The effect of packaging and storage of vitamin-C in fresh and commercial orange juices

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Abstract

Vitamin-C in commercial and fresh orange juice samples was investigated. The commercially available juices included packed and powdered orange juices. Different brands of commercial liquid and powdered orange juices were included in this study. The seasonal variation of vitamin-C in fresh oranges was also explored. Fresh cultivar of oranges including Ambersweet, Tangarin and Mandrin were selected for vitamin-C content. Furthermore the effect of storage, packing material lined and unlined with different fabrics and preservatives on the stability of vitamin-C in orange juice samples were investigated. Redox titration was used for measurement of vitamin-C content in all samples. Maximum vitamin-C content was observed in end of the season fresh juice samples. Some of the commercially available orange juices (within expiry date) showed highest vitamin-C content, while mostly showed loss of vitamin-C during preservation process.

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Introduction

Vitamin-C, also called L-ascorbic acid (AA) is an important vitamin present in almost all fruits and vegetables. It is an important antioxidant, water soluble vitamin, sensitive to oxygen, heat, alkali and many other factors (Bolling et al., 2013; Lavelli et al., 2011;Licciardello & Muratore, 2011). It has been used to prevent and control scurvy, but is also vital for many other important biological functions such as modulation of the immune system and control of inflammatory diseases (Sigusch, 2013; Sorice et al., 2014).

The amount of vitamin-C depends on the cultivar and ripeness of the citrus fruit and storage conditions (Andrews & Driscoll, 1977; Djordjevic et al., 2013; Ramaiya et al., 2013). Moreover the packaging material has a direct effect on the availability of vitamin-C in orange juice. Vitamin-C containing juices stored in metal or glass containers is stable, but when stored in plastic bottles its shelf-life decreases. Bacigalupi and colleagues (2013) showed that orange juices packed in PET bottles had a loss of 53% vitamin-C. During processing of juices, temperature and oxygen also play a major role in the loss of vitamin-C. Non-thermally processing of juices give higher levels of vitamin-C, but financial factors have minimized the use of such methods in the citrus industry. Another emerging problem in the fruit juice industry is the adulteration of orange juice with less expensive juices and sugars, artificial sweeteners, colors and other materials such as organic phosphate, calcium, added sugar, sodium chloride, magnesium sulphate, which also reduces the stability of vitamin-C (Ribeiro et al., 2011; Stander et al., 2013).

The presence of vitamin-C in fruits, juices, fruit pulps etc has been evaluated by many different methods. HPLC is the method of choice for this purpose (Valente et al., 2014). Recently there has been an explosion of new methodologies including automated methodologies for the determination of pharmaceutical formulations and fruit juices for vitamin-C content (Llorent-Martinez et al., 2013). Thus multi-commutated flow injection analysis has been used for the evaluation of vitamin-C in pharmaceuticals (Llorent-Martinez et al., 2013). A molecularly imprinted copolymer has been described as a sensor for vitamin-C with a concentration sensitivity of 0.1-10 mM (Kong et al., 2014). Similarly a redox method involving polyanalin film has been developed that is sensitive to vitamin-C (Bossi et al., 2000). Spectroscopic methods for the determination of vitamin-C are commonly used. This involves oxidation of vitamin-C with manganese III following reduction of the unreacted manganese with diphenylamine, detected at λ570 nm (Shyla & Nagendrappa, 2013). Voltammetric iodometric titration of vitamin-C in pharmaceutical preparations has also been reported (Verdini & Lagier, 2000). Redox methods for an evaluation of vitamin-C are also commonly used where sophisticated methods may not be easily available (Justi et al., 2000; Muszalska et al., 2000).

There are some certified reference materials available for the estimation of vitamin-C in food matrices, fruits and juices (Valente et al., 2014).

