MICROBIAL AND CHEMICAL QUALITY ANALYSIS OF INDUSTRIALLY PROCESSED MANGO JUICE AVAILABLE IN TANGAIL SADAR UAZILA, BANGLADESH

Natasha Khanam[1], Md. Younus Mia[1*], Abu Zubair[2], Md. Khalid Hassan Real[1]
[1]Department of Environmental Science and Resource Management, Mawlana Bhashani Science and Technology University, Tangail, Bangladesh
[2]Department of Food Technology and Nutritional Science, Mawlana Bhashani Science and Technology University Tangail, Bangladesh
*E-mail: mdmia1998@gmail.com

Abstract
The present study was performed to analyze the microbial (total viable bacteria, total coliforms and total fungal) quality and chemical quality (pH, total soluble solids, acidity, ash content and vitamin C) of industrially processed mango (Mangifera indica) juices. Three bottled (Brand-1, Brand-2 and Brand-3) and two tetra packed (Brand-4, and Brand-5) juice samples were collected from three different locations namely Tangail bus stand (Point-1), Super shop market (Point-2) and Porabari bazar (Point-3) under the Tangail Sadar Upazilla, Bangladesh. Total viable bacteria were high in all brands of juice and values ranged from 1.3×10^5-1.2×10^8 cfu/ml. The mean values of total bacteria count were ranged from 4.63×10^5-5.6×10^7 cfu/ml. Total coliforms bacteria were ranged from 0-2×10^4cfu/ml with a range of mean value 0-7.7×10^3 cfu/ml in different brands. The mean value of total fungal found ranging from 8.2×10^5-1.12 to 1.6×10^7 cfu/ml. The pH was acidic and mean value ranged from 3.7-4.06. Study found that total soluble solids was ranged from 12%-13%. The concentration of acidity ranged between 0.16-0.39%.Very low amount of ash content (0.04-0.07%) indicated that mineral content was low. Concentration of vitamin C was also low and the mean value was ranged from 0.05-0.18 mg/100ml. The current study reveals that industrially processed mango juices are not conforming with microbiological as well as chemical specifications and thus conferred that industrially processed juices may not be safe for consumption. The manufacturing companies should develop the quality by maintaining hygiene and use the good quality ingredients in preparing different types of juices.

Key words: Mango Juice, Bacteria, Fungi, Ash Content, Ascorbic Acid, Hygiene

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1. INTRODUCTION

Fruit juices play an important role of the modern diet in many communities (Tasnim *et al*., 2010). These are well liked throughout the world due to very nutritive, invigorating and non-alcoholic beverage (FDA, 1999). They offer good taste and a variety of nutrients and play a significant part in a healthy diet. Mango (*Mangifera indica* L.) is one of the most widely eaten tropical fruit because of its taste, attractive color and flavor and nutritional qualities. Vitamins, organic acids, carbohydrates, amino acids, phenolic acids (e.g., gallic acid, caffeic acid, and tannic acid) and certain volatile compounds are rich in mango (Pal, 1998; Singh *et al*., 2004; Pino *et al*., 2005).

In foods microbial quality is very important since bacteria, fungi, viruses and protozoa are known to cause food-borne diseases while other cause for spoilage. Water used in the processing of juices is major sources of microbial contamination. Several factors such as pH, hygienic practice and temperature and concentration of preservative encourage can prevent or limit the growth of microorganisms in juices (Esteve *et al*., 2005). The total viable bacteria (TVB) indicate total number of bacteria present in a sample (Bowden, 1977). The presence of coliforms bacteria in mango juice has been attributed to their being natural flora of fruits which may be introduced into the juice if improperly processed (Frazier and Westhoff, 1998). The growth of yeast in fruit

