimum was (0.21%). The TSS content of all samples varied from 19% to 12 %. The highest quantity of reducing sugar (6.87%) and the lowest (3.62%) was recorded in different company's mango juices. Most of the mango juices are low in protein content and very low in fat content which is negligible. Total viable count of different types of fruit juices varied from 1×10^3 - 3×10^3cfu/ml. No *E. coli* and fecal coliform were detected in these juices.

This work has shown that the nutritional quality varied from company to company juices. From the data presented in the current study, it can be concluded that the locally available mango juices contain safe levels of nutritional and microbial elements for human consumption, but not in very good position.

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INTRODUCTION

1.1 Significance of the study:

There are many local fruits grow in Bangladesh, but mango is the most popular because it is very delicious and nutritious for our health. We can enjoy fresh mango in one season for some months or days in the whole year. Many people want to take taste of mango around the year, so the way of mango juice is becoming very popular day by day to all. As a result many fruit juice company was built up in Bangladesh. Mango juices are considered as the most preferred non-alcoholic beverage worldwide to all age groups (Rahman et al., 2010). Each cup of mango juice adds a more than feasible source of both vitamins A and C to any diet. Without added sugars, pure mango juice contains about 30 grams of sugary carbohydrates per cup. Although mangos contain moderate to high levels of carbohydrates cause little fluctuation in blood-sugar levels. This process lets the body maintain appetite and metabolic processes much easier. None of the calories found in mangos comes from fat, making the juice easily digestible, energy providing drink. The important nutrient from mango juice helps maintain healthy eye function and growth, gene transcribing and the maintenance of healthy skin tissue. Mango juice also provides a good source of calcium and iron. Iron helps the body eliminate free radicals, while calcium assists with the formation of healthy teeth and bones. Phytochemicals & antioxidants in Mango Juice are so beneficial to prevent many diseases along with cancer. A study done in New Zealand tried to determine why cancer rates were lower in the native Maori people compared to New Zealanders of European descent. The Maori eat 25 foods in greater quantities, six showed strong anti-cancer effects. They included watercress, papaya, taro leaves, green banana and mango, all of which contained carotenoids and flavonoids which might be cause of lower rate of cancer (Percival et al., 2006). Most of Health Benefits of Mango Juice are: Provide antioxidant, protects against arteriosclerosis, and reduces risk of cancer.

Processed or packed mango juice may not always be safe due to chemical hazard in used ingredients and heavy load of microbes. In a situation storage of juice is important too. Sometimes producer do not maintain proper parameter of quality like pH, acidity, specific carbohydrate, total fat content, total soluble solids, and aseptic

condition also. So the juice supplier cannot maintain their declared nutrition value and taste or deliciousness. Producers apply chemical preservatives that can inhibit all types of microbial growth. It is well known that the manufacturers commonly use sulphur dioxide (SO_2) and sodium benzoate as preservatives in processed fruit juices. sulphurdioxide and benzoate can significantly damage the vegetative cells. The sulphites inhibit yeasts, moulds and bacteria are most effective as inhibitors of browning in foods. They also reduce the number of growth of microbes and increase the shelf life of juice products (Rahman et al., 2010). Those preservatives are very harmful for health.

Pasteurization, refrigeration and sterilization are popular methods for preservation of mango juice used to destroy pathogenic microorganisms and to preserve the color, aroma and chemical quality. The major ingredients of the juice are water, sugar, glucose, natural fruit pulp; sodium CMC may also carry some microbial contaminants. Food-borne illness is commonly caused by some chemicals and certain bacteria or their toxins, which are poisonous proteins produced by these bacteria. The most common food borne pathogenic bacteria are *Bacillus cereus, Clostridium botulinum, Escherichia coli, Shigella spp., Salmonella spp., Vibrio parahaemolyticus, Staphylococcus aureus, Campylobacter jejuni, Streptococcus pyogenes, Mycobacterium bovis, Listeria monocytogenes* etc (Rahman et al., 2010). In pregnant women, the fetus is heavily infected, leading to spontaneous abortion, stillbirths, or sepsis in infancy. Contamination of juices with pathogenic microorganisms such as *E. coli* and *Salmonella spp.* has caused numerous illness and even some fatalities. (Durgesh et al., 2008)

Consumption of fruits juice continues to increase in Bangladesh because of its taste, nutrition value and meeting the needs of busier lifestyles. In addition to their increasing popularity in consumption juice has also become a source of increasing food borne disease.

When the chemical quality parameter is not maintained and pathogenic microorganisms involved with our food such as mango juice, can lead to food poisoning occurrences. The presence of some indicator organisms in mango juice is responsible for causing food-borne diseases and even death. So, from public health point of view, it is quite important to know the physico-chemical and microbiological quality of the fruit juices available in the market.

1.2 OBJECTIVES OF THE PRESENT STUDY:

The purposes of this study:

In recent years, the increasing consumer awareness has emphasized the need for chemically and microbiologically safe food. Our food supply can contain microorganisms in interaction with the microbial and chemical hazard because the human food supply consists basically of plants and animals or products derived from them. Primary objective of this study was to assess the physico-chemical and microbiological quality of commercially packed available juices collected from different locations of Dhaka city.

1.3 RESEARCH HYPOTHESIS

Mango juices have always been considered as delicious, nutritious, healthful popular drink, but processed mango juice may not always be safe due to chemical and microbiological hazard. Determination of Physico-chemical and microbiological qualities of some mango juice of Bangladesh will help consumer to know present scenario or condition of Bangladeshi processed juice.

CHAPTER: 02

LITERATURE REVIEW

There is no doubt that fruit juice has been a staple beverage for many decades. Fruit juice is popular for its many nutrients. Fruit juice has vitamin C and perhaps vitamin A, potassium and calcium, depending on the brand and product. A glass of fruit juice is a great way to start the day. It takes a couple minutes to eat a 60-calorie orange but only a couple seconds to guzzle down a 110-calorie glass of orange juice. Fruit juice, in particular, is a highly concentrated source of fruit sugar. This can raise blood sugar quickly. The vitamin C in some juices is largely responsible for the health of collagen, a protein that helps maintain healthy skin and cartilage. Vitamin C also aids in joint flexibility and maintenance of healthy hair. Lastly, vitamin C may help prevent cataracts and macular degeneration. B vitamins like foliate may reduce your risk of cardiovascular disease. Some juices are fortified with the mineral calcium, which helps keep your bones healthy. Juices that contain calcium may be able to help lower blood pressure, reduce anyone's risk of cardiovascular disease and prevent osteoporosis. Calcium may also help alleviate PMS ramping. Juices rich in potassium may further help prevent osteoporosis by forming osteocalcin, a protein found only in the bone. Drinking potassium-rich juice can also help keep blood pressure low. Fruit juices contain fiber, pulp and skin. These are valuable for health. Fiber: The body needs fiber to function well fiber is important if anyone is making efforts to stay fit and avoid excess weight. Pulp: When we juice" fresh fruit, we remove the pulp, which is valuable because of its flavonoids. Researchers find that flavonoids may decrease anyone's risk of cancer. Skin: Nutrition in fruit resides not only in the flesh but in the skin also. Grapes, apples and pears are good examples of fruit with health-bearing skins. The skin of grapes reduces risk of cancer. (Campbell, 2012)

Apple Juice: apples contain the substance pectin which can effectively absorb cholesterol in the body. Apple juice can also help to relieve from chronic pain like arthritis and rheumatic symptoms. This juice can effectively cleanse digestive system and even help treat bowel movement disorders like diarrhea and constipation. Orange Juice: pure orange juice is bursting with vitamin C. orange juice can help in strengthening anyone's immune system at the time of suffering in colds, infections and other viruses. Fruit juice also helps

to soothe the nervous system. Orange juice is also a good remedy for digestive problems. Grape Juice: grape juice is a great beverage that can boost anyone's energy. This juice is also rich in antioxidants which can help anyone fight against wrinkles, age spots and other signs of aging. Gout, liver problems and allergies are some of the ailments that grape juice can also help with. Grapefruit Juice: grapefruits have great detoxifying properties. This juice makes a great antiseptic which can cleanse blood and rid anyone's system of toxic build-up. Pineapple Juice: Rich in vitamins C and B. Pineapple juice is a great antidote for the common cold and sore throat. Pineapple juice can also help with digestive problems. This juice is also a rich source of bromelain, an enzyme which effectively fights inflammation.

Among many fruit juice, mango juice is very popular in Bangladesh because it is very delicious and nutritious for our health. Each cup of mango juice adds a more than feasible source of both vitamins A and C to any diet. Without added sugars, pure mango juice contains about 30 grams of sugary carbohydrates per cup. Most of Health Benefits of Mango Juice are: Provide Antioxidant, Protects against Arteriosclerosis, Reduces Risk of Cancer. Mango Juice is an excellent source of Vitamin C, Vitamin A (beta-carotene), potassium, niacin, quercetin, gallic acid. Phytochemicals & antioxidants in Mango Juice is so beneficial to prevent many diseases along with Cancer. Mango juice also provides a good source of calcium and iron. Iron helps the body eliminate free radicals, while calcium assists with the formation of healthy teeth and bones. Mangoes are perfect to replenish salts, vitamins and energy after physical exercise. The famous Unani physician Hakeen Hashmi teaches that mangoes strengthens and invigorates the nerve tissues in muscles, heart and brain and other parts of the body. The enzymes of the Mango, such as magneferin, katechol oxidase and lactase, clean the bowel of the "filth" within and are an ideal antidote for all toxic effects inside the body. They also provide sufficient resistance to fight any germs and afflictions.

Hartwell claims in his book "Plants Against Cancer," that the phenols in mangoes, such as quercetin, isoquercitrin, astragalin, fisetin, gallic acid and methylgallat, as well as the abundant enzymes, have healing and cancer-preventing capacities. In gall bladder cancer a protective effect of mango consumes had been proven (Pandey, 2010).

Mangos contain also a lot of tryptophan, the precursor of the "happiness-hormone" serotonin. (Campbell, 2012)

pap made of Finger millet (Eleusine coracana), kidney beans (Phaseolus vulgaris), peanuts (Arachis hypogoea), and mango (Mangifera indica) has been proven to be a good complementary food for children of weaning age. It meets the vitamin and energy requirements of children of 6-24 months of age at three servings a day and at the FAO average breast-feeding frequency (Mwikya, 2010)

Freshly extracted fruit juices have always been considered as healthful drink, but processed mango juice may not always be safe due to heavy load of microbes and chemical hazard. In a situation where storage of juice is indispensable may be for a short duration, then simple processing techniques is essential for improving the keeping quality to ensure it's safety, nutritive quality and acceptability.

Figure 001, mango juice photograph

2.1 Fundamental raw material of Mango juice, Mango in Bangladesh:

Mango has been cultivated in this sub-continent from 4000 years ago (Candole, 1984). The wild mangoes particularly, *M. sylvaticaRoxb*., is still found in the Chittagong Hill tracts of Bangladesh. The mango varieties on the contrary belong to only *M. indica L.*, which are predominantly mono embryonic in nature. In Bangladesh, only a small percentage of mango trees are grafted plants. The grafted mango plants are concentrated in a few places in the North-Western region of Bangladesh and mangoes of unknown varieties (seedling mangoes) are grown in the southern and other parts of Bangladesh (Bhuiyan, 1995). In Bangladesh 90% of existing mango plants are raised from seeds (Hossain, 1994) and for the lack of suitable variety Bangladesh now is in a decreasing trend in production (Sardar et al., 1995). Bangladesh climate and soils are suitable for wide range of horticultural crops cultivation. High and medium high lands are mostly suitable for fruits and vegetables production. Mango (*Mangiferaindica L*) is one of the important fruits of Bangladesh. Mango grows in all parts of Bangladesh, but juicy mangoes with good tastes and quality are mostly grown in the northwestern Rajshahi region, especially in Chapainawabganj district. Chapainawabganj, known as the "mango capital" of Bangladesh, has a long tradition of producing around 150 varieties of sweet mangoes. Media reports quoting agriculture department sources said, there are almost two million mango trees on 23 thousand hectres of land in Chapainawabganj district. The district alone with around 50,000 mango groves produced a total of 172 thousand tons of

mango in 2010. (Saleque, 2013). Rajshahi zone especially Dinajpur, ChapaiNwabgonj and Meherpur districts is famous for mango production. The area under mango cultivation during 2003-04 was about 50991 hectares with a total production of about 242605 metric tons (BBS, 2004). In 2009-2010 mango was cultivated in 79066 acres and produced 1047849 M. tons (Agriculture Statistics, B.B.S. 2013). Fruits of the varieties were harvested between 24 May and 10 July. The mangoes of Bangladesh belong mainly to two groups. The elite mango varieties propagated through grafting and other vegetative means Locally known as"KalamerAam" and fruits are born by seedling trees locally known as "GutiAam". Some of the common varieties grown in Bangladesh are listed below:

Early variety:

The varieties which can be harvested within mid-May to mid-June. e.g, Gopalbhog, Himsagar, Khirsapat, Brindabani and BARI Aam-1.

Mid-season varieties:

The varieties which can be harvested within mid- June to late-June, e g. Langra, Misribhog, Krisanbhog, Kohitoor, Lakhanbhog, Daseri and BARI Aam 2-3.

Late varieties:

The varieties which can be harvested within July to mid August , e.g. Fazli, Ashwina, Kuapahari, Mohanbhog, Chausa and BARI Aam 4.

Regular bearing varieties: Neelum, Mollika (Neelum Daseri), Amropali/BARI Aam-3 (Daseri Neelum). (Firoj Ali, 2012)

Beside those some varieties are popular like urjapuri, Ashwina, Kalia, Deori, Fazli, Langra, LotaBombai, Bombai, Khirsapat, RaniPassand. Khisanbogh, LataBombai, Foria, Bombai, Kohitoor, Laksmanbhog, Mohanbhog, Misribhog etc.

Among the top ten mango producing countries Mexico, Philippines, Pakistan, Brazil and India share 36.9 per cent of the total global export. Interestingly, the remaining five top mango producing countries-China, Thailand, Indonesia, Bangladesh and Nigeria fail to occupy any position among the top ten mango exporters. India despite being the highest producer remains at the fifth position with a share of only 5.20 per cent of global exports. Mexico is the top exporter contributing 10.30 percent. Netherlands, Peru, Guatemala,

France and Haiti show their brilliance in the global exports despite the fact that they are not in the top ten producers' list (Saleque, 2013).

Export of fresh fruits and vegetables from Bangladesh significantly increased from 50.71 million $ in Financial Year 2008-09 to 182.23 million $ in Financial Year 2012-13.

Table 1: Export growth of Fresh Fruits and Vegetables

Fiscal Year	Quantity Exported (MT)	Export Value (in Million US$)	Export Growth (%)
2008-09	24670	50.71	-
2009-10	29370	64.21	(+) 26.62
2010-11	48428	109.41	(+) 70.39
2011-12	59573*	134.59	(+) 23.01
2012-13	80660*	182.23	(+) 35.39

Growth of export value from the last five years has significantly increased. The export value during Financial Year 2012-13 increased to 35.39% over the previous Financial Year 2011-12. Some Food industries of Bangladesh like PRAN currently exports processed agro products including mango juice to more than 100 countries. Mangos are bulky and highly perishable in nature and preserving them in the cold storage is not always possible on account of high cost involved with it. Hence, spoilage of mango during transportation as well as during sales is quite high. In the peak period, there is an excess supply creating a glut in the market and causing a fall in the price and affecting the incomes of the farmers. As a result, the growers are not getting their due returns for their produce and the country is being deprived of potential resources. So mango processing or pulp producing industries is the blessings of mango producer.

