Study on the storage stability of fruit juice concentrates

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ABSTRACT
Fruits are perishable commodities and their quality is deteriorated during transportation due to the action of environmental factor (temperature and sunlight) and enzymes. With the application of innovative technologies deterioration can be minimized and juices can be stored for longer period of time. In this research project fresh, properly ripe fruits were selected and their juice was extracted. The extracted filtered juices were pasteurized at 72°C for 15 minutes following by condensation using evaporation. The product was stored at refrigeration temperature and evaluated for chemical and organoleptic analyses like ascorbic acid, malic acid, tartaric acid, acidity, pH, total soluble solids, reducing and non-reducing sugars at zero to thirty days after every ten days interval. Results showed that the loss of ascorbic acid was minimum in mango which was 11.42 %. The maximum loss of ascorbic acid was recorded in mango which was 16.05 % at the end of storage period of 30 days. In case of malic acid it had been increased slightly. The minimum increase of malic acid was found in AJC (Apple juice concentrate) which is 2.78 % while the maximum increase of malic acid was recorded in PJC (Peach juice concentrate) which is 5 % at the end of storage period. Tartaric acid was detected in least quantity as compared to other organic acids because it was present in trace amounts. The minimum decrease of tartaric acid was in peach juice concentrate which was 0.26% while the maximum decrease of tartaric acid was recorded in mango juice concentrate which was 0.93%. Overall the stability of organic acids was minimum in mango juice concentrates and maximum stability was observed in peach juice concentrates.

Key words: fruit juice, juice concentrate, juice stability, juice storage, Organic acids

INTRODUCTION
In Pakistan production estimates for various fruits are: over 1.9 million metric tons (GOP, 2013). Juice concentrates have many advantages over non-concentrated juices; Six to seven times the more quantity can be stored in a storage tank under simplified storage conditions than normal juices. Concentrate can be preserved without being cooled due to its high sugar content. Changes in quantity and quality at different periods can be monitored and adapted to the market situation. Concentrate as commodity is easier to market and the transport of concentrate is simpler and cheaper. The necessary high final concentrations, which depend on the type of fruit, degree of ripeness, juice production method, pretreatment and the fiber and pulp proportion, can be achieved in this way.

Apple juice contains sufficient amount of Vitamin A and C. Vitamin A helps to improve vision as it contains beta-carotene. On the other hand Vitamin C is a good source of antioxidant and makes our immune system strong. The antioxidant helps in cancer prevention. Other vitamins found in apple juice which are comparatively in small proportion are Vitamin K, Vitamin E, Vitamin B1, B2 and B3 (Riaz, 1983). Mangos are rich in Vitamins A and C and have very high fiber content, which encourages good digestion. Mango juice prevents mental weakness due to high content of beta carotene, it helps to prevent heart and eye disease. Regular consumption of mango juice improves complexion and skin health. One cup of mango juice contains over 60% of the daily recommended intake of vitamin C, which helps the body regulate enzymes and metabolic processes (Decio, 1993). Guava fruit contains vitamin C which functions as an antioxidant. In addition, guava also contains vitamins B, A, calcium, phosphorus, iron, and pectin. High fiber content in guava is good for improving digestion (Owen et al., 2004). Vitamin C is one vitamin in peaches that helps a healthy immune system. Peach juice also contains a good amount of vitamins A and K. Peach juice contains trace amounts of many minerals that are beneficial for health. A 3/4 cup glass of peach juice provides a small amount of calcium, which is required by our body to make and maintain strong bones and teeth. A single serving of peach juice also contains a good amount of potassium, as well as small amounts of iron, magnesium, phosphorus and zinc (Elizabeth et al., 2011).