Prolonged use of preservatives such as sodium nitrite is not without consequences. oxidative properties of sodium nitrite are known for hepatic damage and several other organs (Salama et al., 2013; Shinn et al., 2013). No regulatory control has been imposed by the Government of Pakistan for use of these preservatives in orange juices. Manufacturers of the packed orange juice use any preservative any amount at their free will causing public health hazard. Stability of vitamin-C can be maintained in an acidic environment or in the presence of other additives such as sodium nitrite or rosemary extract (Doolaege et al., 2012). Other preservatives such as perchloric and metaphosphoric acids have been used to increase the shelf life of vitamin-C (Valente et al., 2014). The stability of vitamin-C incorporated in whey protein and thermal decomposition kinetics and shelf life of vitamin-C in air and nitrogen have been reported (Janjarasskul et al., 2011; Juhasz et al., 2012). These studies have indicated that the atmosphere influences the stability of vitamin-C. The industrial processing and long-term storage of food material also has strong impact on the stability of vitamin-C. The long-term storage at
ambient temperatures results in the loss of vitamin-C and only 13% of the total vitamin-C remains after 12 months of its storage (Koh et al., 2012).

With innumerable orange juice brands on Pakistan market with little control on quantity and quality of preservatives, storage conditions and packing materials, public health is severely at risk. Therefore, this study was designed to investigate the loss of vitamin-C in different orange juices and different citrus fruits produced locally.

**Material and methods**
The determination of vitamin-C content in fresh oranges of different cultivar and commercial orange juices was conducted. All samples were collected to meet the requirement for analyzing the vitamin-C concentration from fresh oranges and commercial juice samples. Commercial juices samples were collected from the local market of Lahore, Pakistan. Samples were stored at 4ºC, for further analysis. All the packed samples were ensured to get analyzed at least 4-5 weeks before the marked expiry date on the pack. vitamin-C tablets were used as a control.

**Evaluation of vitamin-C content in standard solution**
Vitamin-C tablets (Cawood-C or CaC-1000) were obtained from a local pharmacy. The vitamin-C tablets were ground to a fine powder using a mortar and pestle. 250 mg of the powdered material was poured into a 250 mL Erlenmeyer flask and dissolved in 100 mL of deionized water. 20 mL of the solution was taken into a 250 mL conical flask and 1mL 0.1M starch solution and 150 mL of water were added and mixed well. For titration potassium Iodide 5 mM solution was dropwise added from the burette, until a dark blue color appeared. The titration was repeated three times and means reading was taken. Titration was repeated for different concentrations of vitamin-C (2-100 mg/mL) and a standard curve was drawn and the linear equation was generated and used for determining the vitamin-C concentration in the unknown samples. The concentration of vitamin-C (mg/mL) in the unknown sample was calculated from the linear equation generated for the standard curve.

**Sample preparation and procedure for analysis of vitamin-C content in fresh orange juice**
A piece of fresh orange (Mandarin, Ambersweet or Tangrin) was taken and juice was squeezed out and collected in three plastic bottles. The juice was passed through double layer of cheese cloth to remove any suspended material. To prevent oxidation of vitamin-C, oxalic acid (1mg/mL, final concentration) was added to samples. After addition of starch solution (50 μl), samples were titrated with 5mM solution of potassium iodide solution.

The endpoint of the titration was identified as the first permanent dark blue-black colour formed by the starch-iodine complex. Titration was repeated three times and means volume was taken. As KI is added to the sample, the ascorbic acid is oxidised to dehydroascorbic acid, while the iodine is reduced to iodide ions. The iodine formed is immediately reduced to iodide, as long as there is any ascorbic acid present. Once all the ascorbic acid has been oxidised, the remaining iodine is free to react with the starch indicator, which forms the blue-black starch-iodine complex.