Physical characteristics of Mango:

Mullah and Siddique (1973) studied the physiological characteristics of some mango varieties of Bangladesh and found that the variety Langra had the highest pulp content (77.47%). The highest fruit weight (315.00g) was obtained from Fazli followed by Ashwina (220.00g), Bombai (207.00g) and Langra (200.00g). The highest percentage of edible portion was recorded in Ashwina (76.00%) followed by Langra (73.00%), Fazli (72.00%), Kalia (71.00%), Bombai (70.50%), Khirsapat (68.50%), Gopalbhog (68.50), and BARI Aam-1 (68.00%). TSS was found to be the highest in Khirsapat (22.00%) which was followed by Bombai (19.50%). Langra (18.50%), Gopalbhog and BARI Aam-1 (18.00%). (Bhuyan and Kobra, 2007)

2.2 Health benefits of eating Mango and its Juice:

Mango fruit is one of the most popular and nutritionally rich fruit. It is often called the king of fruit or super fruit for its taste, flavor, fragrance, and heath promoting qualities. It is delicious tropical seasonal fruit and believed to be originated in the sub-Himalayan plains of Indian subcontinent. Botanically, this exotic fruit belongs to the family of *Anacardiaceae*. Mangoes are grown in Asia including Bangladesh, India, and Pakistan, South and Central America, Florida, California, and the Caribbean. Each fruit measures 5 to 15 cm in length and about 4 to 10 cm in width, and has typical "mango" shape, or sometimes oval or round. Its weight ranges from 150 gm to around 750 gm. Outer skin is smooth and is green in un-ripe mangoes but turns into golden yellow, bright yellow or orange-red when ripen depending on the cultivar (Campbell, 2012).

Figure 002, photograph of mango on the tree

Mangoes are perfect to replenish salts, vitamins and energy after physical exercise. In gall bladder cancer a protective effect of mango consumes has been proven. Mangos contain also a lot of tryptophan, the precursor of the "*happiness-hormone*" *serotonin. Mangiferin* from the leaves has been reported to possess anti-inflammatory, diuretic, chlorotic and cardio tonic activities and displays a high antibacterial activity against gram positive bacteria. It has been recommended as a drug in preventing dental plaques. Mangiferin shows antiviral effect against type I herpes simplex virus which could be useful in anti-herpes ointments. The bark of the mango tree contains 16 – 20% tannin and also mangiferine. It acts as stringent and is believed to possess a tonic action on the mucous membrane. It is anathematic, useful in hemoptysis, hemorrhage, nasal catarrh, diarrhea, ulcers, diphtheria, rheumatism and for lumbrici. It is also used in diphtheria and rheumatism. Vitamin A plays an important role in the development

of the placenta and the fetus. It influences the growth, the metabolism of skin, mucus membranes, teeth and retina. Mango is an excellent natural source for pro-vitamin A (Campbell, 2012).

2.3 The following mango remedies are partially adopted from the Unani medicine system:

Beauty Aids: Taking mango regularly makes the complexion fair and the skin soft and shining.

Bleeding: The mango tightens the capillary vessels and cures bleedings of inner parts because of its content in vitamin C and calcium.

Burns: Burnt ashes of mango leaves applied on the burnt parts give quick relief.

Children's eating soil: The habit of eating soil can be cured by feeding the powder of dried kernel of mango seeds with fresh water.

Diarrhea: Sun dried mango leaves powdered, half teaspoon 2-3 times a day with water gives relief.

Dry cough: After having roasted ripe mango on hot sand in a pan, the juice of this mango eliminates all the bronchial congestion and gives relief in cough. According to hakeem hashmi sucking the juice and not eating cut mangos is better for health and some note of caution about mango eating.

Fever: Paste of mango roots applied on palms & soles cures fever.

Gall & Kidney Stones: Dried and powdered mango leaves, 10g a day in water (kept overnight in a tumbler) helps throwing stones out.

Gastritis: In India a decoction of the mango skin is given to people with inflammation of the stomach mucus membranes.

Mental weakness overcomes with mango juice:

Mango contains a lot of glutamine acid – an important amino acid for concentration and memory. Taking one cup sweet mango juice with 25 grams curd and ginger juice 2 or 3 times a day controls loose motions. 20 grams boiled mango powder bark in a liter of water and reduced in to 250 gram with 1 gram of black salt cures diarrhea.

Pregnancy: A pregnant woman should eat at least one mango a day – the calcium and magnesium of the mango relaxes the muscles, relieves stress and prevents abortion.

Teeth Problems: Dried Mango seeds are good toothpaste strengthens the gums and helps in curing dental problems & foul smell pyorrhea. (Campbell, 2012)

Mangoes are a rich source of Iron: Patients suffering from anemia are recommended to consume mangoes regularly. Women lose a large amount iron during their monthly period, so mangoes are a rich source of iron and should form a composite part of daily diet.

Mangoes can help in prevention in Acne: Mango help in relief from acne by helping in unclogging of congested skin pores.

Mangoes are high in Antioxidants: Like any other fruits mangoes are high in antioxidants, which benefits our lives in many ways. By regular intake of mangoes, any one will be able to fight cancer and heart diseases. Patients suffering from high blood pressure and high cholesterol can benefit from eating food that is rich in antioxidants.

Mangoes can boost anyone sex life: Mangoes contain vitamin E which works to regulate and boost anyone's sex hormones. Vitamin E is said to be one of the most beneficial vitamins in order to boost anyone's sex life. So in order to boost anyone's sex drive, should eat plenty of mangoes regularly.

Anti-Inflammatory Fruit: Mangoes offer anti-inflammatory properties which benefits the body in plenty of ways. It can reduce pain from diseases such as arthritis. Because of the anti-inflammatory properties, mangoes can also relief from symptoms of asthma.

Phenols in Mangoes have strong antioxidant and anticancer activities. (Campbell, 2012)

2.4 Summery of benefits:

- Mango Fruit are rich in iron, thus patients with anemia and pregnant women are advised to consume it regularly.

- Eating mangoes helps in relieving congested pores of the skin.

 Patients suffering from acidity and weak digestion can benefit from the consumption of mangoes.

 It is a fruit which is low in carbohydrates and is an rich antioxidant.

 The rich source of vitamin A (beta-carotene), E and selenium present in mangoes helps in protection from heart diseases and other problems.

 Mango fruit is rich in pre-biotic dietary fiber, vitamins, minerals, and poly-phenolic flavonoid antioxidant compounds.

 According to new research study, mango fruit has been found to protect against colon, breast, leukemia and prostate cancers. Several trial studies suggest that polyphenolic anti-oxidant compounds in mango are known to offer protection against breast and colon cancers.

 Mango fruit is an excellent source of Vitamin-A and flavonoids like beta-carotene, alpha-carotene and beta-cryptoxanthin. 100 g of fresh fruit provides 765 mg or 25% of recommended daily levels of vitamin A. These compounds together have antioxidant properties and are essential for vision. Vitamin A is also required for maintaining healthy mucus membranes and skin. Consumption of natural fruits rich in carotenes is known to protect body from lung and oral cavity cancers.

 Fresh mango is a very rich source of potassium. Potassium is an important component of cell and body fluids that helps controlling heart rate and blood pressure.

 It is also a very good source of vitamin-B6 (pyridoxine), vitamin-C and vitamin-E. Consumption of foods rich in vitamin C helps body develop resistance against infectious agents and scavenge harmful oxygen free radicals. Vitamin B-6 or pyridoxine is required for GABA hormone production in the brain. It also controls homocysteine levels in the blood, which may otherwise be harmful to blood vessels resulting in CAD and stroke.

 Copper is a co-factor for many vital enzymes, including cytochrome c-oxidase and superoxide dismutase (other minerals function as co-factors for this enzyme are manganese and zinc). Copper is also required for the production of red blood cells.

 Mango peel is also rich in phytonutrients, such as the pigment antioxidants like carotenoids and polyphenols (Campbell, 2012).

2.5 Different type of mango juice in Bangladesh: In food & beverage market there are many renowned companies having their juice products. We got many brands of mango juice, some of which are given below:

Acme Juice

Pran Juice

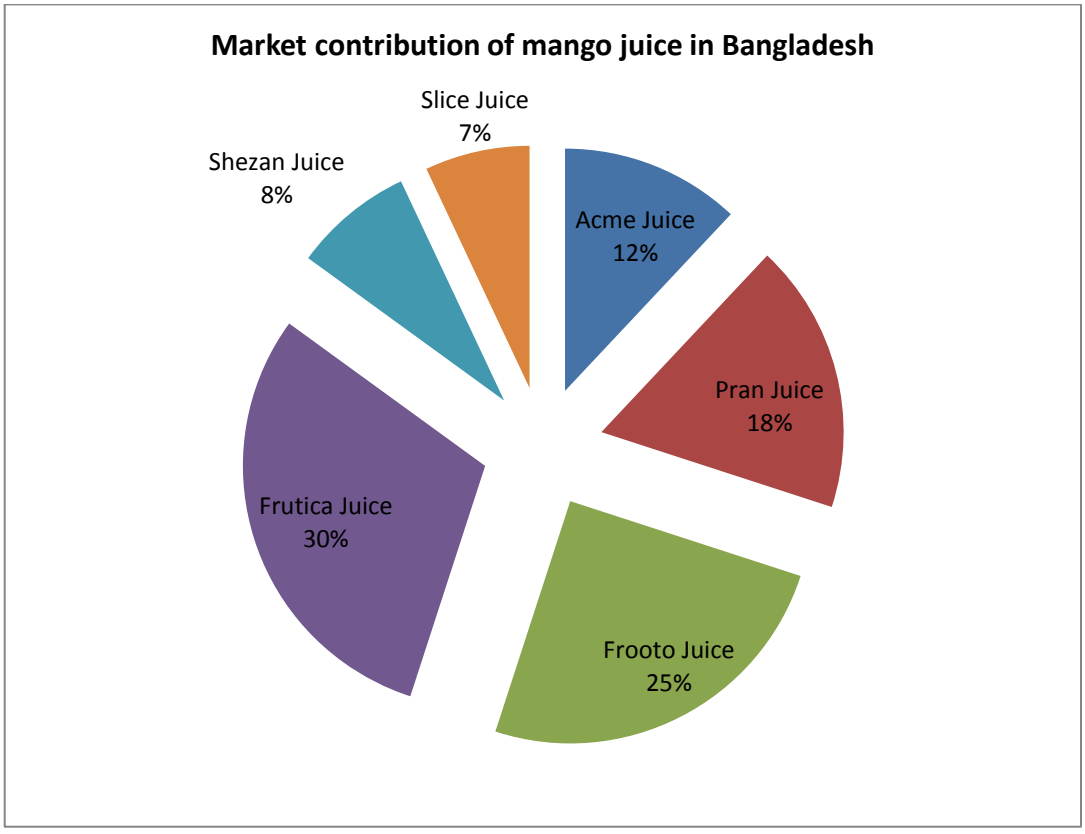
Frooto Juice

Frutica Juice

Shezan Juice

Slice Juice

Figure 03: Pie graph shows the contribution in Bangladesh market as per sale volume (as per one survey)



(Ronald Kimmons, Demand Media)

2.5 How to start a bottled juice company:

Increasing demand for bottled juice is primarily due to a shift in consumer tastes. Traditional artificially-produced juices are seemingly less desirable than natural bottled juice products. This shift may be due to an increase in health concerns and the promise of better nutrition without artificial ingredients. Individuals interested in capitalizing on such an emerging market need to do several things to start a bottled juice company.

Step 1

Organizer should determine the kind of juice products to sell. Aside from bottled and freshly-squeezed or natural juice, the company can also sell energy juice drinks or organic juice.

Step 2

Organizer should create a business plan detailing information such as the target market, operating expenses, marketing approaches, profit projections, public relations strategies and SWOT analysis. The SWOT analysis identifies the strengths and weaknesses of the planned business, along with opportunities and threats within the business environment. It helps you Organizer to determine and analyze Organizers bottled juice competitors and distributors.

Step 3

Organizer should formulate several juice recipes or research possible juice offerings. Organizer should build a successful business means offering the market something not currently available or innovative. A varied menu can help create a following for the business. Creative products and interesting alternatives can promise a head start for a new business.

Step 4

Organizer should locate or identify suppliers for the business. These include wholesale bottle contractors, produce vendors for ingredients and even farmers' markets. Fresh fruit providers should be find out to supply regular materials to create juices. Some suppliers may focus on a particular fruit or group of fruits, such as citrus, while others may provide a full range of fruit. Organizer should find providers for the other materials. Organizer will need to use when manufacturing juice, such as bottle providers.

Step 5

Organizer should decide on the kind of juice to use. Manufacturing bottled juice requires a juicer to extract fresh juice from the fruit. Organizer should decide to produce juice from fruit or using pulp and other chemical ingredients. Regardless of Organizer business' scale, the company needs a juicer to start bottling juice extracts. Some types of juicers incorporate centrifuges or hydraulic presses.

Step 6

Organizer should learn about the health permits that correspond to Organizers type of business. These may vary by location. The government usually requires certain health permits for a food-oriented business. Some necessary licensure includes BSTI certificate, HACCP certification and food enterprise or distribution licenses. Satisfying permit requirements makes it easier to set up the business immediately.

Step 7

Organizer should contact local offices to find out the licenses required to start a retail business. Organizer will need atax identification number, VAT registration and perhaps a license to sell specific products.

Step 8

Organizer should organize the management, marketing and production units of the company or business. Each unit should have clear tasks and objectives. (Kimmons,2006)

2.6 Fruit/juice general composition and Microbiological standards:

The structure and functional aspects of fruits dictate their composition. Table 1 shows some typical constituents of fruit and subsequently juices and the range of values dependent upon fruit, cultivar, cultivation, maturity and other factors.

Table 2: Fruit edible portion composition ranges (Fresh weight basis).

Component	Range (%)	Comments
Water	97 - 70	Influenced by cultivation and post-harvest conditions
Carbohydrates	25 - 3	Sugars and polymers - pectin, hemicellulose, cellulose
Protein	5 - trace	More in oily fruit and seeds
Lipids	25 - trace	Traces in cell membrane, in seeds, high in avocado
Acids	3 - trace	Citric, tartaric, malic, lactic, acetic, ascorbic + minor
Phenolics	0.5 - trace	Tannins and complex phenols
Vitamins	0.2 - trace	Water soluble > fat soluble
Minerals	0.2 - trace	Soil and species dependent
Dietary fibre	<1 to >15	Peel and core dependent
Pigments	0.1 - trace	Carotenoids, anthocyanins, chlorophyll

(FAO Corporate Document Repository, 2010)

The recommended Microbiological standards for any fruit juices sold in the Gulf Region (Gulf Standard 2000)

Table 3: Microbiological standards for any fruit juices:

Test	Total aerobic bacterial count (cfu/ml)	Total coliforms (cfu/ml)	Yeasts and moulds (cfu/ml)
Maximum count anticipated	5.0×10^{3}	10	100
Maximum count permitted	5.0×10^{4}	100	1.0×10^{3}

(Rashed et al., 2012)

Most of food & beverage companies of Bangladesh produce juice from mango pulp so that first they produce pulp from mango, then they use those pulp for manufacturing juice. The juice have been concentrated and later reconstituted with water suitable for the purpose of maintaining the essential composition and quality factor of the juice. The addition of sugar or acids can be permitted but must be endorsed in the individual standard.