Organic acids are a useful index of authenticity in fruit products, since they have lower susceptibility to change during processing and storage than other components of fruits (Camara et al., 1994). Accurate knowledge of organic acids levels and ratio might be useful for determining the percentage juice content of juices and beverages, and also for detecting misbranding and adulteration in this food class (Coppola and Starr, 1986). The nature and concentration of organic acids in fruits have been of interest because of their important influence on the organoleptic properties of fruit juices. Since each fruit has a unique pattern of organic acids, chromatographic analysis of organic acid could be applied for the verification of juice authenticity (Wrolstad, 1981). Keeping in view all the beneficial aspects of juice concentrates the study was planned to study the behavior of organic acids and sugars in juice concentrates with passage of time.
MATERIALS AND METHODS

Procurement and Fruit Processing
Different fruits (Mango, Apple, Guava and Peach) were taken from local market and they were named as MJC, AJC, GJC and PJC respectively. All the fruit samples were stored at 5°C and were processed into fruit juices till further utilization. Fruits of mango, apple, guava and peaches were peeled and cut into slices then juices were extracted and diluted with treated water. Juices were filtered by using a muslin cloth. After juice preparation, juices were heated at 96°C for one minute to inactivate enzymes. Following the heating process, the juices were cool down to room temperature rapidly, and then filtered through 8-folded cheese cloth to eliminate particulates. Water of the juices was removed by evaporation method for concentration of the juices. In the evaporation process, the concentration value is typically increased from 10 to 65° Brix at low temperature. After cooling the juice concentrates were put into 250 mL glass bottles. After bottling, all samples were again heated at 96°C for 20 minutes. Then samples were cooled with tap water and were stored at 4°C for 30 days. Treatments were evaluated at 0, 10, 20, and 30 days of storage for analysis.

Analysis of juice concentrate

pH
The pH of the juice concentrates was determined with the help of digital pH meter (Model HI 9020 Microprocessor pH meter). A sufficient quantity 50 mL of the sample was taken in 100 mL beaker and pH was recorded by the pH meter in accordance with the method explained in AOAC 2000.

Total soluble solids
The total soluble solids in the juice samples were directly recorded by Abbe’s stage refractometer (Model RL Nr. 1373) and the results were expressed as per cent soluble solids (°Brix) as describe by Rangana (1991).

Sugars
Reducing, non-reducing and total sugars were determined by using Lane and Eynon method given by Ruck (1963) by using following formulas.

% Reducing Sugars = Fehling’s solution factor × 100 × dilution/ Vol. of Sample used
% Total sugars = Fehling’s solution factor × 100 × dilution/ Vol. of sample used
% non-reducing sugar (sucrose) = (% Total sugar - % reducing sugar) × 0.95

Determination of organic acids using HPLC Analysis
Organic acids (ascorbic acid, citric acid, malic acid, tartaric acid) were determined by High performance liquid chromatography (HPLC) (Akalin et al., 2002). 7 mL of juice was added to 40 mL of buffer-acetonitrile mobile phase (0.5% wt/vol) (NH₄)₂HPO₄ (0.038 M) (0.4% vol/vol) acetonitrile (0.049 M), at pH 2.24 with H₂PO₄, extracted for 1 hour in orbital shaker (model 75, Burrell Scientifc, Pottsburgh, PA) and centrifuged for 5 minutes. The supernatant was collected and filtered once through Whatman # 1 filter paper and twice through a 0.45-μm membrane filter (Satorious SM 11606, Goettingen, Germany) and then used directly for HPLC analysis. Triplicate analysis was performed on all samples.

Analysis was made by HPLC with UV detector (Perkin Elmer-series 200) at 214 nm using RP-18 column (120 x 4.6 mm) the operating conditions were: mobile phase, aqueous 0.5% (wt/vol) (NH₄)₂HPO₄ (0.038 M) 0.2% (vol/vol) acetonitrile (0.049 M) then both solution were added, 50 % of each to make the final mobile phase, adjusted to pH 2.24 with H₂PO₄; flow rate 0.3 mL/min; ambient column temperature. The mobile phase was prepared by dissolving analytical grade (NH₄)₂HPO₄ in distilled water, HPLC-grade acetonitrile, and H₂PO₄. HPLC grade reagents were used as standards (Sigma chemical Co., St Louis, MO). Solvents were degassed under vacuum filter through a 0.45 μm membrane. 20 μL of sample was injected into HPLC for the analysis. First standard were run to see the retention time of peak of specific acid. Four standards were run for four acids. First their separate peaks were taken then combined peak of all the standards was taken. Then the samples were run and the peaks of samples were overlapped to see the desired acid in the sample. Quantification was based on the external as well as internal standard method of accuracy.

