Influence of processing on the levels of amines and proline and on the physico-chemical characteristics of concentrated orange juice

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A B S T R A C T

The influence of processing on the levels of bioactive amines and proline as well as on some physico-chemical characteristics of concentrated orange juice was investigated. Samples were collected at seven points of a production line on three non-consecutive days, and analysed for the levels of 12 bioactive amines, proline, and reducing and non-reducing sugars, and for some physico-chemical characteristics. Among the amines analysed, only putrescine, spermidine and synephrine were found in the samples at all processing stages. Significant differences were observed for spermidine and total amine levels throughout processing. Synephrine and putrescine were resistant to processing. Proline levels changed significantly throughout processing, as did levels of reducing and non-reducing sugars. The pH varied from 3.59 to 3.72, acidity from 0.873 to 0.918 g citric acid/100 mL, and density from 1.0378 to 1.0970 g/mL. Among these parameters, pH, acidity and density were significantly affected by processing.

1. Introduction

Orange is the most abundant crop in the world. The fruits are consumed fresh or used in the production of different processed products and by-products. More than 40% of the oranges produced globally are used for processing. Frozen concentrated orange juice is the most widely traded commodity in the international market, including the New York Board of Trade and the Brazilian Mercantile and Future Exchange. Concentrated orange juice is attractive because it offers many advantages: economy due to reduction in packing, transportation and storage volumes and costs; the possibility of preservation outside harvesting time; and improved preservation due to water activity reduction (Galaverna et al., 2008; Gama & Sylos, 2007; Jesus et al., 2007; Marín, Soler-Rivas, Benavente-García, Castillo, & Pérez-Alvarez, 2007).

Orange juice is obtained by squeezing, pressing or crushing fresh, mature oranges of the species Citrus aurantium, Citrus reticulata, and other Citrus reticulata hybrids that have been graded, sorted, and washed. Concentrated orange juice is prepared by removing water from the unfermented juice. The juice is concentrated at 90 to 95 °C in vacuum evaporators. Normally, the ideal final juice concentration is 65 °Brix. It is then stored at −6.6 °C or lower until it is sold or packaged for sale. The frozen concentrated orange juice can be stored for several years at adequate temperatures. Most of the orange juice sold today throughout the world is reconstituted juice (Clark, 2003; Jesus et al., 2007; Veldhuis, 1961).

Orange juice is probably the most recognized and accepted fruit juice in many parts of the world. One orange will typically produce 90 g of juice. It has a fruity and pleasant acidic taste. It is considered of high beneficial value because it contains natural antioxidants, among them, vitamin C (ascorbic acid), carotenoids, flavonoids and phenylpropanoids (Galaverna et al., 2008). It is also an important natural source of potassium and folic acid. Indeed, according to recent epidemiological studies, high consumption of orange juice is associated with a reduced risk of free-radical-related oxidative damage and diseases such as different types of cancer, cardiovascular and neurological diseases (Franke, Pra, Erdmann, Henriques, & da Silva, 2005).

Recently, orange juice has also been valued by the presence of nitrogenous compounds, among them amino acids and bioactive amines. Proline is the predominant amino acid present in orange juice, representing 50% of the total free amino acids (Niu et al., 2008; Tadeo, Ortiz, Martín, & Estellés, 1988). Among bioactive amines present in orange juice, the health-promoting roles of polyamines and synephrine are valued. Polyamines are essential for cell renewal and growth and have strong antioxidant properties (Tasconi, Germana, & Bagni, 2004); whereas synephrine has been reported to cause vasoconstriction, increased blood pressure and bronchial muscle relaxation (Pellati, Benvenuti, & Melegari, 2005; Vieira, Theodoro, & Glória, 2007). Furthermore, synephrine is useful in reducing fat mass in obese humans since it stimulates...
lipolysis and raises metabolic rate and oxidation of fat through increased thermogenesis (Tsujita & Takaku, 2007).