juices are favored by acidic condition and have sufficient sugar of juices (Hoover, 1997). Spoilage yeasts (*Saccharomyces cerevisiae*, *Candida lipolytica* and *Zygosaccharomyces spp*.) can tolerate acidic conditions (ICMSF, 1989). Mold are unable to grow in abiotic condition and they are considered to be the least important group of micro-organisms causing spoilage in fruit juices (Parish, 1991). *Penicillium* and *Aspergillus* are spore forming and toxic substances (mycotoxins) produced from *Penicillium* lead to health hazards for the consumer (Frazier and Westhoff, 1989; Parish and Higgin, 1989).
The chemical feature of juice considered in quality assessment including pH, total soluble solids, acidity, ash content, ascorbic acid etc. Fruit juices have a low pH (2-5) as these are rich in organic acid (Tasnim *et al*., 2010). It should also be noted that changes in pH could transform a food into one which supports pathogens to grow (FDA, 2001; ICMSF, 1989). Total Soluble Solid (TSS) is one of the most important quality factors for most of fruit juices and it is influenced by the combined effect of stages of maturity and ripening conditions (Hossain *et al*., 2012; Tasnim *et al*., 2010). The determination of acidity is important because the loss of acidity increase the pH. Ash which is gotten from the burning of juices indicate the minerals like calcium, magnesium, phosphorus, and sodium (Boyer and Liu, 2004; Basar and Rahman, 2007) contained in that juice. Determining the ash content may be important because it is a part of proximate analysis for nutritional evaluation (Marshall, 2010). Mango juice is rich in minerals and vitamin A and C. The anti-oxidant components of mango juice have beneficial long term health effects, such as decreasing the risk of cancer and heart disease (Boyer and Liu, 2004). Vitamin C which is easily degenerated by atmospheric oxygen and enzymes has become important for determination (Tyagi *et al*., 2014).
In Bangladesh, people of all ages are consuming mango juice almost every day as it is available in everywhere. Long term consumption of this juice has potentials health hazards to subsequent consumers. This juice is rich of minerals such as calcium, potassium, phosphorus and vitamin A and C which may fulfill the deficiency in human body. But the juice which quality is not so good may contain low level of minerals and vitamin. Ingestion of coliform bacteria causes nausea, diarrhea, vomiting, stomach cramps etc. diseases in body. Fungus causes headache, vomiting, abdominal pain, diarrhea, respiratory and other effects that resemble allergic responses in human body (Patricia *et al*., 1999). *Penicillium sp.* in total fungus causes renal damage/necrosis of the kidney (Nma and Ola, 2013). The high acid content of mango juice can tooth weaken and demineralize of tooth enamel, discolor of tooth, sensitive to extreme temperatures etc. (AGD, 1952). Juices are frequently consumed by most of the people, so the quality of these juices should be known.

2. MATERIAL AND METHODS

2.1. Sample collection

The study was conducted at Tangail district, Bangladesh. A total 45 mango juice samples of five different brands of five different producers were collected. Among them three brands were bottled mango juices (Brand-1, Brand-2 and Brand-3) and two brands were tetra packed (Brand-4 and Brand-5) mango juices. Samples were collected from three different locations namely Tangail bus stand (Point-1), super shop market (Point-2) and porabari bazar (Point-3) under Tangail sadar upazila. The self life mentioned on package of the samples belonging to the same brand of different points were same. Samples were transferred aseptically into the ice box with sufficient ice blocks maintaining temperature around 4^0-6^0 C. To overcome sampling bias at least 3 samples of each brands were analysed.

2.2. Microbial Analysis

Spread plate technique was performed for total plate count of bacteria with serial dilution by following the standard procedure (APHA, 1976; FDA, 2001). Plate Count Agar, MacConkey Agar, Potato Dextrose Agar were used for the growth of Total Viable bacteria, Total Coliform bacteria and Total Fungus respectively (Alam, 2013). Colonies formed in the plates were counted by using digital colony counter after incubation at 37˚C for 48 hours. The actual numbers of bacteria were estimated as colony forming unit (cfu/ml). The bacteria plate counts per ml per dilution were recorded using following equation

$$\text{Total Count} = \frac{\text{total number of colonies}}{\text{number of plates}} \times \frac{1}{dilution\ factor} \times \frac{1}{volume\ inoculated}\ \text{cfu/ml}$$

