MICROBIAL, PHYSICOCHEMICAL AND NUTRITIONAL QUALITY ASSESSMENT OF FRUIT JUICES IN TANGAIL MUNICIPALITY, BANGLADESH

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Abstract
Analysis of the microbial, physicochemical and nutritional qualities of some commercially bottled and handmade fruit juices showed that total viable bacteria in bottle juices ranged from 9.6×107 to 2.0×1011 cfu/100 ml and handmade juices 1.3×105 to 9.6×107 cfu/100 ml. The log mean values of total bacteria count ranged from 9.14-10.19 cfu/100 ml (bottled) and 6.09-9.08 cfu/100 ml (handmade). Total coliform bacteria ranged from 0-7.6×109 cfu/100 ml (bottled) and 0 - 2.8×105 cfu /100 ml (handmade) with a range of log mean values of 3.18-6.95 cfu/100 ml (bottled) and 3.47-3.48 cfu/100 ml (handmade). The pH was acidic and mean value ranged from 3.14-4.03 for bottled juice and 3.72-3.73 (handmade). It was found that total soluble solids ranged from 10-11.33% for bottle and 11.33-12.33% for handmade juices. The concentration of vitamin C in bottled and handmade juices ranged from 0.74-2.22 mg/100 ml and 2.34-3.7 mg/100 ml, respectively, indicated that vitamin C content was very low. It was also revealed that quality of bottled and handmade juices was unsatisfactory and may not be useful for consumption. It is suggested that proper measure must be taken and manufacturing companies should develop the quality by maintaining hygiene and using good quality ingredients in preparing different types of juices.

Key words: Microbial, Physicochemical, Nutritional quality, Fruit juice

Introduction
Fruit juices are becoming an important part of the modern diet and considered to health and nutritional benefits in many communities. All over the world, in everyone’s diet chart it is always included in different forms like as whole fruit, juice, beverage or still drink etc. Fruit juices are considered as a nutritious popular drink, but processed juice may not always be safe due to chemical hazards and microbial risks (Aneja et al. 2014). Nowadays, throughout the country people drink fruit juices without knowing actual safety and nutritional quality of these types of juices.

As a result they suffer from food borne and many other gastrointestinal diseases (Rashed et al. 2013). Microbial quality is very important in food since bacteria, fungi, viruses and

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protozoa which are potential pathogens of human known to cause food borne diseases while cause for spoilage (Acharjee et al. 2013). The quality of fruit juices is strictly maintained in developed countries under several laws and regulations. Most fruit juices contain sufficient nutrients that could support microbial growth. In recent years, the increasing consumer awareness has emphasized the need for chemically and microbiologically safe food (Aneja et al. 2014). But in many developing and under-developed countries, the manufacturers are not conscious about the microbiological safety and hygiene of fruit juices because of lack of commitment and law enforcement system. Therefore, maintaining of the quality of processed fruit juices is an important concern. The chemical feature of juice considered in quality assessment include pH, total soluble solids, acidity, ash content, ascorbic acid etc (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). In Bangladesh, the manufacturers are not concerned about the microbiological safety and hygiene of fruit juices due to lack of proper knowledge. Juices are frequently consumed by most of the people, so the quality of these juices should be known. Considering the importance, this study has been undertaken to assessing the physicochemical, nutritional and microbiological quality of bottled and handmade juices in Tangail municipality, Bangladesh.

Materials and Methods

Study area, time and collection of samples: The study was conducted at Tangail municipality, Bangladesh. A total of 18 juice samples of different brands and handmade of different producers were collected from three different locations, namely New bus stand, Old bus stand and Santosh, Tangail (Fig. 1). Twelve bottles (brand- 1 and 2) of orange and litchi juice samples were collected from street side shops and the handmade juice samples were collected from different fast food during January - May, 2018. Samples were transferred aseptically into the ice box with sufficient ice blocks.

