Production and Investigation of Biochemical and Organoleptic Changes of Mixed Fruit Juice during Storage Period

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Abstract

A blend of different fruits has a great specialty on its splendid color, flavor, taste and nutritional contents. The purpose of this study was to explore the acceptable formulation of mixed fruit juice with better storage stability. The mixed fruit juice samples were prepared in four formulations incorporating pineapple, papaya, banana and orange with the addition of sugar (10%) and sodium benzoate (0.08%). Then the juices were heated for 5 minutes at 65°C, bottled in air tight plastic bottle and stored at 4°C for 180 days and 25°C for 120 days. It was found that TSS, acidity, total sugar, and bacterial load increased slightly, but vitamin C and pH decreased gradually during the storage periods. Fading of color and off flavor was found at the end of storage period. The storage temperature of 4°C was more effective to hold the quality of juice samples against biochemical changes. The sample showed better retention of vitamin C, pH, acidity and total sugar than other samples both at 4°C and 25°C. Panelists preferred the T4 sample most, which showed maximum hedonic scale ‘8’ point on sensory properties. It is also observed that T4 ensured the better shelf life to preserve the color, flavor and taste until 150 days at 4°C and 105 days at 25°C.

Keywords: Mixed Fruit Juice; Biochemical Properties; Sensory Evaluation.

Introduction

Fresh fruits are wonderful source of energy, vitamins, antioxidants, minerals and fibers. It has been a significant part of human diet and food supplement in order to its demanding and effective role in human nutrition [1, 2]. Bangladesh is a plentiful source in fruits production. The most yielded fruits in Bangladesh are mango, papaya, jackfruit, pineapple, banana and orange [3]. Fruits are produced in abundant especially in peak season and consumed locally, but they are seldom processed. The main reasons are physiological injuries, inadequate storage facilities, poor transportation and lacking of knowledge about preservation technique. Fruits have high moisture content and exhibits relatively high metabolic activity compared with other derived foods and continues after harvesting, thus making most fruits highly perishable commodities [4]. Thus, there is need for diversity in commercial utilizations. There are numerous ways of utilizing and processing fruits such as processing into juice, jams, concentrates, pulp and dehydrated products.

Fruit juice is an imperative trade product in most countries for many years, which are available in their natural concentrations or in treated forms [5]. Since different fruits have different nutritional value and rich antioxidant properties, the production of mixed fruit juice in order to combine all the basic nutrients and vital antioxidants might be an excellent food product for human diet. This usually gives a better quality juice nutritionally and organoleptically [6]. Fresh fruit juices are favourable to microbial and enzymatic action. The spoilage of fruit juices is basically reasoned to the presence of osmophillic microflora, which causes fermentation and leads to produce off-flavor happened in the fruit juices [7]. Thermal treatment and uses of preservatives could...
be effective way to inactivate the microbial and enzymatic activity and extend the shelf life [8]. Furthermore, vitamin C becomes unstable in the presence of oxygen and oxidation depends on pH (at pH 4.3 it is very fast), temperature (oxidation rises with increasing temperature to 60°C), the presence of heavy metals (particularly Cu). Storage temperature is also the prime limiting factor for shelf life of orange juice [9]. Thus, there is a need for a thorough study on the quality of mixed fruit juice after production and during storage that how the storage condition influences the final product biochemically and organoleptically. The present study was carried out for the development of mixed fruit juice from pineapple, papaya, orange and banana and investigates its biochemical and organoleptic properties during storage period.

Materials and Methods

Materials
Ripe banana, pineapple, papaya, orange, sugar, plastic bottle etc. were collected from local market of Dinajpur, Bangladesh. Sodium benzoate was used as a preservative from the laboratory of Food processing and preservation, HSTU.

