1. Introduction.

Fruits and vegetables are considered as a very important element of a healthy diet due to their high nutritional value (Vincente et al., 2014). Among fruits, guava (Psidium guajava L.), belonging to the Myrtaceae family, grows well in tropical and subtropical regions. It bears fruit twice in a year (Bal & Dhaliwal, 2004) and is a rich source of ascorbic acid (McCook et al., 2012). With respect to vitamin C contents it is the richest after orange and has vitamin C contents of about 32.05 mg/100g (Muhammad et al., 2014). Due to its nutritional value, it is an important export fruit. Leading exporting countries are Mexico, Thailand, India, Brazil and Philippines while USA, China and UK are the biggest importers of guava (Comtrade, 2014). In Pakistan guava is mostly grown in Punjab and Sindh provinces (Khushk, Memon, & Lashari, 2009) and Punjab represents about 80.7% of the total production (Jain et al., 2001).

Nutritional and postharvest quality of guava depend upon number of pre-harvest factors like soil type, nutrient availability, rainfall, neighbor plants, climate and season (Singh, 2011). It is a perishable commodity due to high water contents and post-harvest changes and physical injures deteriorate the quality or even make the product inappropriate for human consumption (Lee & Kader, 2000; Singh, 2011).

The growing of this fruits is also limited to seasons and localities. In order to meet the demand of the market throughout the year in all areas, the commodities are preserved using different techniques. High moisture content will lead to the drop of quality and, indirectly, to a decrease in quantity. Different techniques are adopted to minimize post-harvest changes in fruit, like temperature management which extends the shelf life of fruits (Aulakh, 2004; Lee & Kader, 2000) by regulating physiological and biochemical processes. But at the same time, lower temperatures may cause chilling damage and higher ones can reduce the storage life of the product (Biolatto et al., 2005; Dixon et al., 2004; Marcilla et al., 2006; Pailly et al., 2004; Tembo et al., 2008; Widayat et al., 2003). The guava is normally marketed as its processed products such as puree, paste, cheese, dehydrated products, jam, jelly, juice, pectin, spread, yoghurt, syrup, squash and canned slices in syrup or nectar (Jagtiani et al., 1988). But temperature, time,
oxygen and light affect the stability of ascorbic acid; the most critical factor in determining L-ascorbic acid stability is storage temperature and thermally processed guava have a lower vitamin C content compared to fresh guava (Nagy, 1980). Vitamins C is also water soluble which may leech during processing.

Keeping in view the importance of guava fruit in human diet and beneficial aspects for health promotion in the Pakistani community, the present study was planned to convert guava juice into concentrate in order to prolonged the shelf life of juice, to facilitate its transportation to far off areas and to ensure the availability of this food commodity round the year. The guava juice concentrate was prepared from the fruit harvested in spring as well as autumn seasons of Pakistan and this concentrate was subjected to storage studies in order to access quality deterioration during storage. The main objectives of the study was to assess the effect of season and cold storage on quality of guava juice concentrate.

2. Material and method

The research was carried out at Citro Pak Private Limited, Sargodha and Institute of Food Science and Nutrition, University of Sargodha, Sargodha.

2.1. Pulp extraction

Good quality, fresh guava fruit was purchased from the local market of Sargodha during various seasons of harvest and pulp was extracted at Citropak Sargodha, Sargodha. The extracted pulp was passed through muslin cloth to remove undesirable suspended solids and to get the clear pulp.

2.2. Preparation of concentrate

The guava pulp was concentrated using quadruple effect evaporators (Ing. A rossi, Viale Europa, Italy) at 65 °C under vacuum (0.81 Psi) to a final 21-23° brix as shown in the Figure 1.

2.3. Physicochemical analysis

Guava pulp and Concentrate was analyzed for refractive brix, moisture, pH, titratable acidity and Ascorbic Acid according to AOAC (1984). Titratable acidity (TA) and total soluble solids (TSS) were determined by using 0.1N NaOH as titrant and hand refractometer respectively. pH of guava pulp and concentrate was measured by using Figure 1. Flow diagram of guava concentrate calibrated pH meter (INOLAB Digital). The ratio between total soluble solids and acid ratio (TSS/TA) was calculated simply by dividing TSS over TA (Hecke, 2006). All analysis were performed in triplicates for accuracy.

