Volatile flavor components of orange juice obtained from major citrus producing cities of Punjab

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Abstract

Orange juice is the most popular fruit beverage worldwide due to its nutritional and sensory properties. Fresh and unique delicate flavor of orange juice is due to the presence of complex combination of several organic components. The following study was aimed to evaluate volatile components of oranges obtained from three major citrus producing cities of Punjab (Faisalabad, Sargodha and Bhalwal). Orange juice was extracted by using rose head machine and different physiochemical tests (pH, Acidity, Brix, Ash, Ascorbic Acid) were performed to evaluate the orange juice. Volatile flavor components were extracted from orange juice by liquid-liquid extraction is the multiple-batch extraction using a separating funnel. Then the quality and quantity of these components was be determined by using GC-FID. There were four major compounds were determine in orange juices that were Carvone, γ–Terpinene, Citronellol, Limonene of different concentration in orange juices.

Keywords: Orange, juice, volatile, flavor, GC

Introduction

Citrus is a common term and genus of flowering plants in the family Rutaceae, originating in tropical and subtropical southeast Asia, it refers to all edible and rootstock species of genus citrus and a few closely related genera of the family rutaceae. Commonly sweet oranges, mandarins, grape fruit, lemon/limes, tangerines, pummelos, kumquats etc. are included in this group. Citrus is a leading tree fruit crop of world with a production of 105 million tonnes. As in the world, citrus also hold number one position with respect to area and production in Pakistan. Citrus covers an area of 640 thousands hectares with 6.3 million tonnes production in the country. Today Pakistan among the 14 citrus growing countries of the world, producing 167 thousands tonnes citrus on an area of 185 thousands hectares (Anonymous, 2005). Citrus fruit is grown in all four provinces of Pakistan but Punjab produces over 95% of the crop because of its greater population, favorable growing conditions and adequate water. Pakistan contributes 2% share in the total production of citrus in the world (Anonymous, 2007).

The citrus flavor is among the most popular fruit flavors for beverages. The flavor of orange juice has been studied more than that of any other type of citrus fruit. This is partly because the orange juice is the most popular fruit beverage worldwide, and its great demand is a result of its nutritional and sensory properties. Its fresh and uniquely delicate flavor is due to complex combinations of several odour components that have interdependent quantitative relationships (Shaw, 1991; Maccarone et al., 1998). Volatile flavor components are the compounds, which are biosynthesized during the normal metabolic process in plants (Orav and Kann, 2001). The major volatile components that impart flavor to orange juices are: Esters, Aldehydes, Alcohols, Terpenes, Terpenols and Ketones (Selli et al., 2004).

Volatile components are important contributors to natural orange flavor and aroma (Moshonas and Shaw, 1987). Essential oils from orange peel are the most important and most widely used flavoring ingredients in many foods, beverages, cosmetics and toothpastes (Orav and Kann, 2001). Volatile flavor components are also a component for the pharmaceutical industry for the preparation of medicines and soaps, perfumes and other cosmetics. Volatile flavor components impart different functions in foods: like they make the food tasty, provide pleasure and satisfaction to the consumer, serve as an indicator of stability or shelf life of the food. Antimicrobial activity, anticancer, antitumor and antioxidant effects are also exhibited by volatile flavor compounds (Goff and Klee, 2006). The species of oranges is very diverse and consists of a considerable number of cultivars. Up to now, numerous investigations have been performed aimed at identifying the volatile flavor components of orange juices. One of the reasons for this is the high quality of its flavor (Moshonas & Shaw, 1994). Limonene is the most abundant component of this fraction for all the aromas examined; its contents vary from 63% in the blood orange aroma to about 90% while the remaining compounds found in varying amount (Moufida et al., 2003). There has been relatively little information published on location wise determination of volatile flavor components.

Materials and Methods
Procurement of Oranges from three different places (Faisalabad, Sargodha and Bhalwal) of same variety.

**Processing of Orange Juice**

Fruit was washed in tap water and then was peeled and divided into halves. Fruit juice was extracted using a citrus hand juice extractor. After juice extraction, raw juice was filtered through 8-folded cheese cloth to eliminate particulates.

**Physiochemical Analysis**

- **pH measurement**
  - pH was determined by pH meter (AOAC, 2000)

- **Acidity**
  - Acidity was determined by titration method (AOAC, 2000)

- **Brix**
  - Brix was determined by hand refractometer (Rangana, 1991)

- **Density**
  - Density was determined by Pycnometer Measurement (AOAC, 2000)

- **Ascorbic Acid determination**
  - Ascorbic Acid was determined by titration method (AOAC, 2000)

- **Estimation of volatile flavor components**
  - Volatile flavor components were determined by GC-FID (Selli et al., 2004)

**Volatile flavor components**

**Extraction Technique**

Different extraction methods can be utilized in flavor isolation. The simplest, but quite tedious method for liquid-liquid extraction is the multiple-batch extraction using a separating funnel (Stevenson, et al., 1996) but more specialized equipment may be required depending on the solvents used. Extraction can be done using for example organic solvents. Solvent extraction using for example pentane, diethyl ether or dichloromethane can be best applied to isolate volatiles from some nonfat foods (Stephan, et al., 2000). After that anhydrous sodium sulphate was added to remove the water from the samples, about 5% of sample. Then keep the sample for 1 hr. so anhydrous sodium sulphate absorbed the whole moisture from the sample. After 1 hr sample was filter through No. 4 Whatman filter paper. The remaining samples were concentrated through rotary evaporator at a temperature of 40°C. That sample was again concentrated by passing nitrogen from it.