Statistical Analysis
Significant difference among the treatments of final data obtained was determined by using analysis of variance technique (ANOVA) under completely randomized design (CRD) with two factor factorial on SPSS (Statistical Package for the Social Sciences, version 10.0.1, 1999). The mean of all treatments were also compared by using Tukey (HSD) test adopting the method as described by Steel et al (1997).

RESULT AND DISCUSSIONS

pH
The significantly highest pH value was observed at the end of research work 30th day followed by 20th day of storage period with mean values of 4.33 and 4.19, respectively (Table 1: A). The significantly lowest pH value was found at the 10th days of storage period with mean value of 4.07. Fruits affected pH of the different juice concentrates significantly and higher pH was observed in PJC (Peach juice concentrate) followed by MJC (Mango juice concentrate) with mean values of 4.91 and 4.09, respectively while significantly lowest pH value 3.81 was...
observed in MJC (Mango juice concentrate) as shown in Table 1: A. At the start of storage, the pH ranges from 4.21 to 4.79 among juices of different fruits. The pH value increased throughout the storage period and ranged from 3.81 to 4.98 after the 30 days of storage period. Over all increasing trend in pH was observed with prolongation of storage period in all the treatments. It is obvious from the results that acidity during storage period decreased in juice concentrates, similarly the pH increased. The increase in pH may be due to acid hydrolysis of some polysaccharides into disaccharides like starch into sucrose, fructose and glucose etc. These reactions increase the sweetness and decreases sourness, as a result of which pH increases. The results of present investigation are in line with the previous finding of Alaka et al., (2003) who observed that the total titratable acidity declined during storage for both fortified and unfortified samples of guava juice stored in different packaging treatments due to the breakdown of ascorbic acid and citric acid.

**Total Soluble Solids**

The significantly highest total soluble solids were observed at the 30\textsuperscript{th} day of research work followed by 20\textsuperscript{th} day of storage period with mean values of 14.05 and 13.49, respectively. The significantly lowest total soluble solids were found at the zero day of storage period with mean value of 12.73 (Table 1: B). Fruits affected total soluble solids of the juice concentrates significantly and higher in MJC (Mango juice concentrate) followed by GJC (Guava juice concentrate) with mean values of 16.55 and 10.63, respectively. The interaction between storage time and treatment showed significantly the higher total soluble solids with mean value of 16.80 in MJC at the end of experiment while the lower total soluble solids were estimated in GJC at initiation of the experiment with mean value of 10.00. The total soluble solids values increase throughout the storage period and ranged from 10.00 to 16.80 after the 30 days of storage period. The present result are in close agreement with the finding of Sattar et al., (1988), who also reported that the total soluble solids increased during 32 days of storage period of pasteurized drink due to formation of water soluble pectin fractions.

**Reducing Sugars**

The significantly highest reducing sugars was observed at the 30\textsuperscript{th} days of research work followed by 20\textsuperscript{th} day of storage period with mean values of 4.19 % and 3.62 %, respectively. The significantly lowest reducing sugars were found at the 20\textsuperscript{th} day of storage period with mean value of 3.41 % as shown in Table 2: A. Fruits affected reducing sugars of juice concentrates significantly and higher was observed in PJC (Peach juice concentrate) followed by GJC (Guava juice concentrate) with mean values of 6.51 % and 3.53 %, respectively while the significantly the lowest reducing sugars value 2.41 % was observed in MJC (Mango juice concentrate).

The interaction between storage time and treatment showed significantly the higher reducing sugars with mean value of 6.94 in PJC in the end of experiment, while the lower reducing sugars were estimated in MJC at 10\textsuperscript{th} day of the experiment with mean value of 1.32. Over all it is clear from the table that there is a gradual increase in reducing sugars has occurred in all the treatments. The increase in reducing sugars have been reported by a number of research worker and it may be due to conversion of non-reducing sugars to reducing sugars with the increased storage duration (Ahmad, 1986).