2.3. Chemical analysis

The pH was determined using digital pH meter (Adwa, AD 1000). TSS content of fruit juices was determined using an Abbe refractrometer whereby a drop of pulp solution was placed on its prism. The percentage of TSS was obtained from direct reading of the refractrometer. Acidity was determined by dissolving a known weight of sample in distilled water and then titrated against 0.01 N NaOH using phenolphthalein as indicator (Srivastava & Sanjeev, 2003). Ash content of mango juices was determined using standard AOAC methods (AOAC, 2004). Vitamin C was estimated by 2, 6-Dichlorophenolindophenol visual titration method according to AOAC. The reagents used for the estimation of vitamin C were as follows: (i) metaphosphoric acid (6%); (ii) standard ascorbic acid solution; and (iii) 2, 6-Dichlorophenolindophenol dye. For estimation of vitamin-C, the following steps were followed: standardization of dye solution, preparation of solution and titration (AOAC, 2004).

2.4. Statistical analysis

MS Excel 2010 and SPSS 20 software were used for calculating average, standard deviation and preparation of graphs. Karl Pearson's correlation coefficients were determined for analysis of correlation among the parameters.

3. RESULTS AND DISCUSSION

3.1. Total Viable Count of Bacteria (TVC)

In the present study total viable bacteria was ranged from 1.3×10^5-1.2×10^8 cfu/ml in different brands of bottled and tetra packed mango juices at different points (Figure 1). In bottled juice, Brand-2 showed the highest load (1.2×10^8 cfu/ml) at Point-2 and Brand-3 showed lowest load (1.1×10^6 cfu/ml) at Point-3. The highest mean value of total viable bacteria of bottled juice was $5.6\times10^7\pm0.48$ in Brand-2 and the lowest value was $4.4\times10^6\pm0.5$ in Brand-3. In case of tetra packed juices there found highest value (2×10^6 cfu/ml) in Brand-5 at Point-3 and the lowest value (1.0×10^5 cfu/ml) was found in Brand-5 at Point-2. Brand-5 showed highest mean value $7.6\times10^5\pm0.69$ and Brand-4 showed lowest mean value $4.63\times10^5\pm0.7$.

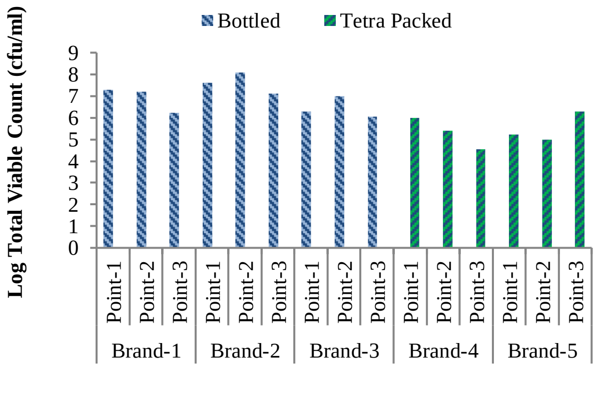

Fig. 1 Variation of Total Viable Count of bacteria in different brands and points

According to Gulf standard for juice, the statdard limit of total viable bacteria count is 1×104 cfu/ml (Gulf standard, 2000). It was revealed that all brands of processed mango juices exceeded this standard limit and the tetra packed juices showed slightly low count than bottled juices. High amount total viable bacteria count may be due to the unhygienic maintenance during preparation of the juices.

3.2. Total Coliforms Count (TCC)

In this study, the range of total coliform bacteria was 0-2×10^4 cfu/ml in different brands of bottled and tetra packed mango juices at different points. In bottled juice, the highest value 2×10^4 cfu/ml was found in Brand-3 at Point-3. The coliforms count was nil in Brand-1 at Point-2 and Point-3 and also nil in Brand-2 at Point-3 and Brand-3 at Point-2. The highest

mean value of total coliforms count of bottled juice was $7.7\times10^3\pm2.28$ in Brand-3 and the lowest value was $9.7\times10^2\pm2.00$ in Brand-1. Again the total coliforms count was nil in tetra packed juices. According to Gulf standard, the approximate limit of total coliform count in juice is 1×10^2 cfu/ml (Gulf standard, 2000). From this study, the mean value of all brands of bottled juices exceeded this standard limit but the tetra packed juices was within this limit. This large number of coliforms detected from bottled juices clearly indicates poor plant management and hygiene in the factory where juice was prepared. This contamination could also be occurred due to lacking of proper quality control system for juice preparation, lacking of right storage conditions
and bad packaging system (Kader *et al*., 2014).

