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A Study on Microbiogolical Status of Some Selected Brands of Mango Juice

Hania Sharmin
Food and Nutrition Department, KCC Women's College
(Affiliated by Khulna University), Khulna- 9100, Bangladesh

Nasrin Nahar Begum
Chairman of the Examination Committee
Assistant professor & Head of Food and Nutrition Department, KCC Women's College
(Affiliated by Khulna University), Khulna- 9100, Bangladesh

Dr. S.M. Mahbubur Rahman*
Head of Biotechnology and Genetic Engineering Discipline, Life Science School
Khulna University, Khulna- 9100, Bangladesh

Abstract
The present investigation was conducted at the Plant Biotechnology Laboratory of Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna, Bangladesh. Seven samples were collected from different manufacturing companies which were commercially packed juices available in the markets. In this study, physicochemical properties (pH, TDS, EC) of juices were determined by using multi meter. Standard culture techniques were followed to assess total viable count (TVC) and confirmation of*Escherichia coli*. In this study, the pH of the fruit juices varied from 2.80 to 3.80. The highest quantity of Electric Conductivity (EC) 1293µs/cm was recorded in Starship Mango juice while the lowest were recorded in Pran and Frutica mango juices.The TDS content of all samples varied from 1.23 to 70.3. Total viable count of different types of fruit juices varied from 1×10^3 - 5×10^3cfu/ml. *E. coli* was 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 microbiological elements for human consumption, but not in very good position.

Introduction

There are many fruits grow in Bangladesh, however mango is the most popular because of its delicious and nutritious properties. 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 mango juice is becoming very popular day by day to all. As a result many fruit juice company were 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 (250ml) 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 (250ml). 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, 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, molds 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, *Clostridiumbotulinum*, *Escherichiacoli*, *Shigellaspp*., *Salmonella spp*., *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Campylobacterjejuni*, *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).

The aim 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. The aim of this study was to assess the physico-chemical and microbiological quality of commercially packed available mango juices collected from different locations of Khulna city.

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. Therefore this thesis work has been taken for the following objectives:

1. To study TVBC (Total Viable Bacterial Count) of some selected brand of mango juices available in local market.
2. To analyze physicochemical properties of some selected brand of mango juice available in local market.

Materials & Methods:

The present investigation was conducted at the Plant Biotechnology Laboratory of Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna, Bangladesh. The materials and methodologies used in this research are presented as follows:

Sample collection:

Among seven juices samples are collecting which are commercially packed juices available in market. They are store in freeze at normal temperature or without freezing.

Table 1: The sampling brands and collection place of mango juices are listed:

Serial No.	Name of Juice	Name of Manufacturer	Collection place
1	PRAN junior (Mango fruit Drinks)200ml	Pran dairy Ltd	Khulna
2	Frutica (Mango fruit Drinks)250 ml	Akij Food and Beverage Ltd.	Khulna
3	Frutoo (Mango fruit Drinks)250 ml	Pran Dairy Ltd	Khulna
4	Mangolee (Mango Juice)250 ml	AST Beverage Ltd.	Khulna
5	Starship (Fruit Juice) 200 ml	AbulKhair Condensed Milk and Beverage Ltd.	Khulna
6	Shezan (Mango Drinks) 250 ml	Hashem Foods Ltd.	Khulna
7	ACME Mango Drinks	ACME agro and Beverage Ltd.	Khulna

Determination of p^H

In chemistry, p^H 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. 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).

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

Total Dissolve Solids

In the juice total dissolve solids was measured. These dissolve solids were primarily sugars; sucrose, fructose, and glucose. Citric acid and minerals in the juice also contributed to the dissolve solids.

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 colors were eliminated by rotating screw head of compensator until sharp, colorless line is obtained. Borderline was adjusted so that it felt on point of intersection of cross – hairs. Dissolve Solid Content (DSC, %) was measured using multi meter calibrated against sucrose. The dissolved solids are measured with the help of a multi meter.

Total Electric Conductivity

In the juice total electric conductivity was measured through multi meter. The meter was set up for measuring samples electric conductivity. First switch on the instrument (multi meter); the sample were placed on the beaker and the meter were read directly on scale of sector. Then the meter gives the accurate result of electric conductivity of every juice samples in unitμs/cm.

Microorganism which are mostly involved with juice

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 mold species. There are different test parameters for different types of samples. For juice samples nutrient agar media was used for counting TVC. 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.

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.

Table 2: Bacterial count pattern of different brands in direct method and Serial dilution method

Process	Conducted media	Procedures	Incubation time	Bacterial count cfu/10-3 ml
Direct method	Nutrient agar	Samples were taken directly to the media and incubated.	121°C for overnight	Pran- 5, Frutica- 2, Frutoo-3, Mangolee-4, Starship-1, Shezan-3, ACME-1
Serial dilution	MacConkey agar	Samples were taken to maintain serial dilution and then incubated	121°C for overnight	Pran>Mangolee> Shezan>Frutoo> Frutica> Starship>ACME

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 plate count agar media. Total viable count (TVC) was performed by pour plate method using Nutrient agar, which was incubated over night at 37^0 C. MacConkey agar is used for confirmation of *E.coli*over night at 37 C.