2.7 Mango pulp:

Mango the delicious fruit is produced in about 90 countries in the world with a production of over 25.1 million tones. Asia is the main producer with 76.9% of the total world production, followed by America with 13.38%, Africa with 9% and less than 1% each for Europe and Oceania. (Edward A. et al, 2007) Mango is a short seasoned fruit and being

highly perishable does not withstand even in cold storage. People like to enjoy mango all time of the year. Therefore, most of the fruit processing industry in Bangladesh preserves mango pulp for the manufacturing of mango products around the year. Mango pulp is not generally consumed directly rather used as fillings for pastries, jams, sauces, fruit juices and drinks. Being the cheapest among several methods of preservation, chemical preservation of mango pulp is the most common and widely used in Bangladesh. The chemical preservatives are used to prevent the food spoilage due to microbial attack and thus are effectively used in combinations for better preservation.

2.7.1 Pulp extraction, packaging and storage:

Mangoes were passed through a mango pulper to separate pulp from the stones and skin and the pulp obtained was mixed with chemical preservatives and stored under ambient conditions (30-40ºC) in the dark for a period of 90 days and analyses were carried out after every 30 days.

2.8THE MOST IMPORTANT STEPS INVOLVED IN PROCESSING OF JUICES FROM PULP ARE:

- Blending or mixing ingredient.
- Straining, Filtration and clarification
- Pasteurization.
- Filling, Sealing and Cooling
- Labeling and Packing.

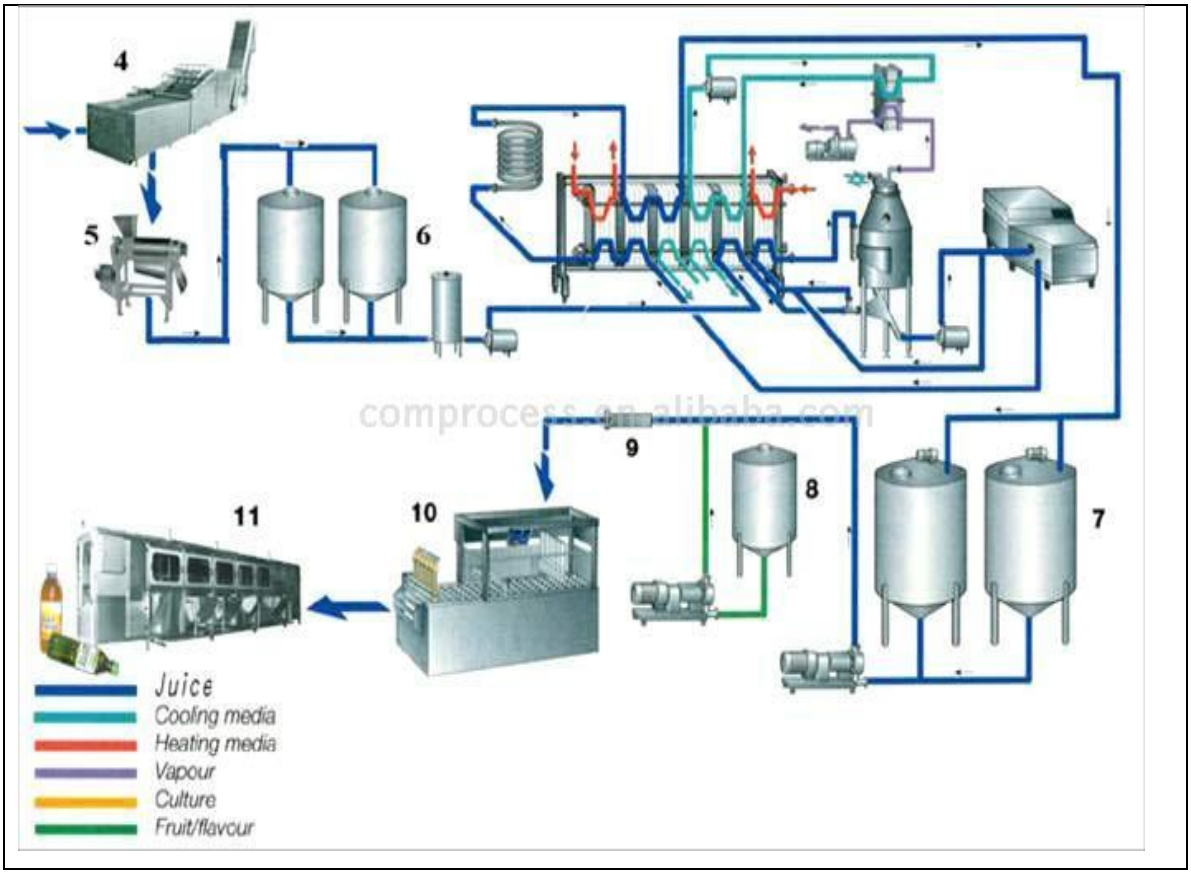


Figure 04, Flow chart of Juice production from pulp

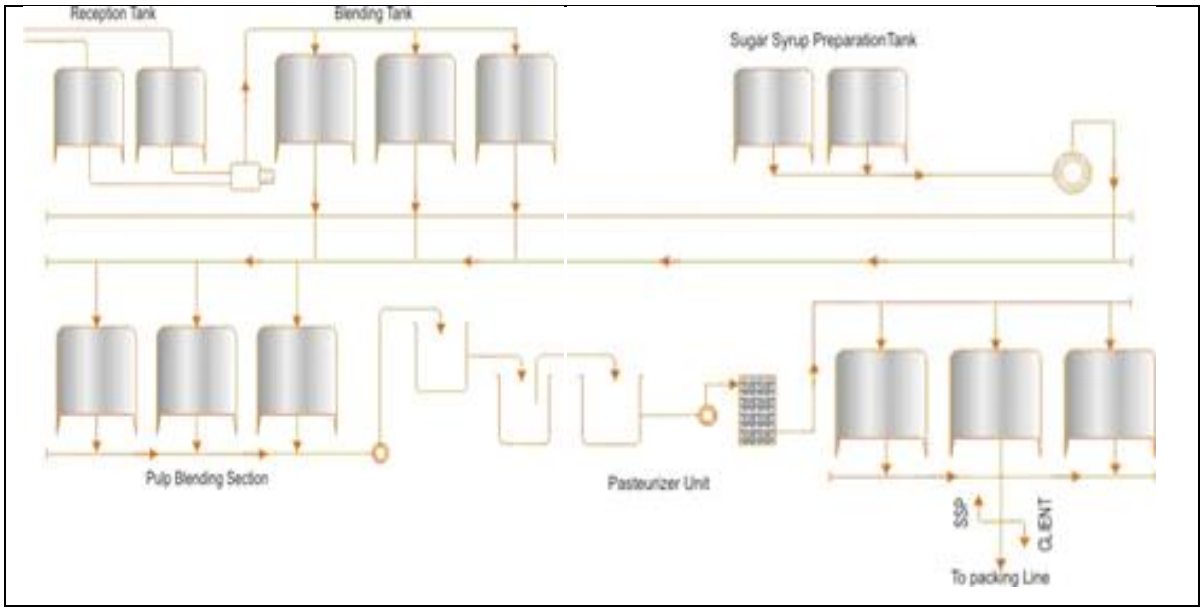


Figure 05, Flow chart of Juice production from pulp-2

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Flow chart of Juice production from fruit pulp

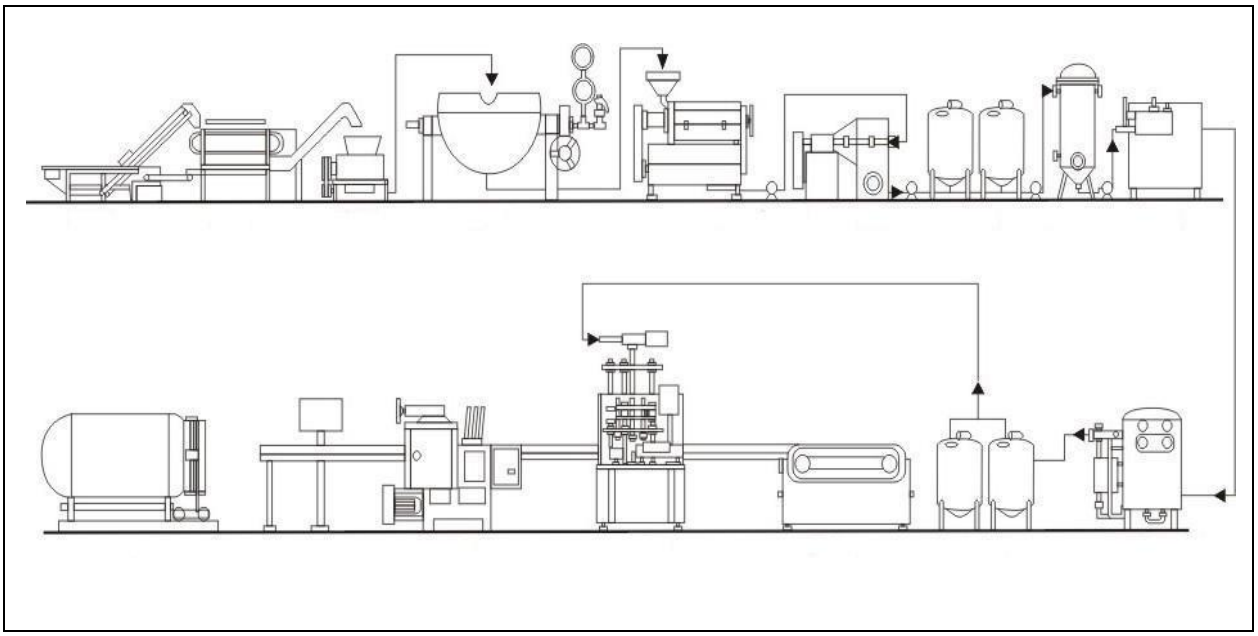


Figure 06, Flow chart of Juice production from pulp-3

2.8.1 Juice Bottling:

The juice is adjusted in the bottling plant with water to reconstitute the liquid back to its "original state". The added ingredients must be shown on the label. There are two types of bottling is available for fruit juice: Hot filling & Aseptic Process.

2.8.2 Hot Fill & Aseptic Process:

Plastic bottles are usually filled using a hot fill process. Tetra Pak cartons are filled with liquid using aseptic processing. The hot fill process heats the liquid juice to a temperature of between 182 degrees F and 192 degrees F for at least 3 minutes. The hot liquid is then poured into the plastic bottles. The high temperature of the liquid sterilizes the plastic bottle as it is being poured in. The bottle is then sealed and it is cooled. One of the main concerns of hot filling plastic bottles is that the chemicals in the plastic bottle may 'leech' into the liquid because of the high temperatures. Many juices also lose some flavor and nutrients during the long heating process. Aseptic processing uses flash heating. The liquid is heated to a temperature of 195 – 285 degrees F but only for 3 – 15 seconds, depending on the product. The liquid is then cooled before filling the cartons. This process reduces energy use and also greatly reduces nutrient loss. The sterile liquid is then poured into the sterile cartons and sealed. This process allows juices to be stored up to a year before opening without refrigeration and eliminates the need for preservatives.

CHAPTER: 03

MATERIALS AND METHODS:

3.1 Sample collection:

Six samples were collected which were commercially packed juices available in market. One juice was prepared at home from mango pulp, water and sugar.

Mango juice samples name and collection area.

Table 4: List of collected samples

Sl no.	Name of Juice	Name of Manufacturer	Collection place
1	Homemade Mango Juice		Home
2	Frutica (Mango fruit Drinks)250 ml	Akij Food and Beverage Ltd.	Dhaka
3	Frutoo (Mango fruit Drinks)250 ml	Pran Dairy Ltd	Dhaka
4	Mangolee (Mango Juice)250 ml	AST Beverage Ltd.	Dhaka
5	Starship (Fruit Juice) 200 ml	AbulKhair Condensed Milk and Beverage Ltd.	Dhaka
6	Shezan Classic (Mango Drinks) 250 ml	Hashem Foods Ltd.	Dhaka
7	ACME Mango Drinks	ACME agro and Beverage Ltd.	Dhaka

3.1.1 Determination of P^H:

In chemistry, pH is a measure of the acidity or basicity of an aqueous solution. Solutions with a P^H less than 7 are said to be acidic and solutions with a P^H greater than 7 are basic or alkaline. Pure water has a P^H very close to 7. In technical terms, P^H is the negative logarithm of the activity of the (solvated) hydronium ion, more often expressed as the measure of the hydronium ion concentration. The P^H scale is traceable to a set of standard solutions whose P^H is established by international agreement. Primary P^H standard values are determined using a concentration cell with transference, by measuring the potential difference between a hydrogen electrode and a standard electrode such as the silver chloride electrode. Measurement of P^H for aqueous solutions can be done with a glass electrode and a P^H meter, or using indicators. P^H is defined as the decimal logarithm of the reciprocal of the hydrogen ion activity in a solution (McClements & Decker, 2009).

3.1.2 Total soluble solids (Brix):

In the juice total soluble solids was measured. These soluble solids were primarily sugars; sucrose, fructose, and glucose. Citric acid and minerals in the juice also contributed to the soluble solids. Brix is reported as "degrees Brix" and is equivalent to percentage. For example, a juice which is 12 degrees Brix has 12% total soluble solids. The National standards state the minimum brix sugar/ acid ratio for navel oranges is 10:1.

3.1.3 Carbohydrates:

Monosaccharaides:

Monosaccharaides are water-soluble crystalline compounds. They are aliphatic aldehydes or ketones which contain one carbonyl group and one or more hydroxyl groups. Most natural monosaccharaides have either five (pentose) or six (hexoses) carbon atoms. Commonly occurring hexoses in foods are glucose, fructose and galactose, whilst commonly occurring pentose are arabinose and xylose. The reactive centers of monosaccharaides are the carbonyl and hydroxyl groups (McClements & Decker, 2009).

Oligosaccharides:

These are relatively low molecular weight polymers of monosaccharaides (< 20) that are covalently bonded through glycosidic linkages. Disaccharides consist of two monomers, whereas trisaccharides consist of three. Oligosaccharides containing glucose, fructose and galactose monomers are the most commonly occurring in juice.

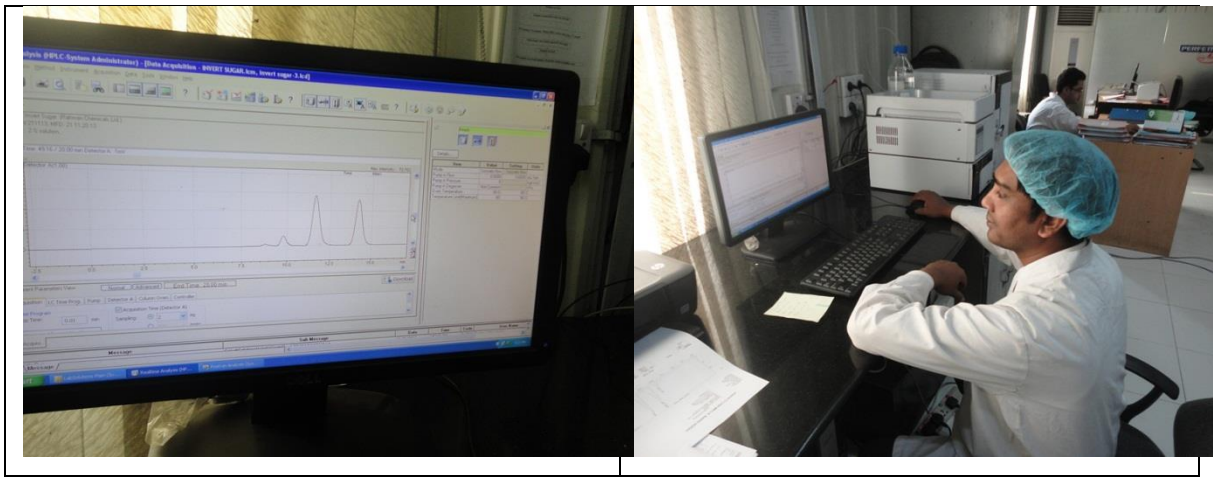


Figure 07: Photograph of HPLC:

3.2 Physico chemical test Method:

All the samples (7) were collected in sterile containers kept in the ice box maintaining 4ºC during transportation to the laboratory and analyzed within 2-3 hours.