3.3. Total Fungal Count (TFC)

The range of total fungal count was 1.1×10^5-4.8×10^7cfu/ml in different brands of bottled and tetra packed mango juices at different points (Figure 2). In bottled juice, the highest value 3×10^7 cfu/ml was found in Brand-2 at Point-1 and the lowest value 1.12×10^5 cfu/ml in Brand-1 at Point-3. The highest mean value of total fungal count of bottled juice was 1.14×10^7 ± 1.06 in Brand-2 and the lowest mean value was 8.2×10^5 ± 1.12 in Brand-3. In case of tetra packed juices there found highest value 4.8×10^7 cfu/ml in Brand-4 at Point-3 and the lowest value 1.3×10^5 cfu/ml was found in Brand-4 at Point-2. Brand-4 showed highest mean value 1.6×10^7 ± 1.46 and Brand-5 showed lowest mean value 1.6×10^6 ± 0.63. According to Gulf standard for juice, the limit of total fungal count is 1×10^3 cfu/ml (Gulf standard, 2000). In this study, all brands of processed mango juice exceeded this standard limit. The tetra packed juices showed slightly high count of total fungus. Poor sanitation, extraction, raw material contaminations (often from insect damage), lack of both proper heat sterilization and adequate quality control during processing of juice may be responsible for the high count of total fungus (Nma and Ola, 2013). The company should maintain hygiene during extraction, use safe water and develop the sanitation condition quality control system.

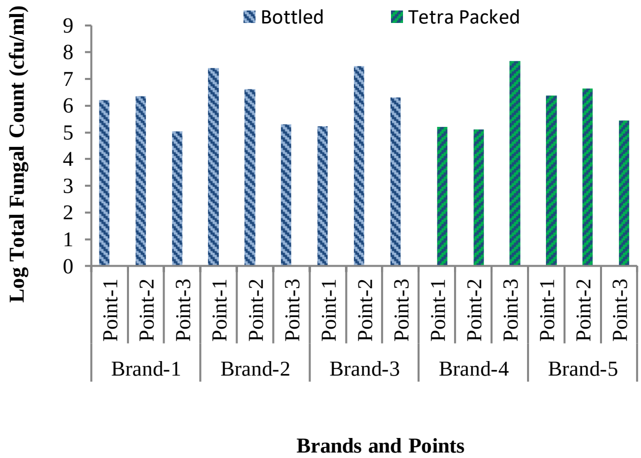

Fig. 2 Variation of Total Fungal Count in different brands and points

3.4. The pH

In this study pH was ranged from 3.3-4.1 in different brands of mango juices at different points (Figure 3). Highest pH value 4.1 was found in Brand-2 at Point-2 whereas lowest pH value 3.3 was found in Brand-4 present study was within the permissible limit.

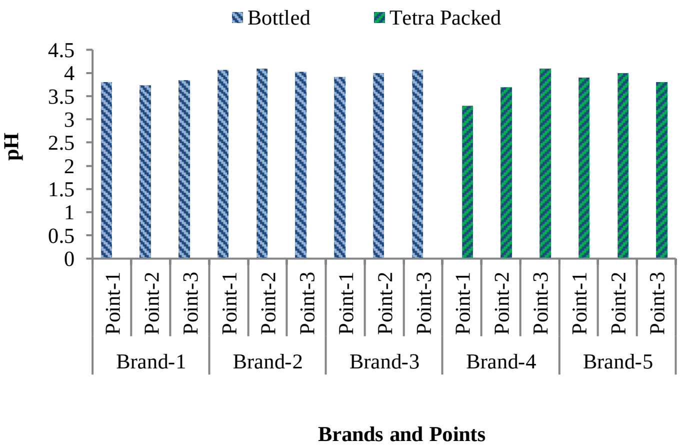

Fig. 3 Variation of concentrations of pH in different brands and points

at Point-1. In bottled juices the highest mean (4.06±0.04) of pH was in Brand-2 and the lowest (3.8±0.06) was in Brand-1. Again in tetra packed juices highest mean concentration (3.90±0.1) was in Brand-5 which was slightly acidic than the bottled juices. The approximate pH for ripe mango juice is 3.4 - 4.8 (Anon, 1962; Bridges *et al*., 1939; Landry *etal*., 1995). According to FAO (2005) and Bates *et al*. (2001), the recommended value of pH for mango juice is 3.5 - 4.0 and samples measured in the