Ascorbic acid was determined by titrating juice sample, diluted with 0.4 % oxalic acid, against 2-6 dichlorophenol indophenols dye (AOAC, 1984). Ascorbic acid was calculated using following equations 1 & 2.

\[
\text{Ascorbic acid (mg/100gm)} = \frac{\text{Factor} \times T \times 100}{S \times D}
\]

\[
\text{Factor from Standardization} = \frac{\text{ml of Ascorbic acid}}{\text{ml of Dye used}}
\]

Where: 
T= ml of dye used for sample
S= ml of diluted sample taken for titration
D= ml of sample taken for dilution

2.4. Microbial Analysis

Only guava concentrate was analyzed for total Plate count and total mold count according to method of Tortorello (2014). Total plate count of viable bacteria was determined by ten-fold dilution of guava concentrate using plate count agar (Oxide Ltd., Basingstoke, Hampshire, England). Mold count was determined by pouring 1mL of the previously prepared dilution on Potato Dextrose Agar (Oxide Ltd., Basingstoke, Hampshire, England) and incubated at 30°C for 72 hours. The colonies of bacteria and mold were counted as follows

Microbial count (CFU/mL) = Number of colonies \times 10
2.5. **Statistical analysis**

All the data obtained during research was statistically analyzed by using MSTAT (version 6.1.4 provided by University of Wisconsin) according to Steel & Torrie (1980).

3. **Results**

3.1. **Fresh juice characteristics**

Refractive brix of fresh guava juice was 9.5º and 11 ºbrix for spring variety and autumn variety respectively while moisture contents of spring and autumn season were 89-90.4% and 89-90%.

Acidity, pH and vitamin “C” contents of guava juice prepared from fruit harvested in spring season were 0.47 (% citric acid), 3.83 and 210 mg/100 mL, respectively. While acidity, pH and “vitamin C” contents of guava juice made from fruit harvested in autumn season were 0.44% (based on citric acid), 3.87 % and 210 mg/100mL, respectively.

3.2. **Analysis of guava concentrate**

3.2.1. **Refractive Brix**

Statistical results regarding refractive brix of guava concentrate indicated that the effect of season and storage period was found to be non-significant whereas the interactive effect of season and storage period was also found to be non-significant on refractive brix of concentrate as shown in Table 1.

The interactive effect of season and storage period on refractive brix of guava juice concentrate revealed that maximum brix was found in concentrate of guava made from fruit of autumn season and after 60 days of storage was 25.379 (mean value) while minimum brix was found in concentrate made from guava of spring season with mean value of 21.35 as shown in Figure 2.

3.2.2. **Moisture Content**

Statistical results regarding moisture content of guava concentrate indicated that the effect of season and storage period was found to be significant whereas the interactive effect of season and storage period was found to be non-significant on acidity of concentrate as shown in Table I.

The interactive effect of season and storage period on moisture of guava juice concentrate revealed that maximum moisture was found in concentrate of guava made from fruit of spring season (78.65) and after 60 days of storage was 76.99 while minimum moisture was found in concentrate made from guava of autumn season, with mean value of 75.66 as shown in Figure III.

3.2.3. **Acidity (% Citric acid)**

Acidity is an important attribute because tartness is a major factor in the acceptability of guava juice concentrate after degree brix. Acid gives the characteristic tartness or sourness to juice and has been acclaimed for their effectiveness as thirst quenchers (McEwan & Colwill, 1996). Citric acid is the major acid in the guava juice and concentrates which enhances flavor and provide stability to the product.
Table 1: Mean sum of squares physiochemical parameters of guava concentrate