Data are scarce on the levels of these nitrogenous compounds in oranges and orange products, and also on how they are affected by processing. Therefore, the objective of this work was to evaluate the influence of processing on the levels of bioactive amines and proline in concentrated orange juice. The effect of processing on some physico-chemical characteristics of the orange juice was also investigated.

2. Materials and methods

2.1. Material

Samples of orange (Citrus sinensis) juice were collected at seven points along the production line of a concentrated orange juice producer located in the state of São Paulo, SP, Brazil, during three non-consecutive days. The samples were comprised of a mixture of four varieties: Hamlin, Pera, Natal, and Valencia. The samples were collected at the following processing points: (i) after juice extraction (original juice), (ii) after filtration, (iii) after centrifugation, (iv) at the first stage of the evaporation process, (v) at the second stage of the evaporation process, (vi) at the third stage of the evaporation process, and (vii) after blending fresh juice and volatile compounds to recover flavour losses during processing.

Spermidine trihydrochloride, spermine tetrahydrochloride, agmatine sulphate, putrescine dihydrochloride, cadaverine dihydrochloride, serotonin hydrochloride, histamine dihydrochloride, tyramine, tryptamine, 2-phenylethylamine dihydrochloride, synephrine, octopamine hydrochloride and proline were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and used as standards.

The reagents used were analytical grade, except for HPLC reagents that were LC grade. Ultrapure water was obtained from Milli-Q (Millipore Corp., Milford, MA, USA). Acetonitrile (HPLC grade) with UV cutoff of 190.0 nm was purchased from J.T. Baker (Phillipsburg, NJ, USA). The mobile phases were filtered in HAWP membranes (Millipore Corp., Milford, MA, USA). A blank was also prepared using ethylene glycol mono-methyl ether instead of ninhydrin.

2.2. Analytical methods

The samples of orange juice collected at different processing points were standardized to soluble solids content of 10.5 °Brix to facilitate comparison of results without interference from the concentration prior to the determination of the levels of bioactive amines, proline and sugars.

2.2.1. Bioactive amines

The samples of orange juice were centrifuged at 11,180 g at 4 °C for 20 min and filtered through qualitative filter paper and through 0.45 μm HAWP membranes (Millipore Corp., Milford, MA, USA). Synephrine and octopamine were separated and quantified by ion-pair HPLC with UV-Vis detection at 275 nm, after purification with C18 Sep pak (Waters, Milford, MA, USA). The other ten amines were determined by ion-pair HPLC, post-column derivatisation with o-phthalaldehyde and fluorimetric detection at 340 nm excitation and 445 nm emission (Vieira et al., 2007).

2.2.2. Proline

The levels of proline were determined by the spectrophotometric method described by Brasil (2005). An aliquot of the sample (10 mL) was transferred to 200 mL volumetric flask and made to volume with distilled water. The diluted sample (1.0 mL) was transferred to a test tube. After addition of 1 mL of formic acid and 2 mL of ninhydrin, the tubes were agitated using a vortex mixer and kept at boiling temperature for 15 min. The tubes were cooled to 20 °C (5 to 10 min) and 10 mL of n-butyl acetate were added. The tubes were covered and agitated vigorously using a vortex mixer. The organic phase was collected, filtered through filter paper containing anhydrous sodium sulphate, and submitted to spectrophotometric analysis at 509 nm for the quantification of proline. A blank was also prepared using ethylene glycol mono-methyl ether instead of ninhydrin. The analyses were performed in duplicate. An analytical curve was prepared using proline solutions at 5, 10, 25, 40 and 50 mg/L. An aliquot (1 mL) of these solutions were transferred to test tubes containing 1 mL of formic acid and 2 mL of ninhydrin and the procedure was followed as described for the samples. The analytical curve was prepared by plotting absorbance values against proline concentrations (mg/L).