Preparation of fruit juice
Fresh, fully ripe and sound fruits were used for extraction of juice. After washing thoroughly with clean water, the fruits were peeled by hand and cut into pieces and then transferred into juicer. The extracted juice was filtered by muslin cloth and then heated for 5 minutes at 65°C and cooled immediately. The juice was stored in a deep freeze at a temperature of -20°C for future analysis. In order to prepare mixed fruit juice, four formulations were prepared for this study as shown in Table 1. The choice of juice-mix ratio was selected from published literature performed by different researchers [10, 8, 11]. All formulations commonly contained 10% banana juice, 20% orange juice, 10% sugar and 0.08% sodium benzoate. All the ingredients were mixed thoroughly and heated at 65°C for 5 minutes in prior to proper mixing. The heated mixed juice was then cooled and filled into plastic bottles [12].

Biochemical Analysis

The stored mixed fruit juice were analyzed for their mineral composition (Na, K, Ca, Mg, S, P), titratable acidity, pH, total soluble solids (TSS), reducing sugar & non-reducing sugar, total sugar, vitamin C and microbail load/total plate count. The determinations were carried out according to published method, such as total soluble solids (TSS) by using a refractometer (HI 96801, Keison International Ltd., Chelmsford, England), acidity, ascorbic acid, reducing sugar & non-reducing sugar followed by the method of Rangana [13], pH by the conventional procedure followed by [8] using a pH meter (HI98190, Hanna Instruments Inc., Limena, Italy), mineral content by the method of Pearson [14] and the total viable count of microorganism followed by Sharf [15]. All the determinations were done in triplicate and the results were expressed as mean value.

Sensory Evaluation

Sensory evaluations of all the samples of mixed fruit juice were done by taste testing panel. The taste testing panel was made up with 10 test panelists. They were asked to evaluate color, flavor, taste and overall acceptability by a scoring rate on a 9 point hedonic scale, means 9 = Like extremely, 8 = Like very much, 7 = Like moderately, 6 = Like slightly, 5 = Neither like nor dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2 = Dislike very much and 1 = Dislike extremely.

Storage stability of prepared mixed fruit juice

The mixed fruit juice samples were filled into air tight plastic bottles. The samples were stored at two temperature such as room temperature (25 ± 2°C) and refrigerated temperature (4º ± 1°C).

Statistical analysis

The data obtained were analyzed and interpreted by analysis of variance (ANOVA) and Duncan’s Multiple Range Test (DMRT) at a level of 5% of significance, using Statistical Analysis System (SAS 9.3 TS L1M2, SAS Institute Inc., NC, USA). Values were presented as mean ± standard errors of 3 observations.

Results and Discussion

Chemical and organoleptic properties of mixed fruit juice

The prepared mixed juice were analyzed for TSS, acidity, pH, vitamin C, reducing sugar, non-reducing sugar, total sugar and mineral contents such as, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S) and phosphorus (P). The compositions of mixed fruit juice on the day of preparation are given on Table 2. The total solid soluble of mixed fruit juice samples was around 10.8-12.3º Brix, which was higher than the findings of Islam et al. [8]. The acidity, pH, vitamin C and total sugar ranged from 0.30-0.36%, 3.84-3.92, 13.21-14.62 mg/10 g and 16.89-17.70%, respectively. The acidity was lower than the findings of Islam et al. [8], which indicate the better stability against biochemical changes. The sample T1 showed the better characteristics having maximum vitamin C of 14.62 mg/100 g and comparatively higher value of pH. Similarly, Sample T1 was better in terms of having higher Ca and Mg and T1 ensured higher k (Table 1).