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>DF</th>
<th>Brix</th>
<th>Moisture</th>
<th>T. A</th>
<th>pH</th>
<th>Brix/Acid</th>
<th>Ascorbic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seasons (A)</td>
<td>1</td>
<td>35.11 **</td>
<td>32.75 **</td>
<td>0.0005 NS</td>
<td>0.0460 **</td>
<td>103.452 **</td>
<td>373.556 **</td>
</tr>
<tr>
<td>Storage (B)</td>
<td>2</td>
<td>2.1633 **</td>
<td>3.1537 **</td>
<td>0.273067 **</td>
<td>0.01502 **</td>
<td>842.035 **</td>
<td>216.222 **</td>
</tr>
<tr>
<td>A * B</td>
<td>2</td>
<td>0.05292 NS</td>
<td>0.14741 NS</td>
<td>0.00207 NS</td>
<td>0.00016 NS</td>
<td>23.7525 **</td>
<td>0.2222 NS</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>0.43524</td>
<td>0.57713</td>
<td>0.00068</td>
<td>0.00176</td>
<td>3.60922</td>
<td>3.11111</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = Highly significant  p value < 0.001  NS = Non-significant  p value > 0.005

Table 2: Microbial Count in guava juice concentrate during Storage (Cfu/ml)

<table>
<thead>
<tr>
<th>Microbial</th>
<th>Seasons</th>
<th>Storage Period (Days)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>0.03</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Autumn</td>
<td>NIL</td>
<td>NIL</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>NIL</td>
<td>NIL</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mold Count</td>
<td>Autumn</td>
<td>NIL</td>
<td>NIL</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>NIL</td>
<td>NIL</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. Effect of season and storage period on acidity of guava juice concentrate

Figure 5. Effect of season and storage period on pH of guava juice concentrate

Statistical results about acidity of guava concentrate showed that the interactive effect of season and storage period was found to be non-significant on pH of concentrate whereas the effect of season and storage period was found to be significant as shown in Table I.

The interactive effect of season and storage period on acidity of guava juice concentrate revealed that maximum acidity was found in concentrate of guava made from fruit of autumn season and spring season after 60 days of storage acidity was 0.88 (% citric acid, mean value) while minimum acidity was found in concentrate made from guava of spring season with mean value of 0.44 (% citric acid) as shown in Figure 4.

3.2.1. pH

The pH is defined as the reciprocal logarithm of hydrogen ion concentration in g/liter. It is an important measure of active acidity which influences the flavor or palatability of a product and affects the processing requirements so pH plays an important role in the preservation and product development.

Statistical results about pH of guava concentrate showed that the interactive effect of season and storage period was found to be non-significant on pH of concentrate whereas the effect of season and storage period was found to be significant as shown in Table 1.

The interactive effect of season and storage period on pH of guava juice concentrate revealed that concentrate of guava made from fruit of autumn season showed maximum pH was 4 which decreased to 3.90 after 60 days of storage .acidity while pH of guava juice concentrate revealed that concentrate of guava made from fruit of autumn season showed maximum pH was 3.89 which decreased to 3.79 after 60 days of storage as shown in Figure 5.

3.2.2. Brix-Acidity Ratio

The brix/acidity ratio is used as an index of maturity. It is an indication of the relative sweetness or tartness of a
Figure 6. Effect of season and storage period on Brix/Acidity ratio of guava juice concentrate

Product. Statistical results regarding brix/acid ratio of guava concentrate indicated that the effect of season and storage period was found to be significant whereas the interactive effect of season and storage period was found to be non-significant on brix/acidity ratio of concentrate as shown in Table I.

The interactive effect of season and storage period on brix/acid ratio of guava juice concentrate revealed that maximum brix/acidity ratio (55.03) was found in concentrate of guava made from fruit of autumn season in the start of storage period and after 60 days of storage brix/acidity ratio was 28.95 in concentrate of autumn season. While minimum brix/acidity ratio, was found in concentrate made from guava of spring season with mean value of 45.7 as shown in Figure 6.

3.2.1. Ascorbic Acid

The statistical results regarding ascorbic acid of guava concentrate (Table I) indicated that the effect of season and storage period on ascorbic acid contents of concentrate was found to be significant whereas the interactive effect of season and storage period was found to be non-significant.