3.2.1 Determining acidity (citric acid content):

Citric acid and small amounts of malic and tartaric acid were added in Juice for its tartness and unique taste. The amount of acid present in the juice was reported as percent citric acid. A titration with sodium hydroxide was used to calculate the value.

Apparatus

Conical flask: 250ml capacity, 50 ml Burette

Reagents

Standard sodium hydroxide solution: 0.1N

Phenolphthalein indicator: 1.0%

Method:

Accurately 2 ml of the sample was taken in a clean dried conical flask. 100 ml of distilled water was added to it. Few drops of 1 % Phenolphthalein indicator were added. The solution was titrated with 0.1N sodium hydroxide using phenolphthalein as indicator until a slight but definite color changed (light pink) persisting for at least 15 seconds.

Calculations

Titrable acidity of the sample

$$\text{Titrable acidity (\%)} = \frac{\text{T.V. x Factor*}}{\text{W}}$$

Where
TV= Titer value of the sample in ml.
W = quantity of the sample taken for the test in ml.

Factor for: - Citric acid: 0.0064 (Citrus fruit)

Malicacid: 0.0067

Tartaricacid: 0.0075

3.2.2 Total reducing sugar (TRS):

A number of chemical methods used to determine monosaccharaides and oligosaccharides which are based on the fact that many of these substances are reducing agents. Those reducing agents can react with other components to yield precipitates or colored

complexes which could be quantified. Acidity was measured according to the AOAC method (AOAC, 1995) and expressed in g/L as citric acid.

Titration Methods:

Apparatus

250ml. Erlenmeyer flask,

Gas Burner,

Wire Gauge,

Vacuum Oven,

200 ml. Volumetric Flask,

Glass Beads

Reagents:

Fehling's Solution A,

Fehling's Solution B,

Dextrose anhydrous GR,

Methylene Blue Indicator Solution

Procedure:

Standardization of Fehling's Solution:

3gms of primary standard dextrose previously dried in vacuum at 100°C for two hours was taken into a 500 ml. volumetric flask. Then dextrose was dissolved and diluted to make volume with water up to the mark. 5 ml. each of Fehling's Solution A and B were taken into a 250 ml. conical flask containing a few glass beads. Then that solution was titrated with standard dextrose solution.

Method:

4-5 ml of juice sample was taken into the 200 ml volumetric flask then water was added in order to make the volume up to 200 ml. Sample was filtered through Whatman filter paper no. 1 and rinsed the residue on filter three times to recover the contents of sample. Filtrate was transferred into 200 ml volumetric flask and made it up to the 200 ml volume mark. This solution was called stock sample solution. 5 ml each of Fehling's solution A and Fehling solution B was taken in a 250 ml conical flask. The content of conical flask

was boiled for two minutes and 1ml of methylene blue solution of conical flux was added without any interruption. While the solution kept on boiling, the stock sample solution was began to added from the burette till the blue colour of the indicator disappeared to a brick red colour. The titration was finished in last one minute so that the contents of the flask boiled altogether for three minutes without any interruption. (United State Pharmacopeia Volume 2, 2012)

Calculations:

% of Reducing Sugars = FX200X100/ (V1XW)

Where, F = Factor for 5 ml. of Fehling's Solution

V1 = Volume of sample solution consumed

W = Weight in gm. of the sample

Figure 08, photograph of homemade mango juice

3.2.3 Determining p^H:

Operation:

p^H value was measured with a p^H meter (Mettler Toledo, Switzerland). Juice sample was taken in an appropriate container; pH-meter was turned in Measure Mode, electrode and temperature sensor probe was dipped into the below level of sample. pH reading of the sample was taken and noted down.

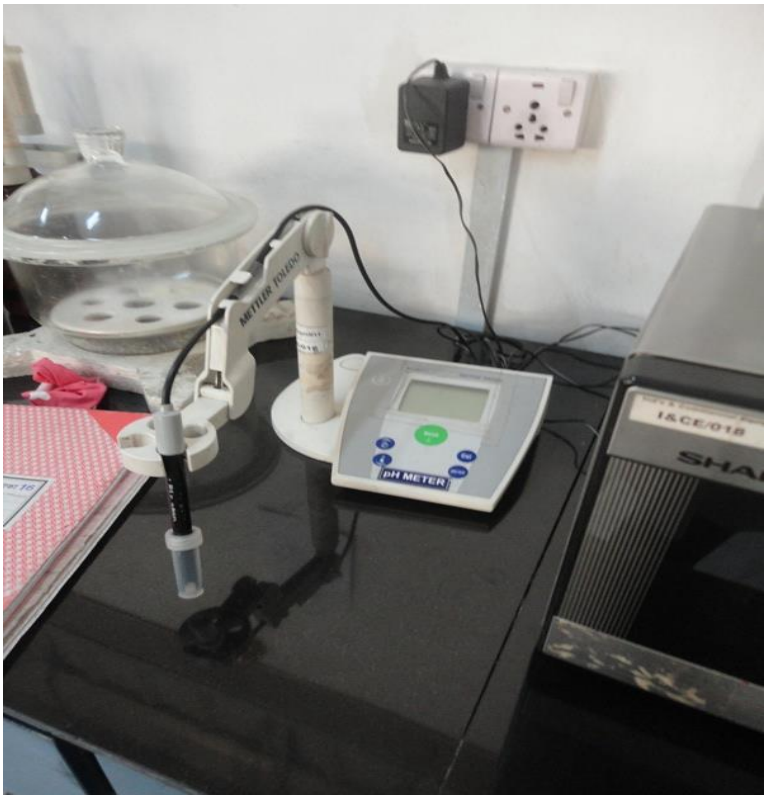


Figure: 09, photograph of p^H Meter.

3.2.4 Procedure determining Protein:

Kjeldahl method:

Juice was digested with a strong acid so that it released nitrogen which could be determined by a suitable titration technique. The amount of protein present was calculated from the nitrogen concentration of the juice. It was usually considered to be the standard method of determining protein concentration; because the Kjeldahl method did not measure the protein content directly; a conversion factor (F) was needed to convert the measured nitrogen concentration to a protein concentration. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) was used for applications. This was only an average value and each protein has a different conversion factor depending on its amino-acid composition. The Kjeldahl method can conveniently be divided into three steps: digestion, neutralization and titration. (McClements, 2005).

Digestion:

The juice sample to be analyzed was weighed into a digestion flask and then digested by heating it in the presence of sulfuric acid, anhydrous sodium sulfate and a catalyst, such as copper was added. Digestion converted any nitrogen in the juice into ammonia, and other organic matter to $C0_2$ and H_20. Ammonia gas was bound to the sulfate ion (SO_4^{2-}) and thus remained in solution: N (The juice) $\rightarrow (NH_4)_2SO_4$

Neutralization:

After the digestion had been completed the digestion flask was connected to a receiving flask by a tube. The solution in the digestion flask was then made alkaline by addition of sodium hydroxide, which converted the ammonium sulfate into ammonia gas. The ammonia gas was pass throw boric acid, so that boric acid was converted to the borate ion.

Titration:

The nitrogen content was estimated by titration of the ammonium borate formed with standard hydrochloric acid, using a suitable indicator (phenolphthalein) to determine the end-point of the reaction.

The concentration of hydrogen ions (in moles) required to reach the end-point is equivalent to the concentration of nitrogen that was in the original juice.

According to this method percentages of crude protein content of a sample

= % nitrogen × 6.25.

3.2.5 Determining soluble solids content (% sugar or °Brix):

Procedure:

First Switch was **on** of the instrument; the sample was placed on the lower surface of prism using the glass rod. Sector was hold firmly and moved backward or forward until field of vision was divided into light and dark portion. The colours were eliminated by rotating screw head of compensator until sharp, colourless line is obtained. Borderline was adjusted so that it felt on point of intersection of cross – hairs. Then Brix of juice was read directly on scale of sector. Soluble Solid Content (SSC, %) was measured using an Abbe refractometer calibrated against sucrose.

Figure 10: photograph of Abbe refractometer.

3.2.6 Carbohydrates profile:

Chromatographic methods:

High Performance Liquid chromatography (HPLC) was commonly used to separate and identify carbohydrates. Carbohydrates were separated on the basis of their differential adsorption characteristics by passing the solution to be analyzed through a column. Carbohydrates could be separated on the basis of their partition coefficients, polarities or sizes, depending on the type of column used. HPLC is currently the most important chromatographic method for analyzing carbohydrates because it is capable of rapid, specific, sensitive and precise measurements.

Sugar content tests were performed using HPLC on a high-pressure SHIMADZU liquid Chromatograph equipped with:-

Model No. CTO20A, Shim-Pack-SCR-101C column, RID-10A refractometric detector, LAB SOLUTION software, Flow Accuracy: ±1% and LC20AD Pump were used.

The amount of the sample injected onto the column was 20 µl. The separation was conducted at a temperature of 80°C with the mobile phase water at a flow rate of 1.3 ml/min. The identification of monosaccharide in Juice was done by comparing retention times of individual sugars in the reference vs. tested solution (standard solution). The quantitative assays were made of the following carbohydrates: fructose, glucose, sucrose, maltose, malt triose and maltotetraose. The contents of those compounds were assayed based on the comparing peak areas obtained in the examined samples with those from the reference solution (standard solution). To make the presentation of the obtained results more comprehensive the following was calculated: total sugars, fructose to glucose ratio and total monosaccharide's. Calculation was done by using the Post Run software.

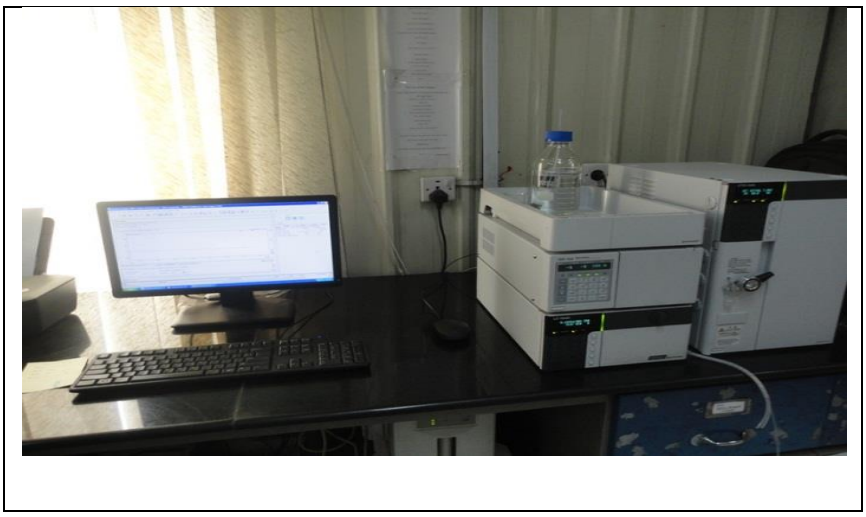


Figure 11: Photograph of HPLC:

3.2.7 Fat Content Determination:

Fat content:

The fat content of the sample was determined as free fat and the total fat (EEC 90/496, 1990). Free fat was extracted from lyophilized sample by Soxhlet using ether as solvent. The total fat content was determined by the acid-hydrolysis method (AOAC948.15, 1995). Samples (1.5 g) were digested with dilute hydrochloric acid (5 ml) for about 45 minutes on a water bath. The mixture obtained was then extracted with a combination of solvents comprising of methanol (2.5 ml), Diethyl ether (7.5 ml) and petroleum ether (7.5 ml). There after the mixture was centrifuged, ether–fat layer was decanted and evaporated and the fat content was measured.

Procedure

25 ml of isopropanol, 25 ml of H_2O with antifoam and 25 ml of sample was added in a 500-ml distillation flask. Heater was turned on and cold water was started through the condenser from bottom to top. 150-ml beaker was kept under the condenser. Flask was attached to the connecting trap of the condenser and placed on the heater making sure the connector would not leak and waited till distillation complete. Completion is indicated by water condensation inside the connecting tubes.

Distillate was titrated with the 0.0247 N KBrO3-KBr solutions to the disappearance of the dye color. Used amount of titrant was recorded. Average blank reagent value was determined and reduced from the total titrant.

Calculations

As 1 mole of *d*-limonene reacted with 2 moles of Br_2 or 4 moles of Br (bromine), 1 ml of 0.0247 N $KBrO_3$-KBr titrant equals 0.001 ml or 0.00084 g of *d*-limonene and equals 0.004% oil by volume for a 25-ml sample.

$$\% \text{ Oil (v/v)} = \frac{\text{Volume of Oil in Sample}}{\text{Volume of Sample}} \times 100$$

Where, (Net ml $KBrO_3$-KBr) = (ml $KBrO_3$-KBr for Sample – ml $KBrO_3$-KBr for Blank)

For 25 ml juice sample titrated with 0.0247 N KBr3-KBr

3.3 Microorganism which are mostly involved with juice:

3.3.1 Total Viable Count:

A TVC is not a specific micro-organism but rather a test which estimates total numbers of viable (for the purposes of this data sheet viable means living) individual micro-organisms present in a set volume of sample. The TVC count may include bacteria, yeasts and mould species. There are different test parameters for different types of samples. For juice samples a different medium was used which is slightly different to Yeast Extract Agar. Clearly it was impossible to have a set of conditions that were ideal for all organisms likely to be present in any one sample. This should be borne in mind when interpreting the results. TVC results were very useful for trend analysis whereby changes in the numbers of microbes present in a system could be monitored over time and multiple samples.

3.3.2 Escherichia coli (*E.coli*):

E.coli is a member of the coliform group which is particularly associated with fecal matter and as such is a good indication of fecal contamination. Many people are confused about the significance of *E.coli* because of recent cases of food poisoning that have been caused by the species. Within species are smaller groups known as serotypes and these groups may have different characteristics. For example, serotype 1 may not cause food poisoning but serotype 2 might cause the disease. *E.coli* has been extensively studied over the years and a lot is known about the organism.

3.3.3 Yeasts and Moulds:

Mold is a type of fungus that grows in multicellular filaments called hyphae. These tubular branches have multiple, genetically identical nuclei, yet form a single organism, known as a colony. In contrast, yeast is a type of fungus that grows as a single cell Yeasts and moulds are both fungal species. They are common spoilage agents, disease causing agents and they are also used for the benefit of mankind in the production of many different substances such as antibiotics, foods and alcohol. Molds are ubiquitous, and mold spores are a common component of household and workplace dust. When mold spores are present in large quantities, they can present a health hazard to humans, potentially causing allergic reactions and respiratory problems. Some molds also produce mycotoxins that can pose serious health risks to humans and animals. Some studies claim that exposure to high levels of mycotoxins can lead to neurological problems and in some cases death. Molds can also pose a hazard to human and animal health when they are consumed following the growth of certain mold species in stored food.