Biochemical changes during storage

Acidity is directly proportional and is a measure of shelf life of the product and guard against the attack of micro-organisms. It also helps to ensure some chemical changes during preparation and storage. Acidity for all the samples at various storage periods was observed (Figure 1). The initial acidity was 0.34%, 0.36%, 0.30% and 0.33% in samples T1, T2, T3 and T4, respectively. After six months of storage at 4°C, the acidity changed to 0.64%, 0.55%, 0.56% and 0.48% in T1, T2, T3 and T4 sample, respectively. After four months of storage at 25°C, the acidity changed to 0.58%, 0.51%, 0.52 % and 0.45% in samples T1, T2, T3 and T4, respectively. The increase of acidity might be due to proportional relation with the increased of storage periods. Similar reports have been investigated by several researchers [16, 12, 17]. The least changes were found in T1 samples around 0.48% at 4°C after 180 days (Figure 1a) and 0.45% at 25°C after 120 days (Figure 1b).
pH is inversely proportional to the acidity of any medium. The results showed that the increase in acidity causes decrease in pH (Figure 1 and 2). The decrease in pH and increase in acidity during storage might be due to degradation of carbohydrates present in mixed fruit juice by the action of microorganisms [18]. After six months of storage at 4ºC, the pH were changed from 3.84 to 3.30, 3.91 to 3.40 and 3.85 to 3.00 in sample T1, T2, T3 and T4, respectively (Figure 2a). On the other hand, after four months of storage at 25ºC, the pH were changed from 3.84 to 3.40, 3.91 to 3.50, 3.85 to 3.30, and 3.92 to 3.60 in sample T1, T2, T3 and T4 respectively (Figure 2b). These finding are almost similar to earlier study by Dhaliwal and Hira [19], Majumdar et al. [17] and Mishra et al. [16]. High acid and low pH may be due to production of acetic acid and lactic acid during storage. However, T4 sample

Table 1. Different formulations for the mixed fruit juice.

| Ingredients          | Sample/Formulation |
|----------------------|--------------------|
| Pineapple juice      | T1 50% T2 20% T3 30% T4 40% |
| Papaya juice         | T1 20% T2 50% T3 40% T4 30% |
| Orange juice         | T1 20% T2 20% T3 20% T4 20% |
| Banana juice         | T1 10% T2 10% T3 10% T4 10% |
| Sugar                | T1 10% T2 10% T3 10% T4 10% |
| Sodium benzoate      | 0.08% 0.08% 0.08% 0.08% |

Table 2. Chemical composition of developed mixed fruit juice.

| Components          | Juice sample |
|---------------------|--------------|
| T1                  | T2           | T3           | T4           |
| TSS (ºBrix)         | 11 ± 0.32    | 12.3 ± 0.41  | 11.5 ± 0.35  | 10.8 ± 0.27  |
| Acidity (% of citric acid) | 0.34 ± 0.02  | 0.36 ± 0.04  | 0.30 ± 0.05  | 0.33 ± 0.03  |
| pH                  | 3.84 ± 0.01  | 3.91 ± 0.03  | 3.85 ± 0.02  | 3.92 ± 0.02  |
| Vitamin C (mg/100 g)| 14.36 ± 0.22 | 13.21 ± 0.16 | 14.25 ± 0.15 | 14.62 ± 0.24 |
| Reducing sugar (%)  | 5.41 ± 0.12  | 4.94 ± 0.09  | 5.21 ± 0.1   | 5.30 ± 0.16  |
| Non-reducing sugar (%) | 12.16 ± 0.07 | 11.95 ± 0.11 | 12.05 ± 0.14 | 12.40 ± 0.21 |
| Total sugar (%)     | 17.57 ± 0.09 | 16.89 ± 0.1  | 17.26 ± 0.12 | 17.70 ± 0.19 |

| Minerals (mg/100 g) | Sodium (Na) | 44.83 ± 0.72 | 45 ± 0.57 | 43.33 ± 0.60 | 41 ± 0.72 |
|                     | Potassium (k) | 44.16 ± 0.60 | 44.16 ± 0.72 | 45 ± 0.57 | 43.16 ± 0.60 |
|                     | Calcium (Ca)  | 14.97 ± 0.31 | 9.05 ± 0.57 | 10.37 ± 0.40 | 7.67 ± 0.30 |
|                     | Magnesium (Mg)| 22.22 ± 0.51 | 18.27 ± 0.63 | 18.49 ± 0.29 | 18.33 ± 0.44 |
|                     | Sulphur (S)   | 16.18 ± 0.58 | 18.59±0.37 | 18.44 ± 0.46 | 18.86 ± 0.40 |
|                     | Phosphorus (P)| 13.39 ± 0.08 | 13.46 ± 0.06 | 13.41 ± 0.06 | 13.41 ± 0.06 |

Values are expressed as the mean ± standard error

Figure 1. Changes of acidity in different mixed fruit juice samples during storage period stored at (a) 4°C and (b) 25°C.
showed the least changes of pH both in 4°C and 25°C during storage (Figure 2).