**Gas Chromatographic method**

By far the most used actual analysis method in flavor research is gas chromatography with a variety of detectors. Different kinds of capillary columns are utilized, and often whole column cryogenics, cryogenic traps, or on-column injections are used in conjunction with further enhancement of resolution, especially for lower boiling point volatiles (Stevenson et al., 1996). The most common instrumental detectors for GC analyses are Mass Spectrometry (MS) Flame Ionization Detection (FID).

The flavor components were identified by GC-FID. Agilent Technologies 6890N was used with the DB-Wax column specified as above. The flow rate of helium carrier gas was 1.5 mL/min. The same oven temperature programming as explained above was applied with an injection volume of 1 mL. The on-column injector temperature was programmed to increase from 20°C to 250°C at 180°C/min. Then, it was held at 250°C for 80 min (Schneider et al., 1998).

**Statistical Analysis**

The data obtained was subjected to statistical analysis by using Completely Randomized Design (one factor factorial) and comparison of means was done by Duncan’s Multiple Range Test (Steel et al., 1997).

**Results & Discussion**

**Chemical Composition**

The chemical composition of the orange juice is given in Table 1. The data obtained from chemical analysis showed different reading of orange juice taken from different locations. Statistical analysis revealed that the results were highly significant of ash, ascorbic acid, acidity, taste and overall acceptability while the other tests like density, brix, pH, flavor and colour show non-significant results.

**Volatile Flavor Compounds**

Volatile flavor compounds of orange juices from three different locations were determined through Gas chromatography-Flame Ionization Detector. Four standards were run on Gas Chromatography Flame Ionization Detector that give different chromatograph’s retention time of different concentration. After the running of standards juice samples (Sargodha, Bhalwal and Faisalabad) were run on Gas Chromatography Flame Ionization Detector that give different chromatograph’s retention time. The concentration was determined through retention time and peak area.

Concentration of standards were observed in different samples like Sargodha, Bhalwal and Faisalabad orange juices showed the concentration of Limonene respectively was 13262.57, 11631.98 and 15520.505. Where as γ-Terpinene concentration was 45.116, 61.99 and 44.64 respectively. Citronellol concentration was 0, 55.60 and 37.55 respectively. Carvone concentration was 169.68, 179.82 and 115.31 respectively. These results are in close confirmatory with findings of (Selli et al., 2004).
Table 1. Mean values of Physicochemical analysis in T1, T2 and T3

<table>
<thead>
<tr>
<th>Physiochemical Test</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidity</td>
<td>1.351</td>
<td>1.354</td>
<td>1.304</td>
</tr>
<tr>
<td>pH</td>
<td>3.730</td>
<td>3.713</td>
<td>3.720</td>
</tr>
<tr>
<td>Brix</td>
<td>12.500</td>
<td>12.333</td>
<td>13.000</td>
</tr>
<tr>
<td>Density</td>
<td>1.054</td>
<td>1.054</td>
<td>1.055</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>33.83</td>
<td>32.44</td>
<td>38.36</td>
</tr>
</tbody>
</table>

T1= Sargodha, T2= Bhalwal, T3=Faisalabad

Table 2 Retention time (R.T) of standards used in Gas-Chromatography

<table>
<thead>
<tr>
<th>Volatile Flavor compounds</th>
<th>R.T (Minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limonene</td>
<td>6.93</td>
</tr>
<tr>
<td>γ -Terpinene</td>
<td>6.39</td>
</tr>
<tr>
<td>Citronellol</td>
<td>35</td>
</tr>
<tr>
<td>Carvone</td>
<td>32.08</td>
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</table>

Table 3 Concentration of standards that are present in orange juice samples.

<table>
<thead>
<tr>
<th>Volatile Flavor compounds</th>
<th>Sarghoda (µg/L)</th>
<th>Bhalwal (µg/L)</th>
<th>Faisalabad (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limonene</td>
<td>13262.57</td>
<td>11631.98</td>
<td>15520.50</td>
</tr>
<tr>
<td>γ -Terpinene</td>
<td>45.116</td>
<td>61.99</td>
<td>44.64</td>
</tr>
<tr>
<td>Citronellol</td>
<td>ND</td>
<td>55.60</td>
<td>37.55</td>
</tr>
<tr>
<td>Carvone</td>
<td>169.68</td>
<td>179.824</td>
<td>115.31</td>
</tr>
</tbody>
</table>
Chromatograph of orange juice sample obtained from Sargodha.

Chromatograph of orange juice sample obtained from Faisalabad.

Chromatograph of orange juice sample obtained from Bhalwal.
Conclusion

The data obtained from chemical analysis showed different reading of orange juice taken from different locations of Punjab. Statistical analysis revealed that the results were highly significant of ash, ascorbic acid, acidity, taste and overall acceptability while the other tests like density, brix, pH, flavor and colour showed non-significant results. Volatile flavor compounds of orange juices from three different locations show minute difference in concentration.

References