The interactive effect of season and storage period on ascorbic acid of guava juice concentrate revealed that concentrate of guava made from fruit of autumn season showed maximum ascorbic acid which was 260.33 g/100mL which decreased to 248.67 g/100mL after 60 days of storage .while ascorbic acid of guava juice concentrate revealed that concentrate of guava made from fruit of spring season showed maximum ascorbic acid which was 251.67 g/100mL which decreased to 239.33 g/100mL after 60 days of storage as shown in Figure 7

3.3. Microbial Analysis

The guava concentrate was also analyzed for viable and mold count. Due to heat treatment the microbial load was nil in all the treatments however after 60 days of storage very few number of microorganisms were detected during storage. This might be due to sanitation and hygienic problems. The results for microbial analysis are shown in Table II.

4. Discussion

In spring season, the weather is in transition stage from winter to summer while in autumn it’s vice versa (Finch, Samuel, & Lane, 2014). Fruits ripening in spring seasons had faced chilling temperatures of winter while fruits ripening in autumn season had under gone through blazing heat of summer. Due to higher temperature in summer season brix content of guava was higher in autumn season fruit as according to Kizildeniz et al. (2015). Temperature also favors the conversion of carbohydrates into sugars and also lower moisture level of fruit due to water evaporation (Gopala-Rao, 2015) may had caused more brix level in autumn season guava juice. Similarly, the results of brix content of guava concentrate are in agreement with the finding of Germain & Linden (1981) who reported an increase in TSS of fruits pulp.
during storage. The high sugar content of pulps from ripened fruits might have contributed to the transformation of starch into soluble sugars under the action of phosphorylase enzyme during ripening (Favier et al., 1993).

Similarly the results of moisture content of guava pulp and concentrate are justified by the findings of previous researchers who observed that moisture content of pulp is affected by season and storage period (Doreyyapa et al., 2001; Fulya et al., 1999). Higher temperature and storage cause lower moisture levels in fruit due to water evaporation (Gopala-Rao, 2015). The results pertaining to the increase in acidity and decrease in pH during storage of guava concentrate are in complete agreement with other researchers (Doreyyapa et al., 2001; Fulya et al., 1999). The increase in acidity may be due to increase in the concentration of weakly ionized acid and their salts during storage (Hussain et al., 2008; Iqbal et al., 2001; Kizildeniz et al. 2015).

The pH is always inversely proportional to acidity and directly proportional to the temperature (Phillips et al., 2016). As acidity has increased during the storage period of concentrates during this research so pH has decreasing trend accordingly. Guava concentrate storage results are in agreement with the finding of (Cecilia & Maia 2002; Doreyyapa et al., 2001; Fulya et al., 1999) who observed a decrease in pH of fruit concentrate during storage. Similarly pH of concentrate made from guava harvested in autumn season have lower pH values as compared to concentrate of guava fruit harvested in spring season. This difference in pH may be due to the formation of free acids and pectin hydrolysis (Imran et al., 2000).

Ascorbic acid (vitamin ‘C’) content is sensitive to oxygen, light and heat. It can easily be oxidized in the presence of oxygen by both enzymatic and non-enzymatic autolysis as described by Kall (2003). So, vitamin C contents in guava concentrate made from autumn season fruit, were higher as compared to concentrate of spring season. It is also justified by the finding of Uddin et al. (2002), Javed (1988), Pruthi et al. (1984) & El-Ashwah et al. (1982) who also observed decline in ascorbic acid contents of concentrates with elevated temperatures while working on various varieties of fruit. The results of guava concentrate are justified by the findings of previous researchers who observed decline in ascorbic acid with storage period (Doreyyapa et al., 2001; Kinh et al. 2001; Fulya et al., 1999).

5. Conclusion

The results showed that guava concentrate made from autumn fruit had 1.13 times brix content, have higher pH and 3.9 % more vitamin C as compared to concentrate made from spring season guava. The concentrate prepared from guava harvested in autumn season was nutritionally better than the concentrate prepared from guava harvested in spring season.

6. Declaration of Interest

It certify that all the authors do not have any affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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