Figure 3 showed that vitamin C or ascorbic acid reduced to significant amount in mixed juices during storage. After six months of storage at 4°C, vitamin C changed to 8.50 mg/100 g, 7.25 mg/100 g, 8.35 mg/100 g and 8.65 mg/100 g in samples T₁, T₂, T₃ and T₄ respectively. After four months of storage at 25°C, vitamin C changed to 8.10 mg/100 g, 7.90 mg/100 g, 8.00 mg/100 g and 8.30 mg/100 g in samples T₁, T₂, T₃ and T₄ respectively. Vitamin C is very sensitive to oxygen, light and heat, which caused to easily oxidize in presence of oxygen by both enzymatic and non-enzymatic catalyst [12, 20, 21].

The reducing sugar content of the formulated samples slightly increased during storage periods, while non-reducing sugar decreased slightly (Table 3). The reducing sugar slightly increased due to hydrolysis of sugar or may be due to the inversion of sucrose under acidic environment. Similar results were found that reducing sugar changed from 2.71% to 8.82% in apple and apricot blend juice during storage [12]. The decrease of non-reducing sugar is similar to the findings of Hussain et al. [22], where non-reducing sugars decreased from 2.56 to 1.88% in apple-apricot blended juice. However, a negligible change in total sugar content of the juice samples was observed both at 4°C and 25°C (Table 3). This finding supports with Bhardwaj and Mukherjee [23]. However, the maximum non-reducing sugar and total sugar were found in T₄ sample around 12.25% and 17.80%, respectively and T₁ showed maximum reducing sugar of around 5.60-5.65%.

In microbiological study, immediately after preparation of juice, the total no. of viable count was not uniform. It also showed that the total colony count increased slightly with the increase of storage periods (Table 4). The initial microbial loads of all samples were found 3.47, 3.53, 3.65, 3.42 log cfu/ml respectively. During storage periods it increased slightly in all samples. The microbial load was very low and far below the safely level up to 5 months storage at 4°C, but microbial load was higher after 3 months storage at 25°C. This result was similar with Zurowietz [24].

**Organoleptic changes during storage**

The sensory evaluation of mixed fruit juice during storage period of 180 days was carried out to evaluate the color, flavor, visual fungal growth and overall acceptability as shown in Table 5. The color, flavor and visual fungal growth of the juice samples was unchanged until 90 days at 25°C and 120 days at 4°C, which...
Table 3. Changes in reducing sugar and non-reducing sugar in mixed fruit juice during storage period.