**Non-Reducing Sugars**

The significantly highest non-reducing sugars were observed at the 10\textsuperscript{th} day of research work followed by 20\textsuperscript{th} day of storage period with mean values of 5.01 % and 4.15 %, respectively. The significantly lowest non-reducing sugars were found at the zero day of storage period with mean value of 3.07 % (Table 2: B). Fruits affected non-reducing sugars of the juice concentrates significantly and higher non-reducing was observed in MJC (Mango juice concentrate) followed by GJC (Guava juice concentrate) with mean value of 7.03 % and 3.29 % respectively while significantly lowest non-reducing value 2.84 % was observed in PJC (Peach juice concentrate).

The interaction between storage time and treatment showed significantly the higher reducing sugars with mean value of 9.06 in MJC at the 10\textsuperscript{th} day of experiment, while the lower non-reducing sugars were estimated in AJC at zero day of the experiment with mean value of 1.56. Non-reducing sugars in some fruits it increase and in some fruits it decreases by the increase in storage duration has been occurred. Much increase was noticed in the treatment GJC (Guava juice concentrate) but decrease was observed in MJC (Mango juice concentrate) as compared to other treatments. The present results were agreed with the values as reported by Babsky et al., (1986) who reported that reducing sugars increased in clarified apple juice concentrate at a rate determined by the inversion of sucrose during storage period of 111 days at 37\textdegree C, Granzer (1982) on the other find that along storage time increasing of strawberry juices, the sucrose contents decrease.

**Total Sugars**

The significantly highest total sugars were observed at the 30\textsuperscript{th} of research work followed by 10\textsuperscript{th} day of storage period with mean values of 8.53 % and 8.42 %, respectively. The significantly lowest total sugars were found at the zero day of storage period with mean value of 7.14 (Table 2: C). Fruits affected total sugars of the juice concentrates significantly and higher total sugars were observed in MJC (Mango juice concentrate) followed by PJC (Peach juice concentrate) with mean values of 9.70 % and 9.35 %, respectively while significantly lowest total sugars value 5.98 % was observed in AJC (Apple juice concentrate).

The interaction between storage time and treatment...
Table 1: Effect of storage on the pH and total soluble solids of juice concentrates

<table>
<thead>
<tr>
<th>Storage Intervals</th>
<th>Treatments</th>
<th>MJC</th>
<th>AJC</th>
<th>GJC</th>
<th>PJC</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>4.21</td>
<td>4.21</td>
<td>4.21</td>
<td>4.21</td>
<td>4.07b</td>
</tr>
<tr>
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<td>4.11</td>
<td>4.11</td>
<td>4.11</td>
<td>4.11</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>3.94</td>
<td>3.94</td>
<td>3.94</td>
<td>3.94</td>
<td>4.19ab</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>3.81</td>
<td>3.81</td>
<td>3.81</td>
<td>3.81</td>
<td>4.33a</td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>4.01b</td>
<td>4.37a</td>
<td>4.07b</td>
<td>4.91a</td>
<td></td>
</tr>
</tbody>
</table>

MJC=Mango juice concentrate
AJC=Apple juice concentrate
GJC= Guava juice concentrate
PJC= Peach juice concentrate

Table 2: Effect of storage on the reducing, non-reducing and total sugars of juice concentrates

<table>
<thead>
<tr>
<th>Storage Intervals</th>
<th>Treatments</th>
<th>MJC</th>
<th>AJC</th>
<th>GJC</th>
<th>PJC</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>2.90</td>
<td>2.84</td>
<td>3.99</td>
<td>6.55</td>
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<td>10</td>
<td></td>
<td>1.32</td>
<td>2.68</td>
<td>3.40</td>
<td>6.23</td>
<td>3.41c</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>2.12</td>
<td>2.84</td>
<td>3.19</td>
<td>6.32</td>
<td>3.62c</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>3.32</td>
<td>2.95</td>
<td>3.56</td>
<td>3.94</td>
<td>4.20a</td>
</tr>
<tr>
<td>Means</td>
<td></td>
<td>2.42d</td>
<td>2.83d</td>
<td>3.54c</td>
<td>6.51a</td>
<td></td>
</tr>
</tbody>
</table>