**Analysis of vitamin-C content in liquid and powdered orange juices**
150 mL of the different commercial juices (Fresher, Pulpy orange, Mitchell’s orange juice, Sammi orange juice, Honest orange juice, Nestle orange juice, Rani orange juice, Vivo orange juice, Tang powder orange juice, RASNA powder orange juice, Aroset powder orange juice, Hi-Nutri orange juice National powder orange juice) were obtained and labelled (the powdered juices were first dissolved in a known volume of water as given on the sachet). 50 mL of filtered juice through cheese cloth was taken into a 250 mL Erlenmeyer flask and 50 μl of starch indicator was added. Iodine solution was drop-wise added to the titration flask. To determine the precise color-change in the solution a splitting drop technique was used. This procedure was repeated in triplicates for the entire orange juice sample and results were calculated using the linear equation generated from the standard solution of vitamin-C.
Statistical analysis

All experiments were performed in triplicates. The results have been analyzed by using ANOVA (SPSS version 16) with a significance level of p≤0.05.

Results and discussion

Vitamin-C is an important anti-oxidant, which functions as a co-substrate for many enzymes and also helps to protect against several diseases such as cancer, heart disease, stress and in building of cartilage (Yokoyama et al., 2000; Riemersma et al., 2000; Sargeant et al., 2000).

Table 1. Vitamin-C content in commercial orange juices (liquid and powder).

<table>
<thead>
<tr>
<th>Brand</th>
<th>Nature of sample</th>
<th>Source of sample</th>
<th>No. of sample</th>
<th>Initial amount</th>
<th>Manufactured Expiry date</th>
<th>Range of values</th>
<th>Mean value</th>
<th>Experiment performed</th>
<th>Packing material</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Commercial Orange Fresher drinks</td>
<td>3</td>
<td>100%/250ml</td>
<td>4/10/09</td>
<td>10/10/10</td>
<td>0.065-0.07</td>
<td>0.068</td>
<td>As opened</td>
<td>12/3/2010</td>
<td>Plastic bottle</td>
</tr>
<tr>
<td>2 Pulpy orange</td>
<td>3</td>
<td>30mg/100ml</td>
<td>---</td>
<td>5 days after opening</td>
<td>0.064-0.068</td>
<td>0.067</td>
<td>As opened</td>
<td>16/3/2010</td>
<td>Plastic bottle</td>
</tr>
<tr>
<td>3 Mitchell’s orange</td>
<td>3</td>
<td>60mg/100ml</td>
<td>2/1/10</td>
<td>0.085±0.09</td>
<td>0.089</td>
<td>20/3/2010</td>
<td>Tin pack</td>
<td>12/4/2010</td>
<td>Plastic bottle</td>
</tr>
<tr>
<td>4 Sammi</td>
<td>3</td>
<td>29mg/100ml</td>
<td>3/9/10</td>
<td>0.056-0.064</td>
<td>0.062</td>
<td>20/3/2010</td>
<td>Tin pack</td>
<td>12/4/2010</td>
<td>Plastic bottle</td>
</tr>
<tr>
<td>5 Honest orange</td>
<td>3</td>
<td>25mg/100ml</td>
<td>13/1/11</td>
<td>0.21</td>
<td>0.21</td>
<td>1/4/2010</td>
<td>Cardboard carton</td>
<td>12/4/2010</td>
<td>Plastic bottle</td>
</tr>
<tr>
<td>6 Nestle orange</td>
<td>3</td>
<td>17mg/100ml</td>
<td>12/6/10</td>
<td>0.21</td>
<td>0.21</td>
<td>12/4/2010</td>
<td>Cardboard carton</td>
<td>12/4/2010</td>
<td>Plastic bottle</td>
</tr>
<tr>
<td>7 Rani float</td>
<td>3</td>
<td>25mg/100ml</td>
<td>2/5/10</td>
<td>0.054</td>
<td>0.054</td>
<td>12/4/2010</td>
<td>Tin pack</td>
<td>12/4/2010</td>
<td>Plastic bottle</td>
</tr>
<tr>
<td>8 Vivo orange</td>
<td>3</td>
<td>26.6mg/100ml</td>
<td>30/1/11</td>
<td>0.49±0.5</td>
<td>0.52</td>
<td>12/4/2010</td>
<td>Plastic bottle</td>
<td>12/4/2010</td>
<td>Plastic bottle</td>
</tr>
<tr>
<td>9 Instant powder juice</td>
<td>3</td>
<td>21mg/25g</td>
<td>1/9/11</td>
<td>0.115±0.117</td>
<td>0.12</td>
<td>12/4/2010</td>
<td>Plastic enamel</td>
<td>12/4/2010</td>
<td>Plastic enamel</td>
</tr>
<tr>
<td>10 Mitchell’s orange</td>
<td>3</td>
<td>41mg/100ml</td>
<td>27/6/11</td>
<td>0.101±0.102</td>
<td>0.101</td>
<td>12/4/2010</td>
<td>Plastic enamel</td>
<td>12/4/2010</td>
<td>Plastic enamel</td>
</tr>
<tr>
<td>11 Rasna orange</td>
<td>3</td>
<td>24mg/25g</td>
<td>27/7/11</td>
<td>0.154±0.155</td>
<td>0.162</td>
<td>15/4/2010</td>
<td>Plastic enamel</td>
<td>12/4/2010</td>
<td>Plastic enamel</td>
</tr>
<tr>
<td>12 Aroset</td>
<td>3</td>
<td>10mg/5g</td>
<td>1/8/08</td>
<td>0.058±0.068</td>
<td>0.079</td>
<td>16/4/2010</td>
<td>Plastic enamel</td>
<td>12/4/2010</td>
<td>Plastic enamel</td>
</tr>
<tr>
<td>13 Hi-Nutrition</td>
<td>3</td>
<td>100%/25g</td>
<td>2/5/10</td>
<td>0.1705±0.015</td>
<td>0.2</td>
<td>17/4/2010</td>
<td>Plastic enamel</td>
<td>12/4/2010</td>
<td>Plastic enamel</td>
</tr>
<tr>
<td>14 National orange</td>
<td>3</td>
<td>13mg/25g</td>
<td>1/11/11</td>
<td>0.157</td>
<td>0.222</td>
<td>17/4/2010</td>
<td>Plastic enamel</td>
<td>12/4/2010</td>
<td>Plastic enamel</td>
</tr>
<tr>
<td>15 Vitamin-C tablets</td>
<td>CaC-1000</td>
<td>500mg/6.6g</td>
<td>10/11/10</td>
<td>497.13±0.09</td>
<td>0.064</td>
<td>1/1/2010</td>
<td>Tin pack</td>
<td>3/12/2010</td>
<td>Alumnum foil</td>
</tr>
<tr>
<td>16 Calwood-C</td>
<td>3</td>
<td>500mg/6.6g</td>
<td>9/11/10</td>
<td>500±0.05</td>
<td>500.06</td>
<td>31/12/2009</td>
<td>Aluminum foil</td>
<td>31/12/2009</td>
<td>Aluminum foil</td>
</tr>
</tbody>
</table>