| Storage period (day) | Juice sample | Reducing sugar | Non-reducing sugar | Total sugar |
|----------------------|--------------|----------------|--------------------|-------------|
|                      |              | 25°C | 4°C  | 25°C | 4°C  | 25°C | 4°C  | 25°C | 4°C  | 25°C | 4°C  |
|                      | T1           | 5.41 | 5.41 | 12.16 | 12.16 | 17.57 | 17.57 | 17.57 | 17.57 |
|                      | T2           | 4.94 | 4.94 | 11.95 | 11.95 | 16.89 | 16.89 | 16.89 | 16.89 |
|                      | T3           | 5.21 | 5.21 | 12.05 | 12.05 | 17.26 | 17.26 | 17.26 | 17.26 |
|                      | T4           | 5.30 | 5.30 | 12.40 | 12.40 | 17.70 | 17.70 | 17.70 | 17.70 |
|                      | T5           | 5.45 | 5.43 | 12.14 | 12.15 | 17.60 | 17.58 | 17.58 | 17.58 |
|                      | T6           | 4.96 | 4.94 | 11.94 | 11.95 | 16.91 | 16.89 | 16.89 | 16.89 |
|                      | T7           | 5.23 | 5.23 | 12.03 | 12.03 | 17.27 | 17.27 | 17.27 | 17.27 |
|                      | T8           | 5.33 | 5.33 | 12.38 | 12.38 | 17.72 | 17.71 | 17.71 | 17.71 |
|                      | T9           | 5.48 | 5.46 | 12.12 | 12.12 | 17.62 | 17.58 | 17.58 | 17.58 |
|                      | T10          | 4.99 | 4.95 | 11.92 | 11.94 | 16.93 | 16.90 | 16.90 | 16.90 |
|                      | T11          | 5.27 | 5.28 | 11.97 | 11.97 | 17.29 | 17.29 | 17.29 | 17.29 |
|                      | T12          | 5.37 | 5.37 | 12.35 | 12.35 | 17.75 | 17.72 | 17.72 | 17.72 |
|                      | T13          | 5.55 | 5.48 | 12.07 | 12.08 | 17.65 | 17.60 | 17.60 | 17.60 |
|                      | T14          | 5.04 | 5.02 | 11.89 | 11.91 | 17.95 | 16.92 | 16.92 | 16.92 |
|                      | T15          | 5.30 | 5.34 | 11.93 | 11.95 | 17.33 | 17.29 | 17.29 | 17.29 |
|                      | T16          | 5.42 | 5.43 | 12.30 | 12.32 | 17.77 | 17.75 | 17.75 | 17.75 |
|                      | T17          | 5.60 | 5.53 | 12.00 | 12.05 | 17.7 | 17.61 | 17.61 | 17.61 |
|                      | T18          | 5.10 | 5.05 | 11.85 | 11.87 | 16.94 | 16.94 | 16.94 | 16.94 |
|                      | T19          | 5.35 | 5.38 | 11.90 | 11.93 | 17.35 | 17.31 | 17.31 | 17.31 |
|                      | T20          | 5.47 | 5.48 | 12.25 | 12.28 | 17.8 | 17.76 | 17.76 | 17.76 |
|                      | T21          | -    | 5.58 | -    | 12.00 | -    | 17.62 | -    | 17.62 |
|                      | T22          | -    | 5.08 | -    | 11.85 | -    | 16.95 | -    | 16.95 |
|                      | T23          | -    | 5.43 | -    | 11.90 | -    | 17.32 | -    | 17.32 |
|                      | T24          | -    | 5.52 | -    | 12.25 | -    | 17.77 | -    | 17.77 |
|                      | T25          | -    | 5.65 | -    | 11.93 | -    | 17.63 | -    | 17.63 |
|                      | T26          | -    | 5.15 | -    | 11.83 | -    | 16.98 | -    | 16.98 |
|                      | T27          | -    | 5.48 | -    | 11.87 | -    | 17.35 | -    | 17.35 |
|                      | T28          | -    | 5.57 | -    | 12.21 | -    | 17.8 | -    | 17.8 |

Table 4. Effect of storage condition on bacterial growth/total plate count (log cfu/ml) of mixed fruit juice samples during storage period.

| Day | T1  | T2  | T3  | T4  | T1  | T2  | T3  | T4  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 0   | 3.47| 3.53| 3.65| 3.42| 3.47| 3.53| 3.65| 3.42|
| 30  | 3.55| 3.78| 3.71| 3.56| 3.62| 3.91| 3.76| 3.69|
| 60  | 3.62| 3.91| 3.76| 3.69| 3.86| 3.19| 3.95| 3.82|
| 90  | 3.67| 4.05| 3.79| 3.72| 4.12| 3.29| 3.98| 4.00|
| 120 | 3.76| 4.13| 3.85| 3.75| 6.15| 6.50| 6.00| 6.10|
| 150 | 3.86| 4.19| 3.95| 3.82| -   | -   | -   | -   |
| 180 | 4.12| 4.29| 3.98| 3.98| -   | -   | -   | -   |
resulted the juice samples to normal (N) overall acceptability (Table 5). Then, the color after 90 days at 25°C and after 120 days at 4°C has become changed, produced off flavor and lost the overall acceptability. This is happened due to the increase of microbial activity and chemical reactions [8], which has been observed by the visual growth of fungal (Table 5) and the increase of total plate count (Table 4). However, T₄ sample was the most effective to hold its sensory property during storage period. It is observed that the color as NC, flavor as NOF, visual fungal growth as NG and overall acceptability as N were found until 150 days at 4°C and 105 days at 25°C (Table 5).