3.3.4 Microbiological test method:

The standard procedure was followed (FDA, 2001) and test were inoculated in appropriate selective media for microbiological analysis of the collected juice samples for the quantitative determination of Total viable count (TVC), fecal coliform, *Escherichia coli* (*E.coli*) (SH. Cody et al., 1999). For presumptive identification of coliform bacteria, samples were inoculated onto MacConkey agar media. Total viable count (TVC) was performed by pour plate method using Nutrient agar, which was incubated at 35 ± 1^{0}C for 48±2h. mFC agar is used for fecal coliform at 44.5 ° C for 22±2h.

3.3.5 Total viable count

Sample Dilution:

0.9 gm Sodium chloride was diluted in 10 ml of water for Sodium chloride – 0.9% saline solution. 1:10 $^{-3}$ dilutions were prepared by aseptically transferring of the well mixed sample to the desired volume of diluent.

Nutrient Agar was used for Total viable count:

1. 2.8gm of dehydrated Nutrient Agar medium was taken in 100 mL of deionized or distilled water into a 250-mL flask.

4. Mixture was stirred well for several minutes to break up clumps and prevent medium from adhering to the flask.

5. Flask was placed in a heated water bath or on a hot plate and heated slowly to reach 90°C. Mixture was stirred constantly to prevent scorching. It was not autoclaved.

6. 1 mL of rosolic acid solution was added per 100 ml of medium with a clean pipette. Heating was continued until media was dissolved.

7. The medium was cooled to a temperature of about 45-50° C. Then medium was poured 20 to 25 mL in 90-mm Petri dish bottoms.

8. When the medium had solidified (about 10 minutes), Petri dishes were closed by pressing firmly with the tops. These plates were suitable for use after the medium had solidified.

9. Then sample was spread on selective Petri dishes. Sterilized forceps was dipped in alcohol and was hold in Bunsen burner for speared out. Forceps was cooled before use.

10. Petri dish was incubated 24 hours at 37 ± 0.5 °C.

3.3.6 Fecal coliform test procedure:

1. 0.2 N NaOH (sodium hydroxide) solution was prepared by dissolving 8.0 g of NaOH in deionized or distilled water and brought the volume up to 1 L.
2. Rosolic acid solution was prepared by adding 0.1 g of rosolic acid crystals to 10 mL of 0.2 N NaOH.
3. 5.210 gm of dehydrated mFC medium was taken in 100 mL of deionized or distilled water into a 250-mL flask.
4. Mixture was stirred well for several minutes to break up clumps and prevent medium from adhering to the flask.
5. Flask was placed in a heated water bath or on a hot plate and heated slowly to reach temperature at 90°C. Mixture was stirred constantly to prevent scorching. It was not autoclaved.
6. 1 mL of rosolic acid solution was added per 100 ml of medium with a clean pipette. Heating was continued until media was dissolved.
7. The medium was cooled to a temperature of about 45-50°C. Then medium was poured 20 to 25 mL in 90-mm Petri dish bottoms.
8. When the medium had solidified (about 10 minutes), Petri dishes were closed by pressing firmly with the tops. Those plates were used after the medium had solidified.
9. Then sample was spread on selective Petri dishes. Sterilized forceps was dipped in alcohol and was hold in Bunsen burner for speared out. Forceps was cooled before use.
10. Petri dish was incubated 24 hours at 44.5° C.

3.3.6 Confirmation of E. coli using MacConkey Agar media:

1. 5.15 gm of dehydrated MacConkey Agar medium was taken in 100 mL of deionized or distilled water into a 250-mL flask.
2. Mixture was stirred well for several minutes to break up clumps and prevent medium from adhering to the flask.
3. Flask was placed in a heated water bath or on a hot plate and heated slowly to reach temperature at 90°C. Mixture was stirred constantly to prevent scorching. It was not autoclaved.
4. 1 mL of rosolic acid solution was added per 100 ml of medium with a clean pipette. Heating was continued until media was dissolved.

5. The medium was cooled to a temperature of about 45-50° C. Then medium was poured 20 to 25 mL in 90-mm Petri dish bottoms.

6. When the medium had solidified (about 10 minutes), Petri dishes were closed by pressing firmly with the tops. These plates were suitable for use after the medium had solidified.

7. Then sample was spread on selective Petri dishes. Sterilized forceps was dipped in alcohol and was hold in Bunsen burner for speared out. Forceps was cooled before use.

8. Petri dish was incubated 24 hours at 37 ± 0.5 °C.

9. Long-wave UV lamp was used for examine the colonies. Fluorescence indicates the presence of E. coli. Some UV lamps do not use the correct wattage and can give false results. UV lamps bulbs were checked in order to be sure of specification.

Calculation and Interpretation of Result: Colony of each plate of same dilution was counted and conformed that selected plates were containing not more than 100 CFU per Petridis. Counted colony for each dilution was averaged and multiplied by the dilution factor.

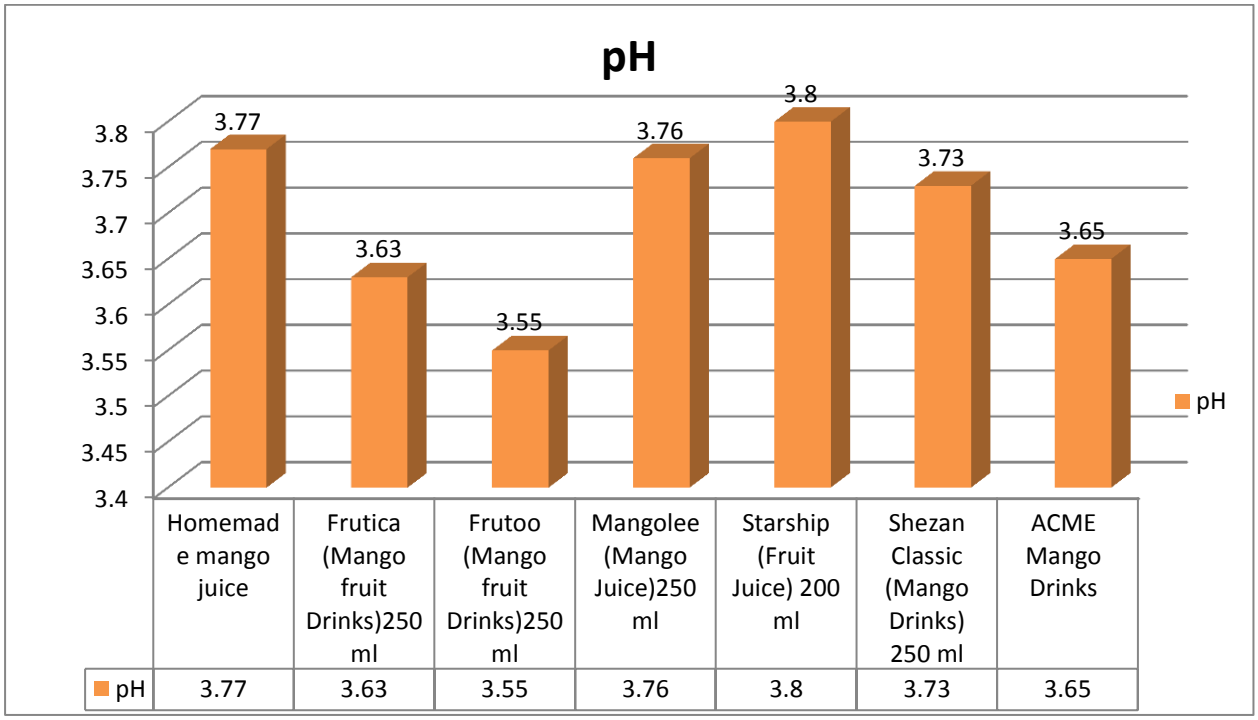
CHAPTER: 04

RESULT

Though mango juices are very popular and potential for human health, but concerns over their safety and quality have been raised. Many company already started production and many also going to launce their mango juice product in Bangladesh, but the level of quality has not been determined. This study was conducted to evaluate the quality of juices by studying their physio-chemical and microbiology parameters.

4.1 pH of various juice samples:

Fruit juices have a low pH because they are comparatively rich in organic acid. The overall range of pH is 2 to 5 for common fruits with the most frequent figures being between 3 and 4. In this study pH of the fruit juices varied from 3.55 to 3.80. The highest pH (3.80) was found in Starship (Fruit Juice) 200 ml pack and followed by Mangolee (Mango Juice) 250 ml pack, Homemade Juice, Shezan Classic (Mango Drinks) 250 ml pack, ACME Mango Drinks, Frutica (Mango fruit Drinks) 250 ml pack, Frutoo (Mango fruit Drinks) 250 ml pack.



	Homemade mango juice	Frutica (Mango fruit Drinks)250 ml	Frutoo (Mango fruit Drinks)250 ml	Mangolee (Mango Juice)250 ml	Starship (Fruit Juice) 200 ml	Shezan Classic (Mango Drinks) 250 ml	ACME Mango Drinks
pH	3.77	3.63	3.55	3.76	3.8	3.73	3.65

Figure 12: pH of various juice samples.

4.2 Total soluble solids of collected juice samples:

The TSS content is significantly influenced by percentage of solid materials dissolved in water in the juice. If mango pulp, sugar, glucose and other ingredient was less in juice then TSS will be less. Sometimes producer added sweetening agent instead of adding sugar and glucose. On the other hand some producer added other ingredient like sodium CMC to increase TSS.TSS of Homemade juice was 19% which was highest value. TSS of Frutica was 12.8%, Frutoo was 12%, Mangolee contained 13.5%, Starship had 12.75%, Shezan Classic had 12.25% and 12 % been recorded in ACME juice. Please find below the figure 13:

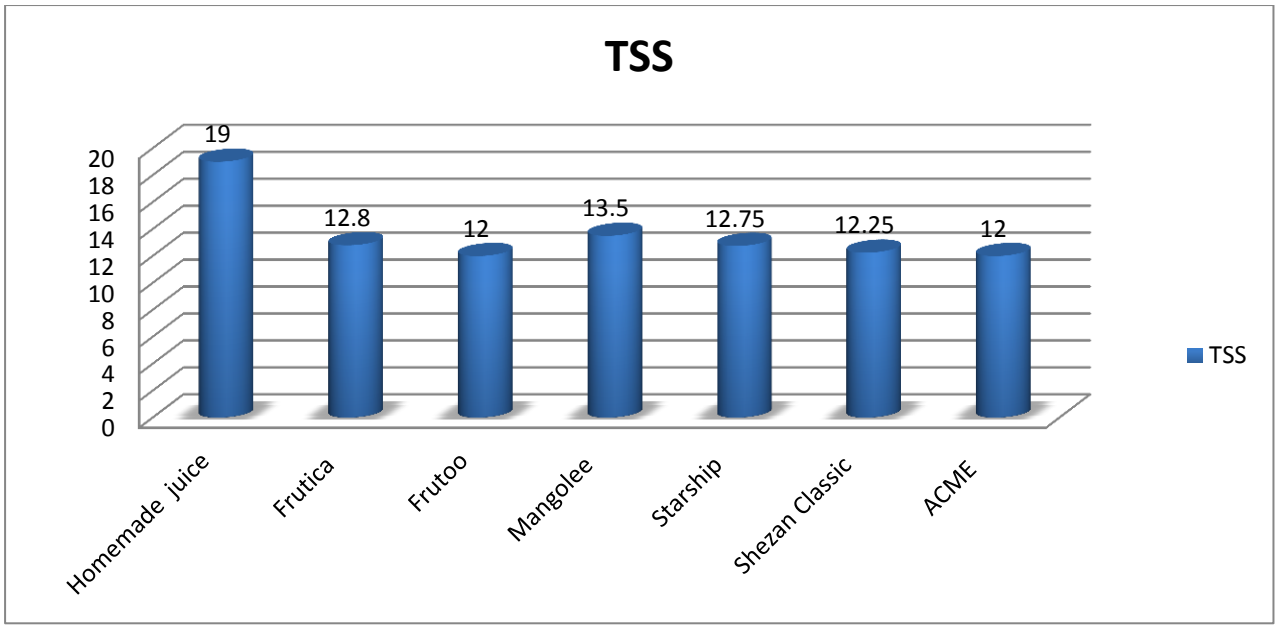


Figure 13: Total soluble solids of collected juice samples.

4.3 Total Acidity of collected juice samples:

The total acidity of fruit juices is due to the presence of a mixture of organic acids, whose composition varies depending on fruit nature and maturity of used pulp or used ingredients which were added during juice processing. Organic acids take the lead in importance for characteristics and nutritive value of fruit juices and confer individual originality among natural beverages. Acidity (as citric acid) varied significantly in different types of fruit juices (Table 5). Acidity (as citric acid) of homemade juice was 0.21, Frutica was 0.23, Frutoo contained 0.24, Mangolee was 0.21, Starship was 0.21, Shezan Classic had 0.23 and ACME juice contained 0.21.

4.3.1 Table 5: Acidity (as citric acid) of collected juice samples:

Sl no	Name of Juice	Acidity (as citric acid)
1	Homemade juice	0.21
2	Frutica	0.23
3	Frutoo	0.24
4	Mangolee	0.21
5	Starship	0.21
6	Shezan Classic	0.23
7	ACME	0.21

4.4 Protein Content in collected juice samples:

Most of the fruit juices were low in protein. Protein is insoluble in fruit juice so a considerable proportion of the protein content in the fruit juices. Homemade juice contained only (0.1 %), Frutica had (0.11%), Frutoo (0.18 %), Starship (0.12 %), ACME juice, Mangolee and Shezan Classic contained (0 %) protein.

4.4.1Table 6: Protein Content of collected juice samples

Sl no	Name of Juice	Protein (%)
1	Homemade juice	0.10
2	Frutica	0.11
3	Frutoo	0.18
4	Mangolee	0
5	Starship	0.12
6	Shezan Classic	0
7	ACME	0

4.5 Fat content in collected juice samples:

Fruit juice does not contain fat; somehow a little percentage of fat could be present in the ingredient used in juice production. We did not find fat in Homemade juice, Frutica, Starship and Shezan Classic juice. Frutoo contained only 0.17 %, Mangolee had 0.13 %, ACME juice contained only 0.11 % shown in the figure 14.