These results were in close agreement to the information provided on the package (500 mg/6.6g).

The vitamin-C content in freshly squeezed oranges (natural) and commercially available orange juice samples are shown in Figure 2 and 3 and tabulated in Table 1 and 2. The amount of vitamin-C did not vary significantly in different brands of vitamin-C available from the market. However, of all orange juice samples included in this study were found to be the best in terms of vitamin-C content. Pasteurization and packaging processes easily destroy vitamin-C. When a container of any type was opened, the vitamin-C content decreased by about 2%.

Different orange cultivars showed different vitamin-C content. Ambersweet had highest vitamin-C followed by Mandarin and Tangrin respectively (Figure 2, Table 2). The vitamin-C contents in Mandarin, Ambersweet and Tangrin orange in the beginning of the season were 66.2±2.58mg/100 ml, 77.13±9.06 mg/100 mL and 37.4±4.97mg/100 mL respectively, while the vitamin-C content of the same cultivar at the end of the season was 105.52±14 mg/100 mL, 117.90±5.43 mg/100 mL and 56.54±8.32 mg/100 mL respectively (Figure 2, Table 2). In commercial fruit juice samples Vivo orange (0.52mg±0.004) and Nestle orange (0.21mg) and in orange instant powder Hi-nutrition (0.2mg±0.017) and National orange
(0.22mg±0.004) showed highest level of vitamin-C (Figure 3, Table 1). Unripe fruit had the highest level of vitamin-C content. Vitamin-C losses during ripening process may be due to higher rate of photosynthesis (Brenes et al., 2005). The position of fruit on the tree also affects vitamin-C content and sunlight exposure enhances vitamin-C content. Fruit positioned on the outside of the tree and on the south side are reported to have higher level of vitamin-C (Aruoma, 1998). The experimental results show that in Ambersweet, Mandarin and Tangrin get more ascorbic acid at certain stages of maturity. The ending season had the highest vitamin-C, while starting season showed lowest vitamin-C content (Figure 3, Table 2).