Microbial analysis: Spread plate technique was performed for bacterial total plate count with serial dilution by following the standard procedure (APHA 1976). Plate count agar, MacConkey agar, were used for the growth of total viable bacteria, total coliform, 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 number of bacteria were
estimated as colony forming unit (cfu/100 ml). The bacteria plate counts per 100 ml per dilution were recorded using the following equation

\[
\text{Total count} = \frac{\text{Total number of colonies}}{\text{Number of plates}} \times \frac{1}{\text{Dilution of actor}} \times \frac{1}{\text{Volume inoculated}} \times \text{cfu/100 ml (Bell et al. 2005).}
\]

**Fig. 1.** Map of the study area.

*Physicochemical analysis:* The pH was determined using digital pH meter. Total soluble solids (TSS) content of fruit juices was determined using an refractometer whereby a drop of pulp solution was placed on its prism. The percentage of TSS was obtained from direct reading of the refractrometer. Vitamin C was estimated by 2,6-dichlorophenolindophenol visual titration method according to AOAC (2004). The reagents used for the estimation of vitamin C were as follows: (i) Metaphosphoric acid (6%), (ii) standard ascorbic acid solutions 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).
Statistical analysis: MS Excel 2010 and SPSS 20 software were used for calculating average, standard deviation and preparation of graphs. Pearson’s correlation coefficients were determined for analysis of correlation among the parameters.

Results and Discussion

Microbial analysis

Total viable count of bacteria: The range of total viable count was $2.0 \times 10^{11}$ to $9.6 \times 10^7$ cfu/100 ml in different brands of bottled juices at different locations. In bottled juices litchi (Pran) showed the highest value ($2.0 \times 10^{11}$ cfu/100 ml) at New bus stand and the lowest value ($9.6 \times 10^7$ cfu/100 ml) in Orange (Pran and BD Food) at both New and Old bus stand. The number of bacteria is very high because storage of products at refrigerator temperature or below is not always best for the maintenance of desirable quality of some fruits. Total viable count is shown in the graph as logarithm value.

The highest log of total viable count value was 11.505 cfu/100 ml in litchi (BD food) at Old bus stand and the lowest Log total viable count value was 7.98 cfu/100 ml in litchi (Pran) at New bus stand (Fig. 2). For handmade juices, the range of total viable count was $1.3 \times 10^5$ to $9.6 \times 10^9$ cfu/100 ml at Tangail fast food and Santosh. In case of orange juice the highest value of total viable count was found at Santosh, and the lowest value was found in litchi juices at New bus stand.

The range of total viable count was $1 \times 10^5$ cfu/ml (Gulf Standard 2000). In other study it was reported that the highest total bacterial load ($2.66 \times 10^6$ cfu/ml) for packed fruit juice sample was found in an orange juice (sample P-12), collected from Farmgate, lemon ($3.94 \times 10^5$ cfu/100 ml) and papaya ($1.98 \times 10^6$ cfu/100 ml) from Mouchack, Dhaka,
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respectively (Rashed et al. 2013). Total viable bacterial count in most of the fresh juice samples was higher than the commercially packed juice, as the highest count was found as $2.4\times10^4$ cfu/ml and $3.2\times10^3$ cfu/ml in fresh and packed juice, respectively which was found to be lower than this study (Rahman et al. 2010). The load of viable bacteria in processed juice samples within the standard limit in the average of $10^3$ cfu/ml (Tasnim et al. 2010). Total viable count of commercially produced litchi juices in a range of $5\times10^5$ (cfu/ml) and total viable count for handmade orange juices in a range of $1\times10^5$ cfu/ml (Mortuza 2016). In a similar study, Fatema et al. (2016) estimated the total viable count of minerals in Allo vera, grapes and papaya. The highest total viable count ranged from $1.4\times10^6$ - $1.2\times10^6$ cfu/ml was present in aloevera and mango juice sample and lowest total viable count was present in papaya $9.0\times10^5$ cfu/ml and Malta $5.5\times10^5$ cfu/ml.

All brands of processed juices exceed the standard limit of Gulf Standard (2000) in the present study. Total viable bacteria was found in high amount in these juices may be due to the unhygienic maintenance during preparation of the juices.