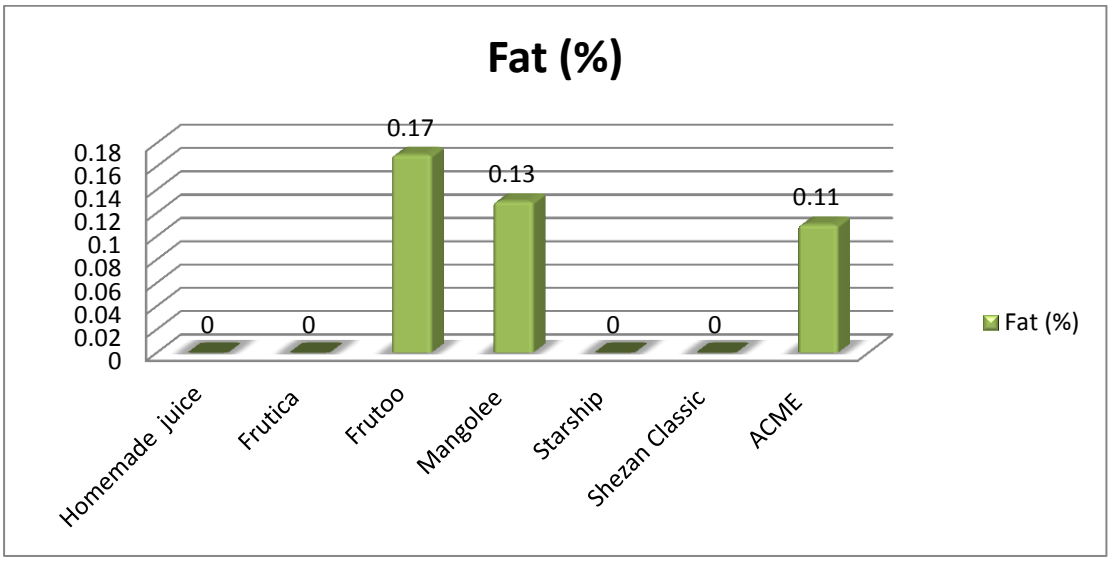


Figure 14: Fat content in collected juice samples.

4.6 Total Reducing Sugar of collected juice samples:

It is estimated that reducing sugar and total sugar content were increased with the advanced ripening of fruits. The combined effect of stages of maturity and ripening conditions significantly affected the reducing sugar and total sugar content of the fruit pulp and juices. Homemade juice contained 3.76%. TRS determined in Frutica was 5.85, Frutoo (Mango fruit Drinks) 250 ml contained 6.87%, 3.6 % TRS was in Mangolee, 4.39 % was in Starship, 6.8 % in Shezan Classic and ACME juice contained 5.3 % reducing sugar . TRS chart is given below (Table 07).

4.6.1 Table 07: Total Reducing Sugar of collected juice samples:

Name of Juice	TRS (%)
Homemade juice	3.76
Frutica	5.85
Frutoo	6.87
Mangolee	3.6
Starship	4.39
Shezan Classic	6.8
ACME	5.2

4.7 Carbohydrate Profile of collected juice samples:

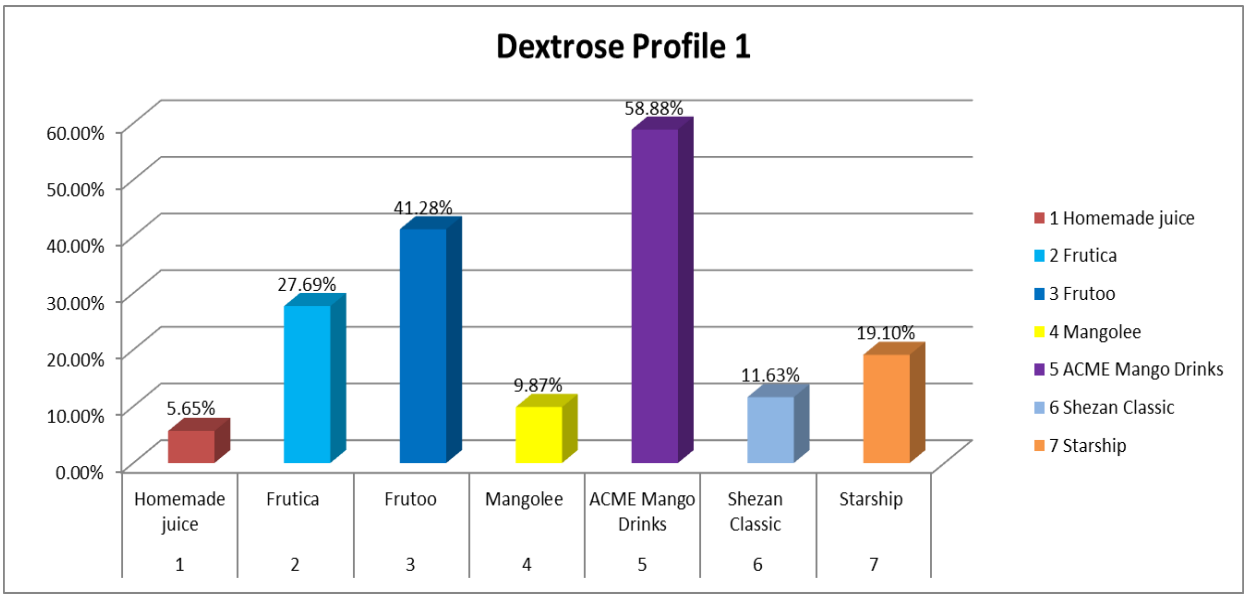
Mango juice contained carbohydrate like sugar, glucose maltose, maltotriose which provide consumer energy. Water is the predominant component of fruit juice. Carbohydrate including sucrose, fructose, glucose and sorbitol varies. Glucose or fructose is called monosacride is more effective from above all type of carbohydrate because of its simple atomic structure and it is absorbed by the Human body instantly rapidly and quickly. Those mango juice contain more monosaccharides are more suitable for consumer. 1-cup serving of mango juice has 128 calories, with less than 1 g each of fat, protein and cholesterol. The juice provides 0.8 g of fiber and has 33 g of carbohydrates, with a sugar content of 31 g. (Luther, 2013). In this study Carbohydrate Profile was analyzed by the HPLC and dextrose profile was segregated from others Oligosaccharides. Homemade juice contained 5.648 % monosaccharides which were very low percentage and it came from only mango pulp because only sugar was when Homemade juice was produced. Frutica (Mango fruit Drinks) 250 ml pack contained 27.69% monosaccharides .Frutoo (Mango fruit Drinks) 250 ml pack contained 41.27%. Mangolee (Mango Juice) 250 ml pack contained 9.867%. ACME Mango Drinks contained 58.88%. Shezan Classic (Mango Drinks) 250 ml pack contained 11.626 % and Starship (Fruit Juice) 200 ml pack contained 19.103% monosaccharides. Below table (Table 08) shows the compression of Dextrose profile between the all mango juice samples.

4.7.1 Table 08: Compression of Dextrose profile between the all mango juice samples:

Sl no.	Name of Juice	Description of Parameter	Value
1	Homemade juice	D P 1	5.65%
2	Frutica (Mango fruit Drinks)250 ml	D P 1	27.69%
3	Frutoo (Mango fruit Drinks)250 ml	D P 1	41.28%
4	Mangolee (Mango Juice)250 ml	D P 1	9.87%
5	ACME Mango Drinks	D P 1	58.88%
6	Shezan Classic (Mango Drinks) 250 ml	D P 1	11.63%
7	Starship (Fruit Juice) 200 ml	D P 1	19.10%

Here DP 1 means Dextrose profile 1 (Glucose or Fructose)

4.7.2 Figure 15: Dextrose profile between the all mango juice samples:



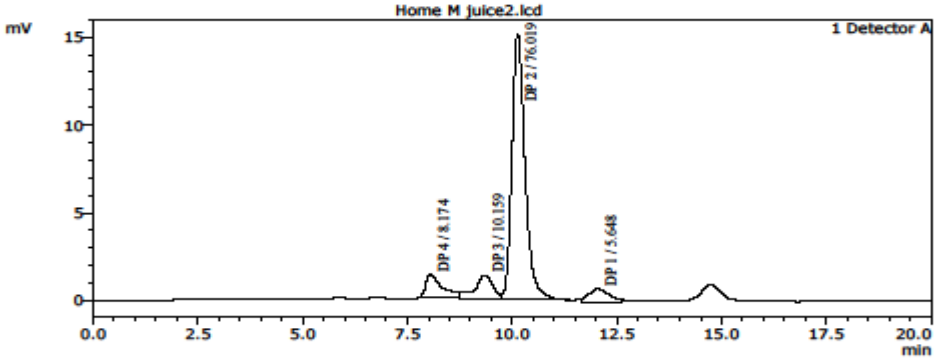
4.7.3 Table 09: Carbohydrate Profile of collected juice samples:

Sl no.	Name of Juice	Description of Parameter	Value
1	Homemade juice	Dextrose Profile 1	5.648
		Dextrose Profile 2	76.019
		Dextrose Profile 3	10.159
		Dextrose Profile 4	8.174
2	Frutica (Mango fruit Drinks)250 ml	Dextrose Profile 1	27.69
		Dextrose Profile 2	72.31
		Dextrose Profile 3	0
		Dextrose Profile 4	0
3	Frutoo (Mango fruit Drinks)250 ml	Dextrose Profile 1	20.24
		Dextrose Profile 1	21.038
		Dextrose Profile 2	58.722
		Dextrose Profile 3	0
		Dextrose Profile 4	0
4	Mangolee (Mango Juice)250 ml	Dextrose Profile 1	9.867
		Dextrose Profile 2	90.016
		Dextrose Profile 3	0
		Dextrose Profile 4	0.118
5	ACME Mango Drinks	Dextrose Profile 1	29.459
		Dextrose Profile 1	29.422
		Dextrose Profile 2	41.119
		Dextrose Profile 3	0
		Dextrose Profile 4	0
6	Shezan Classic (Mango Drinks) 250 ml	Dextrose Profile 1	11.626
		Dextrose Profile 2	81.821
		Dextrose Profile 3	6.553
		Dextrose Profile 4	0
7	Starship (Fruit Juice) 200 ml	Dextrose Profile 1	19.103
		Dextrose Profile 2	68.033
		Dextrose Profile 3	12.864
		Dextrose Profile 4	0

Carbohydrate profile of some selected brand of fruit juice of BD.

Sample Information

Acquired by	: System Administrator
Sample Name	: Home made Mango Juice
Sample ID	: MFD: 25.02.2014
Injection Volume	: 20
Data File	: Home M juice2.lcd
Method File	: iNVERT SUGAR.lcm
Date Acquired	: 2/26/2014 1:01:43 PM
Date Processed	: 2/27/2014 2:07:34 PM
Comment	: 1 % solution.



Peak Table

Detector A

Peak#	Name	Ret. Time	Area	Height	Area%
1	DP 4	8.053	35211	1305	8.174
2	DP 3	9.360	43762	1394	10.159
3	DP 2	10.137	327460	15088	76.019
4	DP 1	12.053	24327	733	5.648
Total			430761	18520	100.000

Description of the above report: In homemade juice; Tetraose (Dextrose Profile 4) percentage was 8.174, Triose (DP3) percentage was 10.150. Sucrose (DP2) percentage was 76.019 and Glucose or fructose (DP 1) percentage was 5.648.

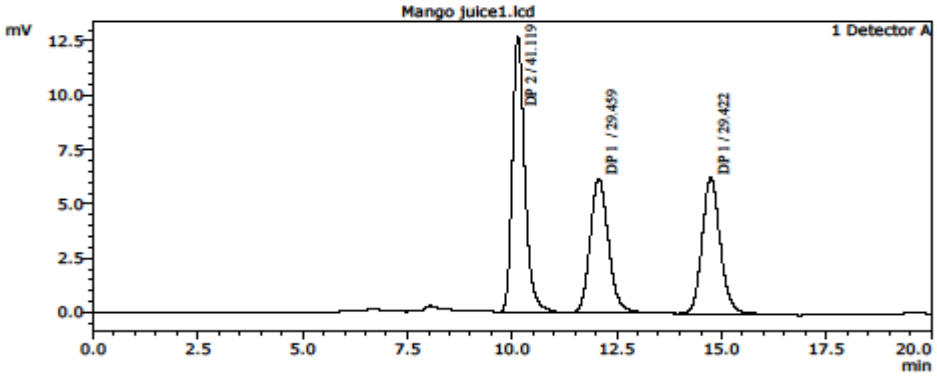
Note: Here DP means Dextrose Profile.

Figure 16: HPLC report (carbohydrate profile) of homemade mango juice:

Carbohydrate profile of some selected brand of fruit juice of BD.

Sample Information

Acquired by	: System Administrator
Sample Name	: ACME Classic Mango Drinks (The ACME Agro & Beverage Ltd.)
Sample ID	: BN:13023 MFD: 18.07.2013
Injection Volume	: 20
Data File	: Mango juice1.lcd
Method File	: iNVERT SUGAR.lcm
Date Acquired	: 2/7/2014 11:37:26 AM
Date Processed	: 2/7/2014 2:56:55 PM
Comment	: 1 % solution.



Peak Table

Detector A

Peak#	Name	Ret. Time	Area	Height	Area%
1	DP 2	10.144	263353	12673	41.119
2	DP 1	12.069	188678	6179	29.459
3	DP 1	14.743	188439	6245	29.422
Total			640469	25097	100.000

Description of the above report: In Acme Classic Mango juice; Sucrose (DP2) percentage was 41.119 and Glucose or fructose (DP 1) percentage was 58.881.

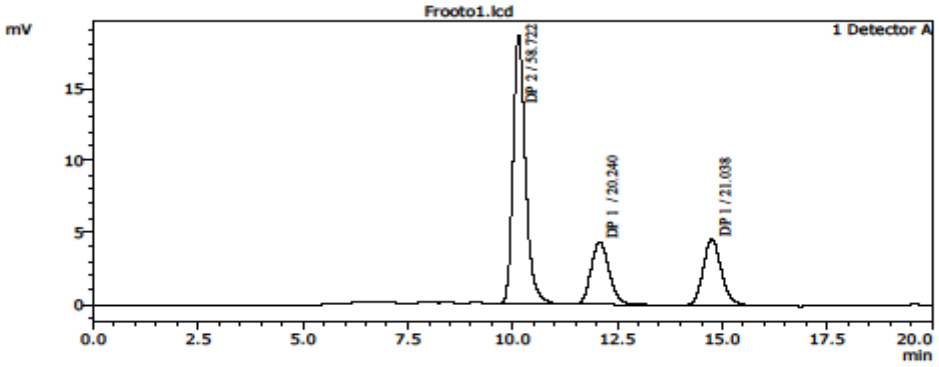
Note: Here DP means Dextrose Profile.

Figure 17: HPLC report (carbohydrate profile) of ACME Mango Drinks:

Carbohydrate profile of some selected brand of fruit juice of BD.

Sample Information

Acquired by	: System Administrator
Sample Name	: Pran Frooto Mango Fruit Drink (PRAN DAIRY Ltd.)
Sample ID	: BN:02 MFD: 03.10.2013
Injection Volume	: 20
Data File	: Frooto1.lcd
Method File	: iNVERT SUGAR.lcm
Date Acquired	: 2/7/2014 1:08:18 PM
Date Processed	: 2/7/2014 3:06:59 PM
Comment	: 1 % solution.



Peak Table

Detector A

Peak#	Name	Ret. Time	Area	Height	Area%
1	DP 2	10.148	387239	18614	58.722
2	DP 1	12.073	133472	4347	20.240
3	DP 1	14.751	138737	4560	21.038
Total			659449	27521	100.000

Description of the above report: In Frutoo (Mango fruit Drinks) juice; Sucrose (DP2) percentage was 58.722 and Glucose or fructose (DP 1) percentage was 41.32.