Table 2. Vitamin-C content in fresh oranges (mandarin, ambersweet and tangrin) with the seasonal variation.

<table>
<thead>
<tr>
<th>Brand</th>
<th>Nature of sample</th>
<th>Source of sample</th>
<th>No. of sample</th>
<th>Range of values</th>
<th>Mean value</th>
<th>Oxalic acid</th>
<th>Experiment performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mandarin</td>
<td>Starting season</td>
<td>3</td>
<td>63-71.3</td>
<td>66.2</td>
<td>Added</td>
<td>As plucked</td>
</tr>
<tr>
<td>2</td>
<td>Mandarin</td>
<td>Mid season</td>
<td>3</td>
<td>54-66</td>
<td>61</td>
<td>Not added</td>
<td>preserved for 4-5 days at room temp</td>
</tr>
<tr>
<td>3</td>
<td>Mandarin</td>
<td>Ending season</td>
<td>3</td>
<td>122-165</td>
<td>150</td>
<td>Not added</td>
<td>As plucked</td>
</tr>
<tr>
<td>4</td>
<td>Ambersweet</td>
<td>Starting season</td>
<td>3</td>
<td>65.6-95</td>
<td>77.1</td>
<td>Added</td>
<td>As plucked</td>
</tr>
<tr>
<td>5</td>
<td>Ambersweet</td>
<td>Mid season</td>
<td>3</td>
<td>55.5-95</td>
<td>72.57</td>
<td>Not added</td>
<td>preserved for 4-5 days at room temp</td>
</tr>
<tr>
<td>6</td>
<td>Tangrin</td>
<td>Starting season</td>
<td>3</td>
<td>109-128</td>
<td>117</td>
<td>Not added</td>
<td>As plucked</td>
</tr>
<tr>
<td>7</td>
<td>Tangrin</td>
<td>Mid season</td>
<td>3</td>
<td>28.8-46</td>
<td>37.4</td>
<td>Added</td>
<td>As plucked</td>
</tr>
<tr>
<td>8</td>
<td>Tangrin</td>
<td>Ending season</td>
<td>3</td>
<td>21.8-32</td>
<td>27</td>
<td>Not added</td>
<td>preserved for 4-5 days at room temp</td>
</tr>
<tr>
<td>9</td>
<td>Tangrin</td>
<td>Ending season</td>
<td>3</td>
<td>44.8-72.6</td>
<td>56</td>
<td>Not added</td>
<td>As plucked</td>
</tr>
</tbody>
</table>

Moisture contents of the fruit vary from 82.8 to 90%, when just ripe, and it decreases, when the fruit is over-ripe. The ripe oranges contain 4.0 to 11-5% fibre and the value increases as fruit matures (Davalos et al., 2005). When different varieties of citrus are stored in pyramidal structure under ambient conditions, the shelf-life extension is observed upto 28 days (La Vecchia et al., 2001). This is because of the reduction in the rate of evaporation of water and respiratory gases (CO₂ and O₂), which improve the appearance of fruit shelf-life of the citrus fruit. It can be further extended by wax coating of the fruit or by using lining material like polyethylene, butter paper and cellophane as packing material (Ramadan et al., 2003).