**Total coliforms count (TCC):** In this study, the range of total coliform bacteria was 0-7.6 $\times10^9$ cfu/100 ml in different brands of bottled juices at different locations. In bottled juices, the highest value $7.6\times10^9$ cfu/100 ml was found in litchi (Pran) juices collected from New bus stand. The coliform count was nil in orange (Pran and BD Food) of Old bus stand and Santosh. The highest log total coliform count 11.38 cfu/100 ml was found in litchi (BD Food) juices collected from Santosh (Fig. 3). On the other hand, for handmade juices, the range of total coliform count was 0 - $2.8\times10^5$ cfu/100 ml at different locations. The highest value of total coliform count was found ($2.8\times10^5$ cfu/100 ml) in litchi juices of Santosh and the total coliform count was nil in orange and litchi juices at different points.

The range of total coliform count is $1\times10^2$ cfu/ml (Gulf standard 2000). The presence of coliform in fruit juice is not allowed by safe food consumption standard (Andres et al. 2004). In another study, total coliform count for handmade lemon juices in a range of lemon $2.8\times10^4$ cfu/ml (Oranusi et al. 2012). Total coliform count found in litchi juices in a range of $4\times10^3$ cfu/ml and total coliform count for handmade orange juices in a range of $5\times10^3$ cfu/ml (Mortuza 2016).

In the present study it is showed that the total coliform was nil in some samples of bottled and handmade juices whereas some bottled and handmade juices exceeded the Gulf standard limit. 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). It is also found that the total coliform bacteria
were found in high concentration of some juices due to unhygienic maintenance during preparation of handmade juices.

Fig. 3. Variation of total coliform count in different samples. NBS = New bus stand, OBS = Old bus stand.

Physicochemical analysis

pH: The highest pH (4.05) was found in litchi juice (Brand-1) in 200 ml bottled and the lowest pH (3.03) was found in litchi juice (Brand-2), Fig. 4. On the other hand, for handmade juices the highest pH (3.8) was found in orange juices and the lowest pH (3.68) was found in litchi juices (Fig. 4). The highest pH was 5.45±0.09 in papaya juices and the lowest of pH 2.40±0.07 was in lemon juices.

Fig. 4. Variation in concentrations of pH in different samples. NBS = New bus stand, OBS = Old bus stand.

In other study, the pH for ripe mango juice was 3.4-4.8 (Anon. 1962). The mean pH value of total fruit juices was 4.9 with range of 3.88-5.71. The pH of both mango and papaya was 3.8 and 4.9, respectively and more acidic. FAO (2005) recommended value of pH for mango juice is 3.5-4.0. In present study authors have found that commercially
bottled litchi juices of Brand-1 are slightly acidic than litchi juices of Brand-2 because Brand-1 (litchi) exceeded the standard limit (FAO 2005). This present study noted that pH values were within the standard limit.

Total soluble solids (TSS): The range of TSS was found from 10 to 12% for bottled juices and from 10 to 13% for handmade juices at different samples (Fig. 5). The highest TSS value was found in bottled (11.33%) orange Brand-2 and litchi Brand-2 juices. On the other hand, highest TSS was 12.33% in orange handmade juices. The lowest concentration (10%) was found in Brand-1 of orange and litchi bottled juices whereas, for handmade juices, lowest concentration of TSS (11.33%) was found in litchi juices. The overall concentration of TSS of bottled juices was 11.33±0.58 and for handmade juices was 12.33±0.58. FAO (2005) estimated that TSS of bottled juices was 9.00±0.02.

![Fig. 5. TSS of different juice samples. NBS = New bus stand, OBS = Old bus stand.](image)

The recommended value of TSS is 11.8% for orange (bottled) and 11.5% for papaya in handmade juices (Tasnim et al. 2010). According to Bangladesh standards specification for fruit or vegetables juice, Brix the TSS in fruit or vegetables juice is minimum (12%). It may be said that TSS content in orange and litchi juices of Brand-1 in this study was comparatively lower than Brand-2 (bottled) and for handmade juices, TSS content in orange and papaya juices was comparatively higher than lemon and litchi juices. In 2001, FDA estimated that TSS of mango juices in a range of 11-13% which are almost similar findings of FAO and FDA. The present study also found that TSS of orange and litchi juices (bottled) is similar to other studies. But TSS ranges of handmade juices such as orange, litchi, papaya are slightly higher than the standard level. It may be concluded that these fruits are mature and ripe that’s why TSS crossed the standard limit.
**Nutritional analysis**