3.5. Total Soluble Solids (TSS)

Total soluble solids was ranging from 12%-13% in different brands at different points (Figure 4). Highest TSS value (13%) was found in Brand-1 at Point-3, Brand-2 at Point-2 and Brand-3 at Point-2. The lowest TSS (12%) was found in Brand-1 at Point-1 and Point-2, Brand-2 at Point-1 and Point-3, Brand-3 at Point-1 and Point-3 in bottled juices. The overall mean concentration of total soluble solids of bottled juices was 12.33±0.58. In case of tetra packed juices there found highest concentration of total soluble solids 13% in Brand-4 at Point-3 and Brand-5 at Point-2 and the lowest concentration 12% was found in Brand-4 at Point-1 and Point-2, and Brand-5 at Point-1 and Point-2. The overall mean concentration was 12.33±0.58% for tetra packed juices. The recommended value of total soluble solids is 13.5% for mango juice (FAO, 2005; Bates *et al.*, 2001)). According to Bangladesh Standards Specification for Fruits or Vegetables Juice, Brix (TSS) percent in fruits or vegetables juice is minimum 12% (BDS 513, 2013). TSS values measured in the present study were within the standard limit according to FAO and BDS 513.

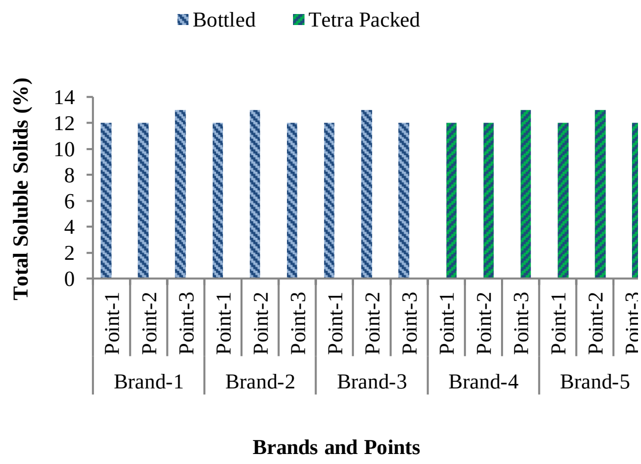

Fig. 4 Variation of concentrations of Total Soluble Solids in different brands and points

3.6. Acidity

In this study acidity was ranged from 0.16% - 0.39% in different brands of bottled and tetra packed mango juices at different points (Figure 5). In case of bottled juice, Brand-1 showed the highest concentration (0.26%) at Point-1 and Brand-2 showed lowest concentration (0.16%) at Point-2. The highest mean concentration of bottled juices was 0.25±0.02% in Brand-1 and the lowest was 0.18±0.01 in Brand-2 and Brand-3 respectively. In case of tetra packed juices there found highest concentration (0.39%) in Brand-4 at Point-1 and the lowest concentration (0.16%) was found in Brand-5 at Point-3. Brand-4 showed highest mean concentration 0.25±0.12% and Brand-5 showed lowest mean concentration 0.20±0.05%. In this study, tetra packed juices showed slightly high mean value of acidity than bottled juices. According to FAO (2005) and Bates *et al*. (2001) the recommended value of acidity for mango juice is 0.25%-0.5%. In this present study, all the brands showed the values of acidity were close to recommended value.