MJC=Mango juice concentrate
AJC=Apple juice concentrate
GJC= Guava juice concentrate
PJC= Peach juice concentrate
Table 3: Effect of storage on the ascorbic acid, maleic acid and tartaric acid of juice concentrates

<table>
<thead>
<tr>
<th>A</th>
<th>Storage Intervals</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic Acid (mg/100mL)</td>
<td></td>
<td>MJC</td>
</tr>
<tr>
<td>0</td>
<td>51.43</td>
<td>46.39</td>
</tr>
<tr>
<td>10</td>
<td>49.44</td>
<td>45.54</td>
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<tr>
<td>20</td>
<td>47.59</td>
<td>43.61</td>
</tr>
<tr>
<td>30</td>
<td>43.18</td>
<td>40.27</td>
</tr>
<tr>
<td>Means</td>
<td>47.91 b</td>
<td>43.95 c</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B</th>
<th>Storage Intervals</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maleic Acid (mg/100mL)</td>
<td></td>
<td>MJC</td>
</tr>
<tr>
<td>0</td>
<td>319.62</td>
<td>971.02</td>
</tr>
<tr>
<td>10</td>
<td>325</td>
<td>972.50</td>
</tr>
<tr>
<td>20</td>
<td>333</td>
<td>975.13</td>
</tr>
<tr>
<td>30</td>
<td>345</td>
<td>975.92</td>
</tr>
<tr>
<td>Means</td>
<td>330.6 b</td>
<td>973.6 a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C</th>
<th>Storage Intervals</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tartaric Acid (mg/100mL)</td>
<td></td>
<td>MJC</td>
</tr>
<tr>
<td>0</td>
<td>0.858</td>
<td>0.669</td>
</tr>
<tr>
<td>10</td>
<td>0.854</td>
<td>0.668</td>
</tr>
<tr>
<td>20</td>
<td>0.848</td>
<td>0.665</td>
</tr>
<tr>
<td>30</td>
<td>0.843</td>
<td>0.663</td>
</tr>
<tr>
<td>Means</td>
<td>0.850</td>
<td>0.666</td>
</tr>
</tbody>
</table>

MJC=Mango juice concentrate
AJC=Apple juice concentrate
GJC= Guava juice concentrate
PJC= Peach juice concentrate

showed significantly the higher total sugars with mean value of 10.34 % in PJC at the end of experiment, while the experiment with mean value of 5.26. The data reveals that total sugars in all juices increases by the increase in storage duration had been occurred. The slightly increase in total sugars may be due to hydrolytic changes and conversion of polysaccharides like starch and pectin etc. into sugars. These findings agreed with the values as reported by Purthi et al., (1984), Babsky et al., (1986) who reported that reducing sugars increased in clarified apple juice concentrate at a rate determined by the inversion of sucrose during storage period of 111 days at 37°C.

Ascorbic Acid
The significantly highest content of ascorbic acid was observed at the start of experiment i.e. at zero day analysis in Mango, Apple, Guava and Peach with mean values of 51.43, 46.39, 36.38 and 43.45 mg/100 mL respectively and similarly the significantly lowest contents of ascorbic acid were observed at the end of storage period of 30 days in Mango, Apple, Guava and Peach with mean values of 43.18, 40.27, 31.74 and 38.49 mg/100 mL (Table 3: A). Fruits effected ascorbic acid of juice concentrates significantly and higher ascorbic acid content were observed in GJC (Guava juice concentrate) followed by MJC (Mango juice concentrate) with mean values of 64.25, 47.91 mg/100 mL and significantly lowest ascorbic acid contents were observed in Peach with mean value of 41.06 mg/100 mL. Similarly comparison of means of storage shows that there maximum degradation of ascorbic acid at storage period of 30 days followed by 20 days with 45.92, 48.77 mg/100 mL mean value of ascorbic acid.