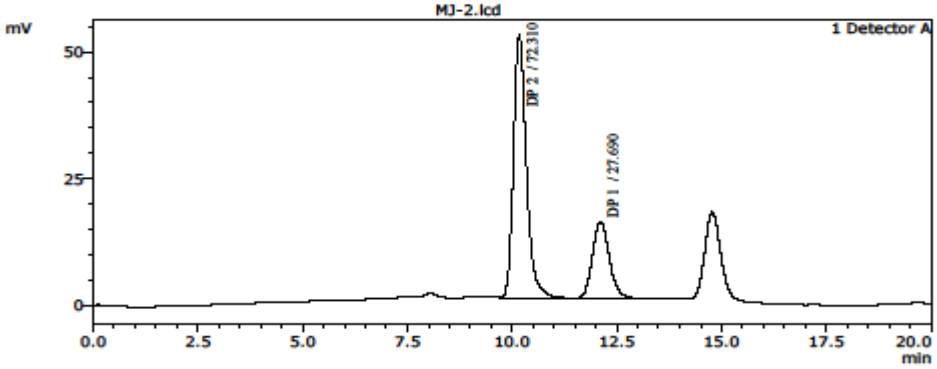
Note: Here DP means Dextrose Profile.

Figure 18: HPLC report (carbohydrate profile) of Frutoo (Mango fruit Drinks) 250 ml:

Carbohydrate profile of some selected brand of fruit juice of BD.

Sample Information

Acquired by	: System Administrator
Sample Name	: Frutika Mango Fruit Drinks
Sample ID	: BN:118FMI3, MFD: 22.05.2013
Injection Volume	: 20
Data File	: MJ-2.lcd
Method File	: LIQUID GLUCOSE.lcm
Date Acquired	: 8/2/2013 11:31:03 AM
Date Processed	: 2/24/2014 1:16:38 PM
Comment	: 2 % solution.



Peak Table

Detector A

Peak#	Name	Ret. Time	Area	Height	Area%
1	DP 2	10.175	1112555	52059	72.310
2	DP 1	12.108	426032	15237	27.690
Total			1538587	67296	100.000

Description of the above report: In Frutica mango juice; Sucrose (DP2) percentage was 72.310 and Glucose or fructose (DP 1) percentage was 27.690.

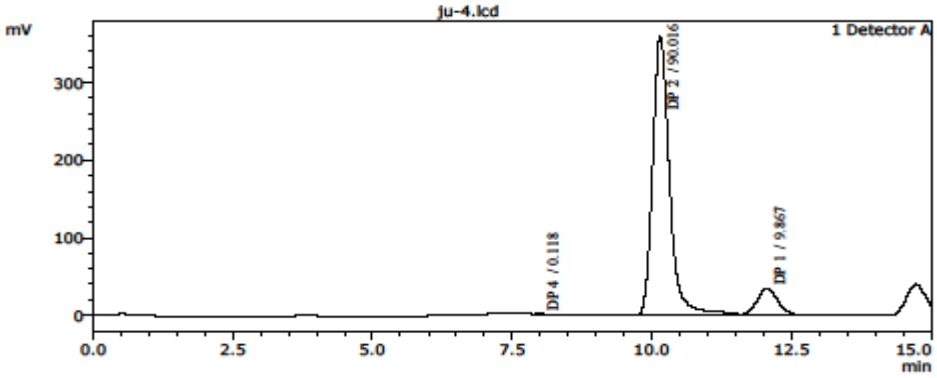
Note: Here DP means Dextrose Profile.

Figure 19: HPLC report (carbohydrate profile) of Frutica mango juice:

Carbohydrate profile of some selected brand of fruit juice of BD.

Sample Information

Acquired by	: System Administrator
Sample Name	: Mangolee Mango juice, AST beverage ltd.
Sample ID	: BN:47600, MFD: 03.06.2013
Injection Volume	: 20
Data File	: ju-4.lcd
Method File	: LIQUID GLUCOSE.lcm
Date Acquired	: 7/12/2013 12:05:22 PM
Date Processed	: 7/12/2013 2:07:59 PM
Comment	: 25 % solution



Peak Table

Detector A

Peak#	Name	Ret. Time	Area	Height	Area%
1	DP 4	8.009	10059	397	0.118
2	DP 2	10.152	7695618	359355	90.016
3	DP 1	12.064	843519	33613	9.867
Total			8549197	393365	100.000

Description of the above report: In homemade juice; Tetraose (Dextrose Profile 4) percentage was 0.118, Sucrose (DP2) percentage was 90.016 and Glucose or fructose (DP 1) percentage was 9.867.

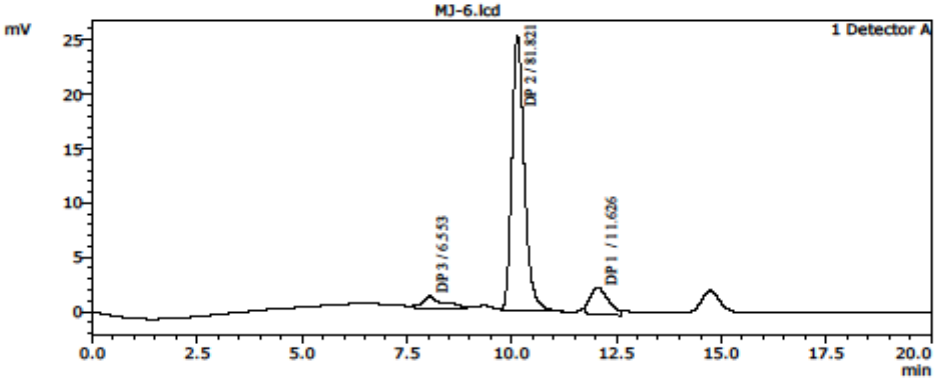
Note: Here DP means Dextrose Profile.

Figure 20: HPLC report (carbohydrate profile) of Mangolee (Mango Juice) 250 ml:

Carbohydrate profile of some selected brand of fruit juice of BD.

Sample Information

Acquired by	: System Administrator
Sample Name	: Shezan Classic Mango Drinks (Hashem Foods Ltd.)
Sample ID	: BN:01 MFD: 14.11.2013
Injection Volume	: 20
Data File	: MJ-6.lcd
Method File	: iNVERT SUGAR.lcm
Date Acquired	: 2/7/2014 10:45:45 AM
Date Processed	: 2/7/2014 2:48:13 PM
Comment	: 1 % solution.



Peak Table

Detector A

Peak#	Name	Ret. Time	Area	Height	Area%
1	DP 3	8.041	41965	1202	6.553
2	DP 2	10.144	523976	25191	81.821
3	DP 1	12.064	74455	2406	11.626
Total			640396	28800	100.000

Description of the above report: In Shezan Classic mango juice; Triose (DP3) percentage was 6.553. Sucrose (DP2) percentage was 81.821 and Glucose or fructose (DP 1) percentage was 11.626.

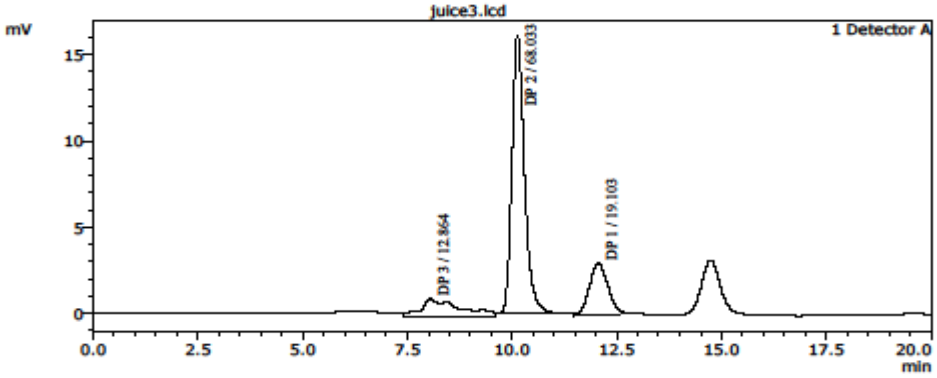
Note: Here DP means Dextrose Profile.

Figure 21: HPLC report (carbohydrate profile) of Shezan Classic mango juice:

Carbohydrate profile of some selected brand of fruit juice of BD.

Sample Information

Acquired by	: System Administrator
Sample Name	: Star Ship Mango (Fruit Juice)
Sample ID	: MFD: 27.04.2013 T13:32
Injection Volume	: 20
Data File	: juice3.lcd
Method File	: iNVERT SUGAR.lcm
Date Acquired	: 2/24/2014 3:06:19 PM
Date Processed	: 2/24/2014 3:57:40 PM
Comment	: 1 % solution.



Peak Table

Detector A

Peak#	Name	Ret. Time	Area	Height	Area%
1	DP 3	8.046	63366	982	12.864
2	DP 2	10.134	335113	16071	68.033
3	DP 1	12.059	94096	2975	19.103
Total			492575	20028	100.000

Description of the above report: In Starship (Fruit Juice) mango juice; Triose (DP3) percentage was12.864. Sucrose (DP2) percentage was 68.033 and Glucose or fructose (DP 1) percentage was 19.103.

Note: Here DP means Dextrose Profile.

Figure 22: HPLC report (carbohydrate profile) of Starship (Fruit Juice) 200 ml:

4.8 Total viable count of collected juice samples:

Microbial count of different fruit juices were shown in the Table 09. From the results it is clear that total viable count (microbial load) showed the presence of bacteria in Maximum samples fruit juices in the range of 1×10^3 - 3×10^3cfu/ml. Maximum samples contained lower load of microbes than the Gulf standard (Gulf Standards. 2000) for foods described in Table 3. Faecal coliform was not present in any samples. From Table 09, it can be found that Frutica (Mango fruit Drinks) 250 ml pack contained 3×10^3 cfu/ml. Frutoo (Mango fruit Drinks) 250 ml pack contained 1×10^3cfu/ml, Mangolee (Mango Juice) 250 ml pack contained 2×10^3cfu/ml, Shezan Classic (Mango Drinks) 250 ml pack contained 3×10^3 cfu/ml respectively. There was no viable count recorded in the sample homemade mango juice, Starship (Fruit Juice) 200 ml pack and ACME Mango Drinks. Fruit & fruit juices are commonly contaminated with bacteria, often from insect damaged. Sweeting agent, thickening agent, mango pulp, flavoring agent, water and other chemical were potential sources of microbial contamination. Bacteria that has been associated with spoilage in soft drink industries include *Acetobacter*, *Bacillus*, *Clostridium*, *Gluconobacter*, *Lactobacillus*, *Leccuonostoc*, *Saccharobacter*, *Zymobacter* and *Zymomonas* . *Gluconobacter* is common spoilage agent of fruit juices. (Neha et al., 2011). It is possible that microorganism may have gained entry through water because some producer do not use treated water or maintain aseptic condition in production floor always. Most of juice manufacturer use mango pulp which was preserved with the chemical preservative like sodium benzoate, so if we do not get any viable count, it doesn't mean that producer maintain their qualities as per GMP or HACCP recommended rules. The entry of some microorganism in juices may be attributed to contact with the outer surface of fruits during juicing, survival with lack of proper production environment and not maintenance of aseptic filling condition. Furthermore, these fruit juices should kept in cool temperature, but most of retail shop keeper have no refrigerator or continuous electricity supply which may have led to the proliferation of contaminating bacteria resulting in increased bacterial counts. It was thus concluded that the lack of hygiene, holding time during the preparation of fruits juice and contaminated source of ingredients were major detrimental for the quality. Poor hygienic quality of these juices and consumers are placed at a risk of contracting clearly food borne infections (Ahmed et al., 2011)

4.8.1 Table 09: Total viable count of collected juice samples:

Sl no.	Name of Juice	TVC (CFU/10 $^{-3}$ml)	TVC (CFU/ml)
1	Homemade mango juice	0	0
2	Frutica (Mango fruit Drinks)250 ml	3	3.0×10^{3}
3	Frutoo (Mango fruit Drinks)250 ml	1	1.0×10^{3}
4	Mangolee (Mango Juice)250 ml	2	2.0×10^{3}
5	Starship (Fruit Juice) 200 ml	0	0
6	Shezan Classic (Mango Drinks) 250 ml	3	3.0×10^{3}
7	ACME Mango Drinks	0	0

4.9 *Escherichia coli* count in collected juice samples:

Escherichia coli were present only in one sample. *Escherichia coli was absent in* Mangolee (Mango Juice) 250 ml pack , Frutica (Mango fruit Drinks) 250 ml pack, Shezan Classic (Mango Drinks) 250 ml pack, Homemade mango juice, Starship (Fruit Juice) 200 ml pack and ACME Mango Drinks pack . Frutoo (Mango fruit Drinks) 250 ml contained 1×10^{3}cfu/ml pack.

4.10 Fecal coliform count in collected juice samples:

Fecal coliform was not present in any samples.

Figure 23: Growth on Nutrient Agar media at 37°C of Frutica mango juice.

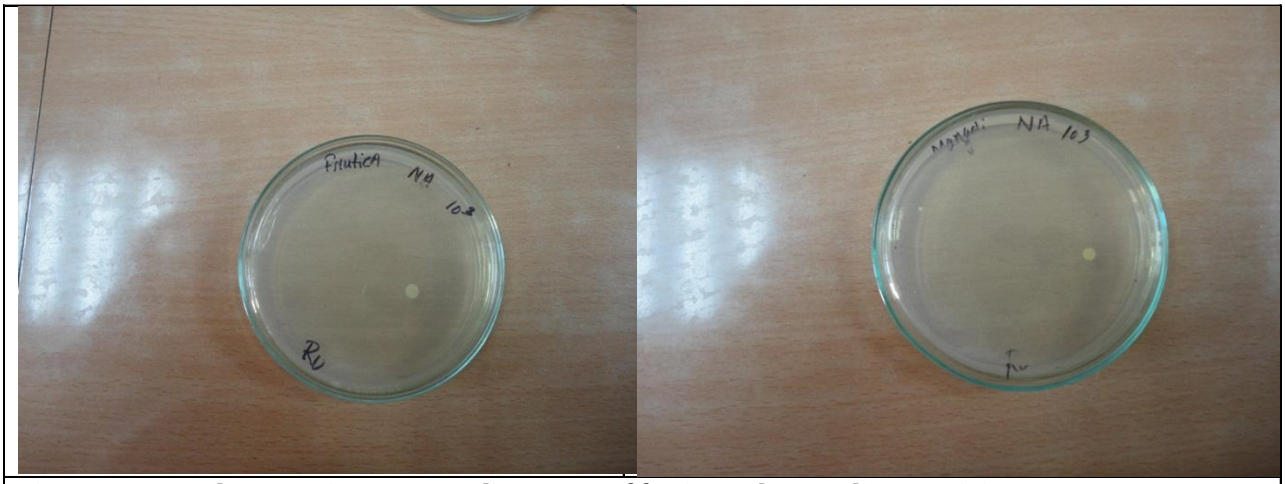


Figure 24: Growth on Nutrient Agar media at 37°C of frutica and Mangoli mango juice.

Figure 25: Growth on Nutrient Agar media at 37°C of frutica and shejan mango juice.