Citrus fruit shows an increase in reducing sugars during storage. The conversion of non-reducing sugar into reducing sugar in different variety of citrus fruit, stored at ambient temperature is known to be more than those stored in pyramidal structure. When fresh orange juice is preserved in containers, vitamin-C loss is due to oxidation by a residual air layer trapped within the container during processing. The loss is faster in the first 2 weeks and is more evident at higher storage temperatures (Proteggente et al., 2002). Therefore, it is recommended that orange juice must be kept cool to prevent vitamin-C degradation (Ramadan & Moersel, 2007).

Studies have shown that orange peel has the highest level of vitamin-C, followed by the pulp, and juice. Only 26% of vitamin-C of a citrus fruit can be found in the juice. The peel has 53% and the pulp and rag has 21%. Vitamin-C content of commercial orange juices ranges from 2.4 to 43 mg/100 mL (Ramadan &
Moersel, 2007). Storage of commercial orange juices in closed containers at room temperature for 4 months results in vitamin-C losses ranging from 29 to 41%. Commercial orange juice, when stored in open containers in the refrigerator for 31 days, lost 60 to 67% of its ascorbic acid, while fresh orange juice lost ascorbic acid at much slower rate of 7 to 13% (Terry et al., 2001). Open containers of commercial orange juice, when stored outside the refrigerator for 10 days, may lose 12.5% of their ascorbic acid content, while refrigerated juice for the same period, the ascorbic acid loss reached - to 9%. Canned orange juices have lower vitamin-C levels due to heating during the canning process (Willett, 1991). Vitamin-C content is less in pasteurized juices than in fresh orange juices. This loss is due to the addition of sugar, which dilute liquid juices. A similar reduction in vitamin-C content of citrus juices and concentration of mineral content increases the final concentration of vitamin-C content. This is probably due to the addition of the sugar which invariably contains certain minerals that complex with vitamin-C (Rodríguez-Bernaldo & Costa, 2006).

In canned orange juice there is residual oxygen that oxidises ascorbic acid to dehydroascorbic acid and also vitamin-C reacts with the tin. Glass packed orange juice provides poor retention of vitamin-C, losing 10% after 4 months of storage, oxidation starts due to photo oxidation through transparent glass. Cardboard carton have specially designed multi-layered oxygen and light barriers to protect both loss of vitamin-C, flavour, and to enhance shelf-life (Richelle et al., 2001). Orange juice packed in foil-lined cardboard cans retain greater than 90% of their vitamin-C after 12 months at -20°C. Mostly sugar found in orange juices is fructose that can cause breakdown of vitamin-C. The higher the fructose content the greater the loss of vitamin-C has been reported (Kahkonen et al., 1999).

The Pharmaceutical preparations e.g. tablets containing vitamin-C have also been analyzed for the determination of vitamin-C content. The determination of vitamin-C content in tablets was 500.06±0.03 mg/tablet in CaCl₂ 1000 in Calwood-C 497.13±0.09 mg/tablet. The same method was used to analyze vitamin-C content in instant powdered juices. However, the results were not reliable. In samples with early manufacturing date the loss of vitamin-C was greater, Tang orange juice had only 0.0283±0.004 mg/100 mL vitamin-C content (Figure 3). For the production of powdered orange juice (Tang) the fresh orange juice is evaporated at high temperature that results in the total loss of vitamin-C (Gillman et al., 1995). The samples having early manufacturing date had low vitamin-C content, while the samples having late manufacturing date had a comparatively high vitamin-C content e.g. Vivo 100% orange inside have 0.512±0.002 mg/100ml vitamin-C content and Sammi orange has least 0.054mg/100mL vitamin-C content. For the purpose of dilution of orange drinks sugar is added, which causes the breakdown of ascorbic acid (Daglia et al., 2000).
Packed juices (liquid and powdered) purchased from the local market of Lahore contain very low content of vitamin-C suggesting the loss of vitamin-C is considerably high due to storage and the process of preservation. The conclusion of this study is that the vitamin-C content of the commercial juices are very low and do not reach the threshold value i.e 2.4mg/serving. When fresh juices are treated by oxalic acid the oxidation process is reduced. Ending season fresh orange has highest amount of vitamin-C in mandarin.

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