2.2.3. Physico-chemical characteristics

The total soluble solids were determined using a refractometer, model RL1-PZO (Warsaw, Poland), equipped with a thermometer. The results were corrected for the temperature and expressed as °Brix. Total titratable acidity was determined by titration with 0.1 mol/L NaOH using phenolphthalein indicator. The results were reported as g of citric acid/100 mL of juice. The Brix/acid ratio was determined by simple division of the respective values. The pH values were measured with a digital potentiometer Digimod model DM-20 (São Paulo, SP, Brazil), after calibration with pH 4.0 and 7.0 standard buffers. The specific gravity was determined by pycnometer at 20 °C (AOAC. Official methods of analysis of AOAC International. 16th ed., 1995).

2.2.4. Reducing, non-reducing and total sugars

The reducing and non-reducing sugars were determined by the Lane and Eynon (1934) method. The clarified samples, containing equal volumes of Fehling A and B solutions, were titrated with 0.5% glucose solution. Inversion for non-reducing sugars was necessary to calculate the total sugars (AOAC, 1995).

2.3. Statistical analysis

Results were given as mean ± standard deviation of three independent determinations. Analysis of variance was used and the means were compared by the Tukey test at 5% of probability using SPSS 12.0.1 (Chicago, IL, USA). The existence of correlation between amines and proline levels and the physicochemical characteristics was determined by Pearson correlation at 1% probability.

3. Results and discussion

3.1. Total soluble solids of samples collected during concentrated orange juice processing

As expected, there was a significant change in the soluble solids content of the samples collected during different stages of concentrated orange juice processing (Fig. 1). Immediately after extraction, the juice was 10.5 °Brix, which is in accordance with the literature (Ruschel, Carvalho, Souza, & Tondo, 2001). Filtration did not significantly affect the soluble solids content; however, after centrifugation there was a significant decrease in total solids (p < 0.05). During filtration there is removal of pieces of seeds, cell membrane residues, peel fragments and large particles of pulp, whereas during centrifugation, mucilage and smaller particles and molecules are removed, contributing to lower final solids content (Robards & Antolovich, 1995).

During the first stage of concentration, there was little change in soluble solids. In fact, the juice is first heated mainly to
inactivate natural enzymes such as pectinases, and to reduce pathogenic microorganisms (Basak & Ramaswany, 1996; Clark, 2003). Later in the processing, there was a significant increase in the concentration of soluble solids at rates that differed depending on the evaporation stage, reaching 68.2 °Brix at the end of the process. The final concentration exceeded 65.0 °Brix, which is the recommended concentration for commercialization and exportation of concentrated orange juice (Varnan & Sutherland, 1997). However, the excessive concentration was compensated by the incorporation of fresh orange juice to recover flavour losses during the concentration step. The values found in the concentrated juice are in accordance with the standard of identity and quality for the product (del Castillo et al., 1999).

Significantly lower pH values were obtained at the second stage of evaporation. At this stage, there was also a significantly higher titratable acidity, probably due to an increased concentration of acids in the second stage of the evaporation process. However, at the third stage of evaporation, there was an increase of pH and concomitant decrease of acidity. These results suggest that the decrease of acidity is probably due to degradation or oxidation of ascorbic acid and its participation in the Maillard reaction (Galaverna et al., 2008; Johnson, Braddock, & Chen, 1995). According to Galaverna et al. (2008), there is usually a 30% degradation of ascorbic acid after thermal concentration of orange juice.

With respect to the density, lower values were observed after centrifugation (p < 0.05), probably associated with the removal of small cell, fragments residues and mucilage, and the significant decrease on total solids. After the second evaporation stage, there was a significant increase in the density; however, after the blending of fresh juice, density decreased reaching values similar to those found in the original juice. Similar density values were reported by Ruschel et al. (2001) for fresh squeezed orange juices. The Brix/acidity ratio did not change throughout the process, with values between 11.05 and 11.52.