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.

Results

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 microbiology parameters.

p^H of various juice samples

Fruit juices have a low p^H because they are comparatively rich in organic acid. The overall range of p^H is 2 to 5 for common fruits with the most frequent figures being between 3 and 4. In this study p^H of the fruit juices varied from 2.80 to 3.80. The highest p^H (3.80) was found in Starship (Fruit Juice) 200ml pack and followed by Mangolee 250ml pack, ACME (Mango Juice) 250 ml pack, Frutica (Mango fruit Drinks) 250 ml pack, Shezan (mango juice) 250ml, Pran junior (mango juice) 200ml pack, Frutoo(Mango fruit Drinks) 250 ml pack respectively.

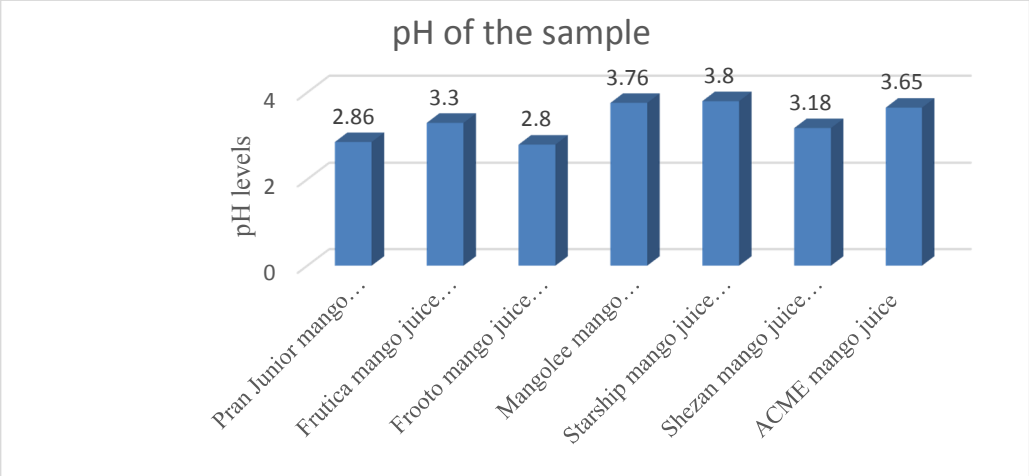

Figure 1: p^H of various juice samples

Total dissolves solids of collected juice samples:

The TDS content is significantly influenced by solid materials dissolved in water in the juice. If any of the ingredients like mango pulp, sugar, glucose was less in juice then TDS will be less. Sometimes producer added sweetening agent instead of adding sugar and glucose.TDS of Starship juice was 1.23 which was the lowest value. TDS of Frutica was 2.63, Frutoo was 1.95, Mangolee contained 53.9, Pran junior 5.99, Shezan Classic had 4.57 and 70.3 been recorded in ACME juice, which was the highest value.

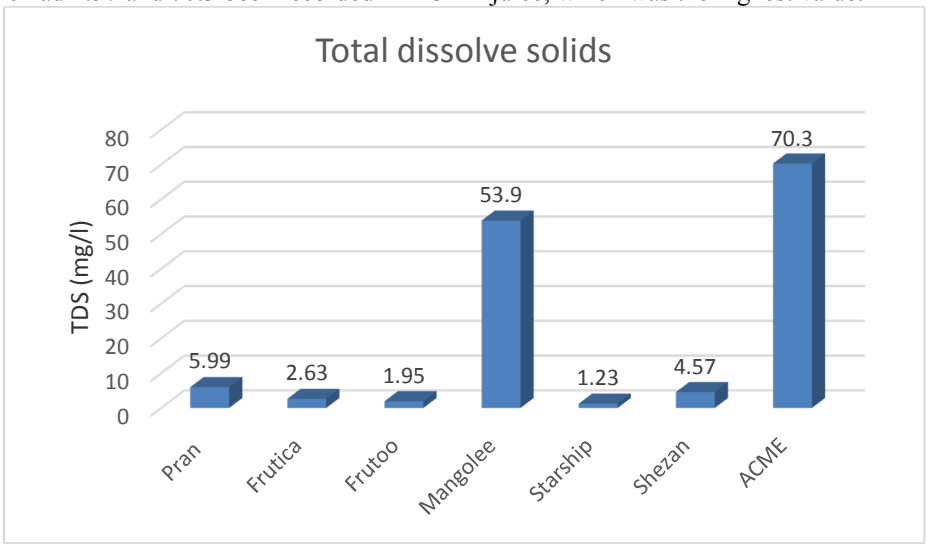

Figure 2: Total dissolve solids of various juice samples

Total Electric Conductivity (EC) of collected juice samples:

In this research the lowest count of electric conductivity was found in Pran mango juice (988µs/cm) and the highest was found in Starship mango juice (1293µs/cm).