*Vitamin C:* The range of concentration of vitamin C both for the bottled and handmade juices was from 0.74 to 2.22 mg/100 ml and 1.1 to 4.1 mg/100 ml, respectively. In bottled juices, highest concentration of vitamin C (2.22 mg/100 ml) in Brand-1 and 2 (orange juice) and the lowest concentration of vitamin C (0.74 mg/100 ml) was found in Brand-1 and 2 (litchi juice) (Fig. 6). In case of handmade juices highest concentration of vitamin C (4.1 mg/100 ml) was found in orange juice and the lowest concentration of vitamin C (1.1 mg/100 ml) was found in litchi juices (Fig. 6). In other study, Tasnim et al. (2010) estimated that vitamin C concentration of processed orange juice is 5.64±0.08. Comparing between the nutritional analysis of vitamin C of bottled and handmade juices, it is assessed that highest concentrations of vitamin C are found in both for brand 1 (bottled) (orange, 2.22 mg/100 ml) and for handmade (4.1 mg/100 ml) juices. In the present study, it is estimated that the amount of vitamin C in bottled juices is less than handmade juices.

*Fig. 6. Variation in concentrations of vitamin C in different samples. NBS = New bus stand, OBS = Old bus stand.*

*Correlation matrix among different microbial, physicochemical and nutritional parameters of fruit juices:* Correlation matrix among the parameters was determined by Pearson’s correlation coefficient along with their significant test (Table 1). Total viable count showed a negative correlation with pH and vitamin C and insignificant positive correlation with TSS and TCC. Total viable count, total coliform count, TSS had negative relation with pH.

Correlation matrix among the parameters was determined by Pearson’s correlation coefficient along with their significant test are given in Table 2. From the table there found that vitamin C showed positive relation with pH, TSS, TVC and TCC but the
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relation is not significant (p>0.05). pH has positive correlation with vitamin C but negative relation with TSS, TVC and TCC. Total coliform count showed a positive relation with TSS and Vit. C and negative relation with pH and TVC.

Table 1. Correlation matrix among the microbial, physicochemical and nutritional parameters of bottle juices.

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>TSS</th>
<th>Vit C</th>
<th>TVC</th>
<th>TCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSS</td>
<td>-0.521</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vit. C</td>
<td>0.492</td>
<td>-0.521</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TVC</td>
<td>-0.257</td>
<td>0.303</td>
<td>-0.127</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>TCC</td>
<td>-0.258</td>
<td>0.038</td>
<td>-0.252</td>
<td>0.073</td>
<td>1</td>
</tr>
</tbody>
</table>

TVC = Total viable count, TCC = Total coliform count, TSS = Total soluble solids.

Table 2. Correlation matrix among the microbial, physicochemical and nutritional parameters of handmade juices.

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>TSS</th>
<th>Vit C</th>
<th>TVC</th>
<th>TCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
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</tr>
<tr>
<td>TSS</td>
<td>-0.395</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vit. C</td>
<td>0.054</td>
<td>0.681</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TVC</td>
<td>-0.142</td>
<td>0.418</td>
<td>0.351</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>TCC</td>
<td>-0.008</td>
<td>0.618</td>
<td>0.323</td>
<td>-0.352</td>
<td>1</td>
</tr>
</tbody>
</table>

The results of the present study showed that, the juices were prepared and served in unhygienic environments, where a number of pathogenic microorganisms were found. However, while collecting juice samples, it was found that chopping board, knives, spoons, glass and jugs were also not frequently washed and a chance of cross contamination was also possible. Since juice samples, collected from different sampling sites of Tangail municipality were not satisfactory as total viable count, total coliform count were detected in large amount, so it could be hardly recommended that, consumption of commercially and handmade processed juices was safe. Most of the brands (bottled) and handmade juices exceeded the standard permissible in this study. Though the chemical parameters (pH, TSS) were within the recommended range, in some cases it exceeded the standard. From nutritional analysis, vitamin C was lower in bottled juices than the handmade juices. In respect to microbial, chemical and nutritional quality,
these juices were not safe for human consumption. In the long run, people may suffer from different diseases such as diarrhea, vomiting, cholera, botulism, nausea etc. However, government health agencies must adopt measures to educate the producers on food safety and hygienic practices. Regular monitoring of the quality of fruit juices for human consumption must also be enforced by authority/law enforcement agencies.

References


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