The interaction between storage time and treatment showed that the combined effect of both factors on ascorbic acid contents is non-significant. Overall decreasing trend in ascorbic acid contents were observed with prolongation of storage period in all the treatments. Similar results were obtained by Maria et al., (2003) who observed that with storage period the ascorbic acid (vitamin C) contents were decreased, they observed that the loss of ascorbic acid was 25.65 and 26.74 % for the hot filled and aseptically bottled cashew apple juice, respectively. Kabasaliki et al., (2000) studied the ascorbic acid content of commercial fruit juices and its rate of loss on storage; they observed the loss of ascorbic acid was 29-41 % in commercial fruit juices stored in closed container at room temperature for 4 months.

Malic Acid
The significantly highest content of malic acid was observed at the 30th day of experiment in Mango, Apple, Guava and Peach with mean values of 345, 975.92, 28.55 and 10.18 mg/100 mL, respectively and similarly the significantly lowest contents of malic acid were observed at the start of storage period at zero days in Mango, Apple, Guava and Peach with mean values of 319.62, 971.02, 281.09 and
9.67 mg/100 mL (Table 3: B). Fruits affected malic acid of the juice concentrates significantly and higher malic acid contents were observed in AJC (Apple juice concentrate) followed by MJC (Mango juice concentrate) with mean values of 973.65 and 330.66 mg/100 mL and significantly the lowest malic acid contents 9.87 mg/100 mL were observed in Peach. Similarly comparison of means of storage shows that there is maximum increase of malic acid at storage period of 30 days followed by 20 days with 407.41, 402.40 mg/100 mL mean value of malic acid.

The interaction between storage time and treatment showed that, the combined effect of both factors on malic acid contents is significant. Significantly the higher malic acid contents was in Apple end of storage while the lower contents of malic acid was estimated in Peach in the initation of storage period with mean value of 975.92 mg/100 mL and 9.67 mg/100 mL, respectively. The data on malic acid contents presented in Table shows that the malic acid contents increase in Mango, Apple, Guava and Peach respectively from zero to 30 days of storage period. The present results are in close agreement with the previous finding of Farnworth et al., (2001) whose results show that there is an increase in malic acid contents in thermally processed apple from 917 to 992 mg/100 mL at 4°C for two or nine months of storage.

Tartaric Acid

The significantly highest content of tartaric acid was observed at the start of experiment i.e. zero day analysis in Mango. Apple, Guava and Peach with mean values of 0.858, 0.669, 0.373 and 0.384 mg/100 mL respectively and similarly the significantly lowest contents of tartaric acid were observed at the end (30 days) of storage period in Mango, Apple, Guava and Peach with mean values of 0.843, 0.663, 0.370 and 0.381 mg/100 mL (Table 3: C). Fruits affected tartaric acid of the juice concentrates non-significantly and higher tartaric acid contents were observed in MJC (Mango juice concentrate) followed by AJC (Apple juice concentrate) with mean values of 0.850 and 0.666 mg/100 mL respectively, while significantly lowest tartaric acid value 0.371 mg/100 mL was Observed in AJC (Apple juice concentrate).

The interaction between storage time and treatment showed that the combined effect of both factors on tartaric acid contents is non-significant. The tartaric acid contents decreased throughout the storage period ranged from 0.843 to 0.370 mg/100 mL after 30 days of storage period. Over all a negligible decreasing found in tartaric acid was observed, with prolongation of storage period in all the treatments. The minimum decrease of tartaric acid was in Peach which was 0.26 % while the maximum decrease of tartaric acid was recorded in Mango which was 0.93 % at the end of storage period of 30 days. The present results are in fine with the previous finding of Ghanim et al., (2010) who worked on the determination of organic acid and effect of storage on their stability in cheese. He observed that there is very minute decrease take place in tartaric acid in cheese during storage at 4°C.

REFERENCES