Table 3: Total Electric Conductivity

Sl. No.	Name of the juices	Total electric conductivity
01	Pran junior	988 µs/cm
02	Frutica	991 µs/cm
03	Frutoo	1046 µs/cm
04	Mangolee	1054 µs/cm
05	Starship	1293 µs/cm
06	Shezan	1072 µs/cm
07	ACME	1069 µs/cm

Total viable count of collected juice samples:

Microbial count of different fruit juices were shown in the Table 08. From the results it is clear that total viable count (microbial load) showed the presence of bacteria in all samples of fruit juices at a range of 1×10^3-5×10^3cfu/ml. All the samples contained similar load of microbes of the Gulf standard (Gulf Standards. 2000) for foods described in Table 3. From Table 08, it can be found that Frutica (Mango fruit Drinks) 250 ml pack contained 2×10^3cfu/ml. Frutoo (Mango fruit Drinks) 250 ml pack contained 3×10^3cfu/ml, Mangolee (Mango Juice) 250 ml pack contained 4×10^3cfu/ml, Shezan Classic (Mango Drinks) 250 ml pack contained 3×10^3cfu/ml, Pran junior contained 5×10^3cfu/ml respectively. There was less viable count recorded in the sample starship mango juice 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.

Table 4: Total viable count of collected juice samples:

Serial No.	Name of the juice	TVC (CFU/10^{-3}ml)	TVC (CFU/ml)
1	Pran junior (Fruit Juice) 200 ml	5	5.0×10^3
2	Frutica (Mango fruit Drinks)250 ml	2	2.0×10^3
3	Frutoo (Mango fruit Drinks)250 ml	3	3.0×10^3
4	Mangolee (Mango Juice)250 ml	4	4.0×10^3
5	Starship (Mango juice)200ml	1	1.0×10^3
6	Shezan Classic (Mango Drinks) 250 ml	3	3.0×10^3
7	ACME Mango Drinks	1	1.0×10^3

Escherichia coli count in collected juice samples:

Escherichia coli were present in four sample such as Pran (Mango juice) 200ml pack, Frutoo (Mango juice) 250ml pack, Mangolee (Mango juice) 250ml pack and Shezan (Mango juice) 250ml pack. *Escherichia coli* was absent in Frutica (Mango fruit Drinks) 250 ml pack, Starship (Mango Drinks) 200 ml pack and ACME Mango Drinks pack.

Manufacturer Company based total viable bacterial count:

Most of juice manufacturer company 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. Poor hygienic quality of these juices and consumers are placed at a risk of contracting clearly food borne infections (Ahmed et al., 2011).