The mean scores for color, flavor, taste and overall acceptability of different juice samples are presented in Table 6. According to two way analysis of variance ANOVA, the juice samples were significantly (P<0.05) different from each other. Panelists preferred the T₄ sample, which showed hedonic scale '8' point in flavor, taste and overall acceptability indicates that the sample has been liked very much.

The color of T₄ sample also showed 7.7 point, which means moderately like and is also higher than the other samples (Table 6). The least hedonic points were found for T₁ sample, which indicates the least preference by panelists in terms of color, flavor, taste and overall acceptability.

### Conclusion

The experiment was carried out to explore the acceptable formulation of mixed fruit juice incorporating pineapple, papaya, orange and banana fruit and its storage stability. The sample T₄ (pineapple 40%, papaya 30%, orange 20%, banana 10%) was more acceptable due to its delightful yellow color and better chemical composition such as, TSS 10.8°Brix, acidity 0.33%, pH 3.92, vitamin C 14.62 mg/100 g, reducing sugar 5.30%, non-reducing sugar 12.40%, potassium 43.16 mg/100 g, calcium 7.67 mg/100 g and magnesium 18.33 mg/100 g. The storage temperature of 4°C

| Storage period (day) | Juice sample | Color | Flavor | Visual fungal growth | Overall acceptability |
|----------------------|--------------|-------|--------|----------------------|-----------------------|
| 0                    | T₁, T₂, T₃, T₄ | NC    | NOF    | NG, NG               | N, N                  |
| 30                   | T₁, T₂, T₃, T₄ | NC    | NOF    | NG, NG               | N, N                  |
| 60                   | T₁, T₂, T₃, T₄ | NC    | NOF    | NG, NG               | N, N                  |
| 90                   | T₁, T₂, T₃, T₄ | NC    | NOF    | NG, NG               | N, N                  |
| 105                  | T₁, T₂, T₃, T₄ | SC    | SOF    | SG, SS               | -                     |
| 120                  | T₁, T₂, T₃, T₄ | SC    | EOF    | EG, S                | N                     |
| 150                  | T₁, T₂, T₃, T₄ | SC    | EOF    | EG, S                | SS                    |
| 180                  | T₁, T₂, T₃, T₄ | MC    | EOF    | EG, S                | SS                    |

N.B: NC = No change, SC = Slightly change, Moderately change, NOF = No off flavor, SOF = Slightly off flavor, EOF = Excessive off flavor, NG = No growth, SG = Slightly growth, EG = Excessive growth, N = Normal, SS = Slightly spoiled, S = Spoiled
was more effective to hold the quality of juice samples against biochemical changes and \( T_4 \) sample showed better retention of vitamin C, pH, acidity and total sugar than other samples both at 4ºC and 25ºC. Panelists preferred the \( T_4 \) sample most, which showed maximum hedonic scale ‘8’ point in flavor, taste and overall acceptability at the level of 5% significance. It is observed that \( T_1 \) ensured the better shelf life to preserve the color, flavor and taste until 150 days at 4ºC and 105 days at 25ºC. The experimental finding concludes that the mixed fruit juice with a simple preservatives sodium benzoate (0.08%) and packed in plastic bottles manually can be easily preserved for three months in room temperature and for up to five months in refrigerated temperature.

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**Table 6. Mean score for color, flavor and overall acceptability of mixed fruit juice.**

| Juice sample | Color | Flavor | Taste | Overall Acceptability |
|--------------|-------|--------|-------|-----------------------|
| \( T_1 \) | 5.5c | 5.7c | 6.1c | 5.6c |
| \( T_2 \) | 6.8b | 7.0b | 6.4b | 7.1b |
| \( T_3 \) | 5.4c | 6.1c | 7.5c | 6.1c |
| \( T_4 \) | 7.7a | 8.1a | 8.4a | 8.1a |
| LSD (P<0.05) | 0.375 | 0.577 | 0.533 | 0.569 |

The same letter in the same column indicates no significant difference at the level of 5% significance.