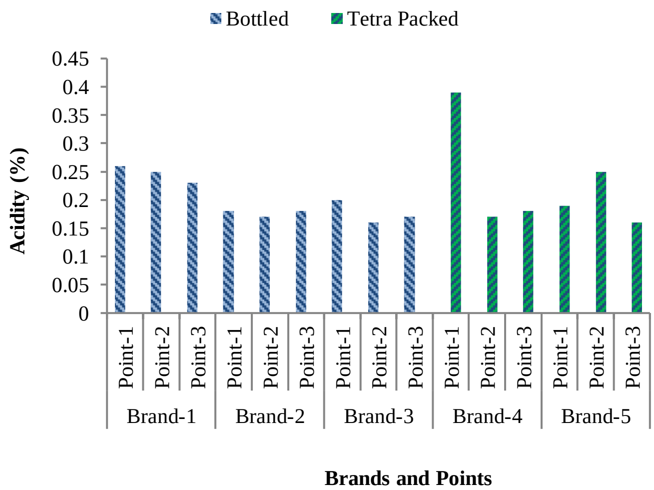

Fig. 5 Variation of concentrations of acidity in different brands and points

3.7. Ash content

In this study, the range of ash content was 0% - 0.11% in different brands at different points (Figure **6**). In case of bottled juice, Brand-3 showed the highest concentration (0.11%) at Point-1 and Brand-2 showed lowest concentration (0%) at Point-1. The highest mean concentration of ash content was 0.07±0.05% in Brand-3 and the lowest was

0.04±0.05% in Brand-2 of bottled juice. In case of tetra packed juices there found highest concentration (0.11%) in Brand-4 at point-3 and the lowest concentration (0.01%) was found in Brand-5 at Point-2. Brand-4 showed highest mean concentration 0.07±0.04% and Brand-5 showed lowest mean concentration 0.04±0.03%. The value of ash content of tetra packed juice was as similar as bottled juice. The concentrations of ash in these juices were not satisfactorily good in quality. The lower ash content indicates low mango contents or raw material, recipe or the ingredients used in the juice (Hussain *et al.*, 1993). The manufacturers should use more mango pulp during preparation of juice.

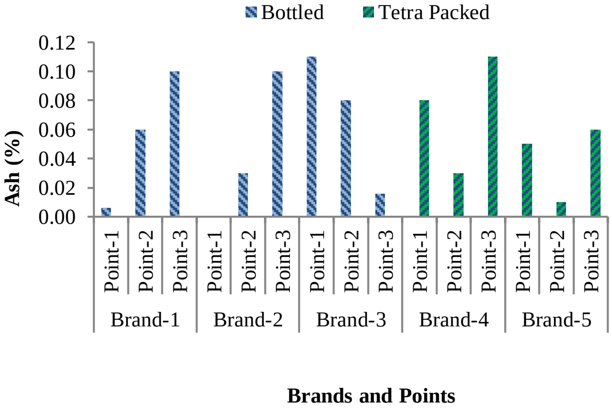

Fig. 6 Variation of concentrations of Ash in different brands and points

3.8. Vitamin C

In this study, the concentration of vitamin C for different brands of bottled and tetra packed juice were ranging from 0.02-0.30 mg/100ml (Figure 7). For bottled juice, Brand-3 showed highest concentration (0.30 mg/100ml) at Point-3 and Brand-1 showed lowest concentration (0.02 mg/100ml) at Point-2. The highest mean concentration 0.18±0.01mg/100ml was found in Brand-3 and the lowest mean concentration 0.05±0.04 mg/100ml was found in Brand-2. In tetra packed juice, highest concentration (0.14 mg/100ml) was found in Brand-5 at Point-2 and the lowest concentration (0.02 mg/100ml) was found in Brand-4 at point-3. The highest mean concentration was 0.07±0.06 mg/100ml in Brand-5 and the lowest mean concentration was 0.05±0.05 mg/100ml in Brand-4. The concentrations of vitamin C encoded by manufacturing companies were 40 mg/100ml in Brand-1, 8.25 mg/100ml in Brand-2, 30.5 mg/100ml in Brand-3, 18 mg/100ml in Brand-4 and 8.25 mg/100ml in Brand-5. In this study, the estimated concentrations of vitamin C were too much lower than that values which were indicated by the companies. These concentrations can't fulfill the demand of the body therefore manufacturers should increase the concentration of vitamin C by using mango pulp in sufficient amount.