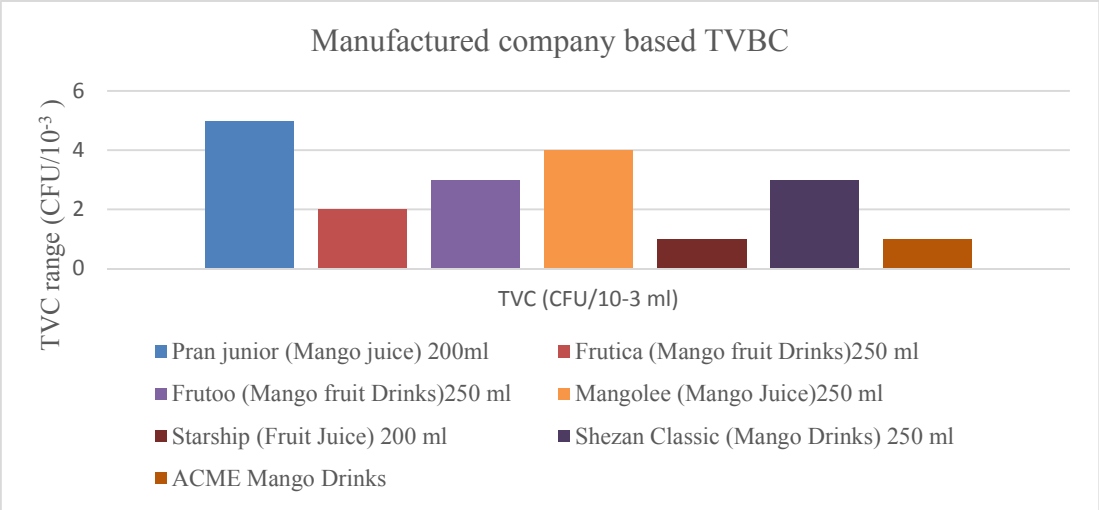

Figure 3: Total viable count (TVBC) in selected Mango Juice Company

The data show that the Pran contained 5 CFU/10^{-3}viable bacteria which manufactured by Pran Dairy Ltd. This juice contained maximum viable bacteria than the other juices selected for this research; it may be their processed fault either this experiment. Mangolee mango juice contained 4 CFU/10^{-3}viablebacteria, which is manufactured by AST Beverage Ltd. Frooto and Shezan Classic mango juice contained 3 CFU/10^{-3} viable bacteria, which are manufactured by Pran Dairy and Hashem Foods Ltd. Frutica, which is manufactured by Akij Food and Beverage Ltd. contained 2 CFU/10^{-3}, other samples of juice Starship and ACME, which are manufactured by Abulkhair condensed Milk and Beverage Ltd. and ACME agro and Beverage Ltd. contained 1 CFU/10^{-3}respectively. So, at the aspect these are better juice than Pran junior and Mangolee mango juice and more preferable than other branded juices which are selected for this research in respect of TVBC.

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. There appear to be some differences between the juices of different companies are taken for this study.

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 2.86, Sample II p^H 3.30, Sample III p^H 2.80, Sample IV p^H 3.76, Sample V p^H 3.80, Sample VI p^H 3.18 and Sample VII pH 3.65. In this study, the pH of the fruit juices varied from 2.80 to 3.80 (Figure 06). The highest pH was shown in Starship Mango Juice (3.80) 200 ml, followed by Mangolee mango juice (3.76), ACME Mango Juice 250 ml (3.65), Frutica Mango Drinks (3.30), Shezan Classic Mango fruit Drinks 250 ml (3.18), Pran Junior Mango fruit Drinks 250 ml (2.86). The levels of p^H of all the samples were within the limits of BSTI standard for fruit juice (BDS 1581, BSTI. 2002). Fruit juice have a low and high TDS. As per the observation of that study, Sample I contained (5.99), Sample II (2.63), Sample III (1.95) Sample IV (53.9), Sample V (1.23), Sample VI contained (4.57), and Sample VII contained (70.3).As per the observation of that study, there was unequal variance in juice sample. Lowest TDS was 1.23 Sample V (Starship) and highest TDS was Sample VII (ACME) was observed in that study. So, the juice samples taken for this study were maintained average TDS which were within limit of Bangladesh regulatory authority (BSTI). The TDS contented of Mangolee (Mango Juice 250 ml) juices and ACME mango juice in this study was higher than that of other juices (Figure 05).The electric conductivity of the juice samples of this research are Pran junior Mango juice was 988µs/cm, Frutica Mango juice was 991µs/cm, Frooto Mango juice was 1046µs/cm, Mangolee Mango juice was 1054µs/cm, Starship Mango juice was 1293µs/cm, Shezan mango juice was 1072µs/cm, ACME Mango juice 1069µs/cm respectively (Table 3)

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 (1×10^3 to 5×10^3cfu/ml) recorded in the fruit juices analyzed in this study was relatively lower than the microbial load reported in some earlier works (Table-08).

From manufacturer company based the highest TVBC count from Pran contained 5 CFU/10^{-3}viable bacteria which manufactured by Pran Dairy Ltd. This juice contained maximum viable bacteria than the other juices selected for this research; it may be their processed fault either this experiment. Mangolee mango juice contained 4 CFU/10^{-3}viablebacteria, which is manufactured by AST Beverage Ltd. Frooto and Shezan Classic mango juice

contained 3 CFU/10^{-3} viable bacteria, which are manufactured by Pran Dairy and Hashem Foods Ltd. The lowest TVBC count from Frutica, which is manufactured by Akij Food and Beverage Ltd. contained 2 CFU/10^{-3}, other samples of juice Starship and ACME, which are manufactured by Abulkhair condensed Milk and Beverage Ltd. and ACME agro and Beverage Ltd. contained 1 CFU/10^{-3}respectively.

Conclusion

This work has shown that the locally available mango juices contain safe levels of physicochemical and microbial elements for human consumption, but quality level is not in very good position. Mango juice provides a different range of nutritional components. Each and every mango juice manufacturer should maintain the quality parameter limit of nutrition and food value. Some branded mango juice maintain lower limit of parameter though those were within limit range which was mentioned by BSTI. Basic quality of physicochemical parameter like P^H, TDS, and Electric Conductivity (EC) was maintained within limit but that was not sufficient. Producer should increase food value of this popular mango drinks by providing new technology, innovation and serving mankind welfare instead of only gaining profit. The microbial growth was found less frequently among some mango 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 physicochemical parameter have a variance for deference companies juice. About 100% of the samples recorded an acceptable range based on the Gulf standards for mango juices. 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. 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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