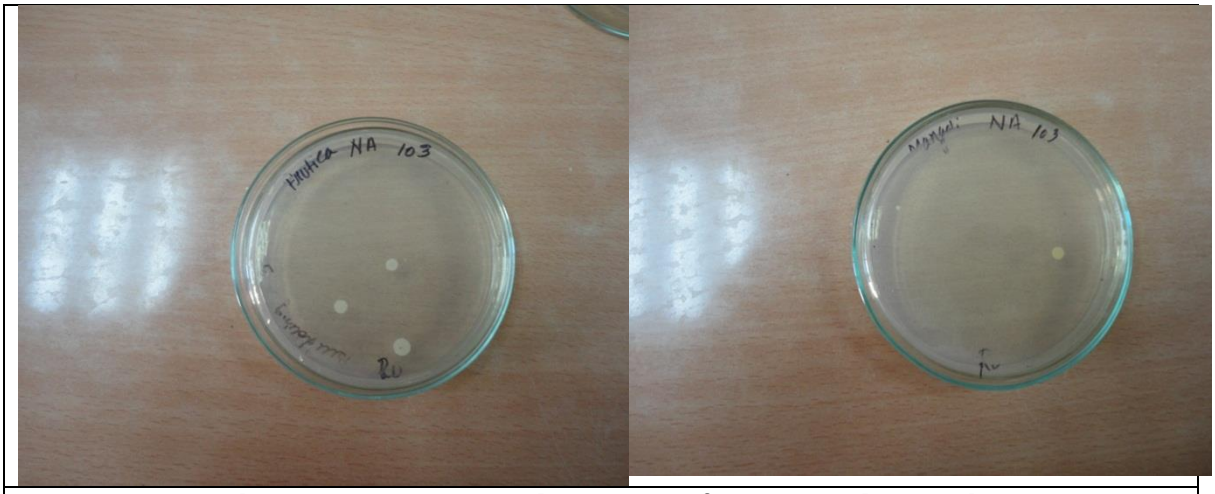


Figure 26: Growth on Nutrient Agar media at 37°C of Frutica and Mangoli mango juice.

CHAPTER- 5

DISCUSSION:

Though there were many different fruit drinks available in Bangladesh market and their test, quality and nutrition properties are also different. For the limitation of time, source of equipment and laboratory facilities, only mango juice was taken into consideration for this study. Mango juices offer the potential benefits for health, their safety and quality need to be maintained. An estimate of the gross nutrient composition of the juices is shown in Table 2. There appear to be some differences between the juices of different companies are taken for this study (Table 4). Most of the common juices were low in protein. In this study, protein content (0.8%) in Frutoo (Mango fruit Drinks) 250 ml was comparatively higher than in other juices (Table 6). Saeed et al. (2012) observed in Sample I contained (0.175 %), in Sample II (0.175 %), in Sample III (0.145 %) Sample IV (0.016 %), Sample V (0.133 %), and in Sample VI (0.179 %.). In this study protein percentage was similar or slightly lower than the result observed that study. As per this study, homemade juice contained only (0.1 %) protein, Frutica had (0.11%), Frutoo contained (0.18 %), Starship had (0.12 %), ACME juice, Mangolee and Shezan Classic contained (0 %) protein or protein content was not detected. There is huge difference between this study result and the result of Saeed et al. (2012), specially for TSS (Total soluble soiled). As per the observation of that study, Sample I contained (5.1 %), Sample II (9.8 %), Sample III (6.5 %) Sample IV (5.1 %), Sample V (12.9 %), and Sample VI contained (10.3 %). As per the observation of that study, there was unequal variance in juice sample. Lowest TSS was 5.1 (sample I and IV) and highest TSS was 19.0 (Sample V) was observed in that study whereas the lowest was found 12.0 and highest TSS was 12.9 in this study. So, the juice samples taken for this study were maintained average TSS which were within limit of Bangladesh regulatory authority (BSTI). The TSS contented of Mangolee (Mango Juice 250 ml) juices and homemade mango juice in this study was higher than that of other juices (Figure 13).

Fruit juices have a low pH because they are comparatively rich in organic acid. As per the observation of that study, p^H of Sample I was 4.21, Sample II p^H 4.26, Sample III p^H 4.56, Sample IV p^H 4.4, Sample V p^H 4.56 , and Sample VI p^H 4.6. In this study, the pH of the fruit juices varied from 3.55 to 3.80 (Figure 10). The highest pH was shown in Starship (Fruit Juice) 200 ml, followed by Homemade mango juice (3.77), Mangolee (Mango Juice) 250 ml (3.76), ACME Mango Drinks (3.65), Frutica (Mango fruit Drinks) 250 ml

(3.63), Frutoo (Mango fruit Drinks) 250 ml (3.55). The levels of p^H of all the samples were within the limits of BSTI standard for fruit juice (BDS 1581, BSTI. 2002).

As per the observation of the study of Saeed et al. (2012) result in $p^{H,}$ juice contained slightly high p^H (4.21 to 4.56) whereas in this study observed (3.55 to 3.80). The total acidity of fruit juices was due to the presence of a mixture of organic acids, whose composition varies depending on fruit nature and maturity of used pulp or used ingredients that were added during juice processing. Organic acids took the lead in importance for characteristics and nutritive value of fruit juices and deliberated individual originality among beverages. For acidity parameter observed by Saeed et al. (2012); Sample I contained 0.189 %, Sample II contained 0.102 %, Sample III 0.098 % Sample IV 0.203 %, Sample V 0.231 %, and Sample VI 0.259 %. In the present study, total titrable acidity varied significantly in different juice samples (Table 5). Maximum content of total titrable acidity (0.24%) was recorded for Frutoo (Mango fruit Drinks) 250 ml while it was minimum (0.21%) in Mangolee (Mango Juice) 250 ml and ACME Mango Drinks. Many producers added ascorbic acid to their products to make up acidity; this could be the cause for similar content of acid (0.21 to 0.24) in mango juices in this study. There was similarity for acidity with others result and the result of this study. The variations in reducing sugar of the samples may be attributed to the formulations of each manufacturer. As per Table 07; Homemade juice contained (3.76%) TRS, (5.85%) TRS was in Frutica, Frutoo (Mango fruit Drinks) 250 ml contained (6.87%) TRS, (3.6 %) TRS was in Mangolee, (4.39 %) TRS was in Starship, (6.8 %) TRS in Shezan Classic and ACME juice contained (5.3 %) TRS. The combined effect of stages of maturity and ripening conditions significantly affected the reducing sugar and total sugar content of the fruit pulp and juices. As per the observation of Tasnim et al. (2010), the highest quantity of reducing sugar (9.99%) in mango juices and (2.24%) was (lowest) in orange juices. In this study (6.87%) reducing sugar was found in Frooto mango juice and 3.6% was lowest in Mangolee mango juice. Carbohydrate Profile was analyzed by the HPLC and dextrose profile was segregated from others Oligosaccharides. Homemade juice contained (5.648 %) monosaccharides which was very low percentage and it came only from mango pulp because only sugar was used when homemade juice was produced and did not use glucose or fructose or any invert sugar. So, it can be said that average (5%) monosaccharides come from mango pulp. So, those juice contained more monosaccharide means were produced with more mango pulp or glucose fructose was used more. Dextrose profile 1 indicates monosaccharide in the graph of HPLC generated result. The juices which contained high quantity of Dextrose profile 1 (DP 1) are more preferable for consumer because consumer

would get instant energy from them. Frutica (Mango fruit Drinks 250 ml) contained (27.69%) monosaccharides. Frutoo (Mango fruit Drinks 250 ml) pack contained (41.27%). Mangolee (Mango Juice 250 ml) contained (9.867%). ACME Mango Drinks contained (58.88%). Shezan Classic (Mango Drinks) 250 ml contained (11.626 %) and Starship (Fruit Juice) 200 ml contained (19.103%) monosaccharides. Plant foods, especially plant juices, tend to have redox potential (Eh) values from 300 to 400 millivolt. Having such a high redox potential is an indication for availability of sufficient amount of free oxygen accessible to aerobic microbes. Thus, the survival and growth of aerobic bacteria and molds in such products are high and with the same microbial groups being responsible for spoilage of the same products. The range of microbial counts (1×10^1 to 3×10^1cfu/ml) recorded in the fruit juices analyzed in this study was relatively lower than the microbial load reported in some earlier works.

As per the study of Tasmina et al. (2011) found TVC $3.2x10^2$ to $2.4x10^4$cfu/ml, *Staphylococcal* count as $4.9x10^2$ cfu/ml with the range from 0 to $2.3x10^3$ cfu/ml, *Bacillus* count was $7.3x10^2$ cfu/ml and it ranged from $1x10^2$ to $2.0x10^3$cfu/ml in different types and brands of fruit juice samples. The study of TsigeKetema found TVC $6.2x10^3$ - $3.1x10^7$ CFU/ml. TVC and other microbes were higher than the recorded TVC of this study. However, the recommended specifications for fruit juices served in the Gulf region suggests that the maximum count permitted for total colony count of coliforms, yeast and molds are $1x10^4$, 100, and $1x10^3$ CFU/ml respectively. On the basis of the Gulf standards, it is clear that the colony counts of almost all the microbial groups in fruit juices collected for this study have not exceeded the standard. In this study, Total viable count of different samples varied from 1×10^1 to 3×10^1cfu/ml. No *Fecal coliform* could be detected in these juices. *E. Coli* was detected only in one sample and it was 1×10^1 cfu/ml. The equipment used for processing of the juices could contribute to the number of bacterial and fungal species. We know that chemical preservatives significantly decreased the microbial load in fruit juices. Regulating the microbial safety of facilities to be used for processing and the use of good quality fruits and surface disinfection besides cleaning with pure water could certainly improve the microbiological quality of the juices. For longer shelf-life and safety of the juices against fungi and moulds, producers generally use of an approved food additives. Many organic acids with Generally Regarded as Safe (GRAS) status have been currently used for preservation of many foods and juices. However, these low counts may not necessarily pose hazard to the health of consumers.

CHAPTER: 06

CONCLUSION:

This work has shown that the locally available fruit juices contain safe levels of nutritional and microbial elements for human consumption, but quality level is not in very good position. Each juice provides a different range of nutritional components. Each and every juice manufacturer should maintain the quality parameter limit of nutrition and food value. Some juice maintain lower limit of parameter though those were within limit range which was mentioned by BSTI. Basic quality parameter like P^H, TSS, Total fat content, protein content, acidity, acid value and carbohydrate profile was maintained within limit but that was not sufficient. Producer should increase food value of this popular fruit drinks by providing new technology, innovation and serving mankind welfare instead of only gaining profit. The microbial growth was found less frequently among some fruit juice samples, all the juice samples are not free from the microbial loads. From the data presented in the current study, it can be concluded that the nutritional quality have a variance for deference companies juice. About 100% of the samples recorded an acceptable range based on the Gulf standards for fruit juices. They had shown that the presence of pathogenic organisms such as *E. coliform*, *Fecal coliform* and fungus were not detected and considered safe for consumption. TVC were found in some juices for the lack of monitoring and maintaining GMP (Good Manufacturing Practice) .Government-authorized institute is responsible for low quality mango juice. Most of the Bangladeshi companies use pulp which was treated with preservative and use hot filling unit or aseptic filling system, but there is many other technology like Hyperbaric Pressure, Hyperbaric + CO_2, Pulsed electric field, Ultrasonic Membrane, Pulsed light, Magnetic field, Irradiation can be used instead of old technology. Some preservative is very harmful for human body and causes cancer. The Government-authorized institute such as BSTI should undertake preventive investigations to check the microbial and chemical quality of the fruit juices to improve the quality of fruit juice. Government authorized department should take initiatives for providing training to the technical person of company to increase producers awareness on maintaining the rules and regulation of GMP, HACCP and FSSC for juices production. They can take initiative for increase awareness of consumer for checking batch manufacturing date before consume juice.

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Appendix –A

Different type of Media used

Nutrient agar (Himedia) Composition: gm/ litter	
Peptic digest of animal tissue	5.000
Sodium chloride	5.000
Beef extract	1.500
Yeast extract	1.500
Agar	15.000

Use: For cultivation of less fastidious microorganism, can be enriched blood or other fluid

P^H at 25 ^{0}C 7.4 ±0.2

MacConkey agar (Himedia) Composition: gm/ litter	
Tryptose	10.000
Proteose peptone	5.000
Yeast extract	3.000
Lactose	12.500
Bile salts mixture	1.500
Sodium chloride	5.000
Aniline blue	0.100
Agar	15.000

Use: For selective isolation and differentiation of coliform organisms and other enteric pathogen.

P^H at 25 ^{0}C 7.1 ±0.2

M-FC Agar Base (Himedia)

Composition: gm/ litter

Tryptose	**10.000**
Proteose peptone 5.000	**5.000**
Yeast extract	**3.000**
Lactose	**12.500**
Bile salts mixture	**1.500**
Sodium chloride	**5.000**
Aniline blue	**0.100**
Agar	**15.000**

Use: For the detection and enumeration of fecal coliforms using membrane filter technique at higher temperature (44.5°C).

P^H at 25 ^{0}C 7.4 ±0.2

Appendix –B

Reagent

Fehling's solution

Composition

Fehling's reagent consists of two solutions.

Solution A contains 7% CUSO4 and solution B contains 25% KOH and 35% sodium potassium tartarate.

When the two solutions are mixed in equal amounts a clear blue solution results for the tartrate forms a soluble complex with the copper hydroxide produced. this solution is

widely used as an oxidizing agent preferably in

Quantitative sugar determinations.

Use: Fehling's solution is a chemical reagent used to differentiate between water-soluble carbohydrate and ketone functional groups, and as a test for reducing sugars

0.5% CuSO4 solution Composition
This is 0.5g of CuSO4 in 100mL of solution

10% NaOH Composition
4g NaOH pellets in 1 liter of water is a 0.1M solution, or a 0.4% solution of NaOH and 10g NaOH pellets in 1 liter of water is a 10% W/V solution.

Use: 0.5% $CuSO_4$ & 10% NaOH solution are chemical reagent used to identification of Protein in juice.

LIST of other Reagent.

1	5% glucose solution
2	5% fructose solution
3	5% sucrose solution
4	5% maltose solution
5	5% malt triose solution
6	5% maltotetraose solution
8	Citric acid
9	Malic acid
10	Tartaric acid
11	Dextrose anhydrous GR
12	Methylene Blue Indicator Solution
13	ammonium sulfate
14	boric acid
15	ammonia gas
16	phenolphthalein
17	hydrochloric acid
18	Ether solution
19	methanol
20	Diethyl ether
21	petroleum ether

Appendix –C

The Important equipment's used through the study are listed below

Name of Equipment	Origen
Autoclave	SAARC
Incubator	SARRC
Micropipette (10-100 µl)	Eppendorf, Germany
Micropipette (20-200 µl)	Eppendorf, Germany
Oven, Model: MH6548SR	LG, China
Safety cabinet Class II Microbiological	SAARC
Vortex Mixture	VWR International
pH meter Model: Mettler Toledo	Switzerland
Abbe refractometer, Model : DR-A1	ATAGO CO, Japan
High performance liquid Chromatograph (HPLC) Model No. CTO20A	SHIMADZU, Japan
Soxhlet extractor	Japan
Weighing Balance	Japan

LIST IF ABBREVIATION & SYMBOLS

Sr.No.	TITLE	ABBREVIATION
1	AOAC	Association of Official Agricultural Chemists
2	CH3CH2OH	Ethanol
3	C6H12O6	Glucose
4	° C	Celsius
5	DP	Dextrose profile
6	H2O	Water
7	H2SO4	Sulfuric Acid
8	TVC	Total viable count
9	TFC	Total Fecal coliform count
10	TSS	Total soluble solids
11	TRS	Total Reducing Sugar
12	w/v, v/v	Weight per Volume, volume per volume
13	(NH4)2SO4	Ammonium sulphate
14	Wt, ml, kg, g	Weight, Milliliter, Kilogram, Gram
15	SO2	Sulfer dioxide
16	TSS	Total soluble solids
17	CFU	colony-forming unit
18	sp., et al	Species , and friends
19	C6H12O6	Glucose
20	US$	United States dollar
21	MT	Metric ton