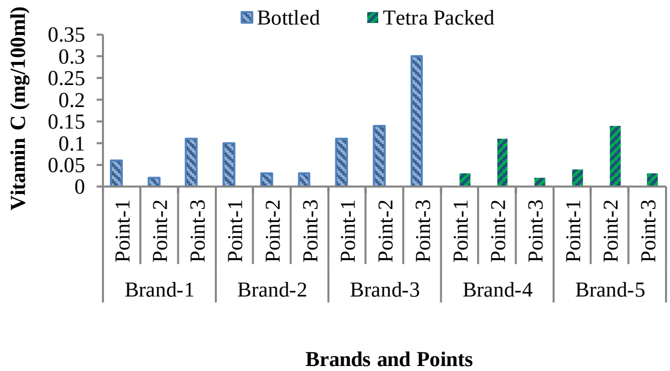

Fig. 7 Variation of concentrations of Vitamin C in different brands and points

Correlation matrix among the microbial and chemical parameters

Correlation matrix among the parameters was determined by Karl Pearson's correlation coefficient along with their significant level (Table 1). From the Table 1 there was found that total coliform and total fungal count were positively correlated with total viable bacteria but the relation was not significant (p>.05). Total Viable Count had positive correlation with pH but negative relation with TSS, acidity, ash content and vitamin C. Total coliform had positive relation with pH and vitamin C but negative relation with TSS, acidity and ash content. Coliform bacteria are neutrophiles therefore the positive correlation with pH and negative correlation with acidity is relevant. Total fungal count showed a significant positive relation with pH (r=0.582, p< .05), positive relation with TSS and vitamin C and negative relation with acidity and ash content. There was seen significant negative relation between acidity and pH at 1% level of significance (r= - 0.772). The pH had positive relation with TSS and vitamin C. There was seen negative relation between pH and ash content. TSS had positive relation with ash content and vitamin C but negative relation with acidity. Acidity showed positive relation with ash content but negative relation with vitamin C. Vitamin C and ash had negative relationship.

Table 1. Correlation matrix among the microbial and chemical parameters of mango juices samples

Parameters	TVC	TCC	TFC	pH	TSS	Acidity	Ash	Vitamin C
TVC	1							
TCC	.458	1						
TFC	.047	.171	1					
pH	.115	.352	**.582***	1				
TSS	-.133	-.248	.428	.419	1			
Acidity	-.081	-.178	-.298	**-.772****	-.137	1		
Ash	-.208	-.448	-.271	-.117	.177	.051	1	
Vitamin C	-.133	.446	.066	.285	.033	-.232	-.319	1

*****Correlation is significant at the 0.05 level (2-tailed)
******Correlation is significant at the 0.01 level (2-tailed)
(TVC= Total Viable Count, TCC= Total Coliforms, TFC= Total Fungal Count, TSS= Total Soluble Solids)

4. CONCLUSIONS

The present study exhibited the microbial and chemical quality of industrially processed mango juice (both bottled and tetra packed) to ensure food safety for a precise control over public health risk. Most of the brands of bottled and tetra packed juice samples collected from different locations of Tangail Sadar Upazila showed poor level of hygiene because total viable count, total coliform and total fungal count exceeded permissible limit. Among chemical parameters, the concentrations of pH, acidity and total soluble solids were within recommended range. The concentrations of mineral and vitamin C were too much lower than the given level of the brands. In regard to microbial and chemical quality, these juices were not safe and suitable for consumption. Long term consumption of these juices may cause different diseases such as diarrhea, vomiting, nausea, stomach cramps etc. in people. Implementations of GMP and HACCP are mandatory for these juice processing industry. A combination of regular monitoring, proper maintenance, use of safe water, sufficient use of raw materials and proper training of workers about health and hygiene could be appropriate choice to develop the quality of mango juice. In addition to this, not only government authorized institution but also some strongly active administrative organization like mobile court should be given more authorization to undertake pre-emptive investigations to check the microbial and chemical quality of juice.

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