### 3.2. Physicochemical characteristics of the samples during concentrated orange juice processing

According to Table 1, the fresh squeezed juice had an average pH of 3.72, acidity of 0.91 g/100 mL and a density of 1.0454 g/L. These values are similar to those reported by several researchers (Basak & Ramaswany, 1996; Cava & Hernández, 1996; del Castillo, Corzo, & Olano, 1999). There were significant changes on some physico-chemical characteristics of the orange juice throughout processing. There were significant differences at 5% probability for pH, acidity and density during the production of concentrated orange juice. However, the final product had similar values for these parameters compared to the original juice, probably due to the incorporation of fresh orange juice to recover flavour losses during the concentration step. The values found in the concentrated juice are in accordance with the standard of identity and quality for the product (del Castillo et al., 1999).

### 3.3. Sugar levels of samples during concentrated orange juice processing

In order to evaluate the influence of processing on the levels of sugars in orange juice, the samples were standardized to 10.5 °Brix to avoid interference of the difference on the moisture contents of the samples throughout the process. The levels and proportion of reducing and non-reducing sugars detected were similar to those reported by Veldhuis (1961) and Robards and Antolovich (1995) in different varieties of fresh squeezed juice. According to Table 2, there was a significant change in the levels of reducing and non-reducing sugars during concentrated orange juice processing. During the first two stages of evaporation there was a significant decrease in reducing sugars. This reduction could possibly be attributed to the participation of reducing sugars in the Maillard browning reaction (Shinoda, Komura, Homma, & Murata, 2005). At these stages, there was a concomitant significant increase in the levels of non-reducing sugars, which could result from the formation of reversion sugars.

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**Table 1**

<table>
<thead>
<tr>
<th>Processing stage</th>
<th>pH</th>
<th>Acidity (g/100 mL)</th>
<th>Ratio °Brix/acidity</th>
<th>Density (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original juice</td>
<td>3.72a</td>
<td>0.91 ± 0.05</td>
<td>11.40 ± 0.05</td>
<td>1.0454 ± 0.002</td>
</tr>
<tr>
<td>Filtration</td>
<td>3.70 ± 0.05</td>
<td>0.93 ± 0.04</td>
<td>11.05 ± 0.04</td>
<td>1.0467 ± 0.001</td>
</tr>
<tr>
<td>Centrifugation</td>
<td>3.71 ± 0.05</td>
<td>0.75 ± 0.05</td>
<td>11.40 ± 0.04</td>
<td>1.0378 ± 0.003</td>
</tr>
<tr>
<td>Evaporation (1st stage)</td>
<td>3.63 ± 0.05</td>
<td>1.18 ± 0.05</td>
<td>11.43 ± 0.05</td>
<td>1.0592 ± 0.002</td>
</tr>
<tr>
<td>Evaporation (2nd stage)</td>
<td>3.59 ± 0.05</td>
<td>1.93 ± 0.05</td>
<td>11.52 ± 0.05</td>
<td>1.0970 ± 0.004</td>
</tr>
<tr>
<td>Evaporation (3rd stage)</td>
<td>3.72 ± 0.05</td>
<td>0.95 ± 0.04</td>
<td>11.11 ± 0.04</td>
<td>1.0473 ± 0.002</td>
</tr>
<tr>
<td>Blending</td>
<td>3.72 ± 0.04</td>
<td>0.93 ± 0.02</td>
<td>11.23 ± 0.02</td>
<td>1.0466 ± 0.001</td>
</tr>
</tbody>
</table>

Mean values (±standard deviation) with different letters in the same column are significantly different (Tukey test, p < 0.05).
After filtration and centrifugation there was a decrease in non-reducing sugars (p < 0.05). Therefore, it is probably associated with the loss of higher molecular weight sugars.

The levels of non-reducing sugars also decreased during the third evaporation stage, and there was an increase in reducing sugars. These changes could result from hydrolysis of non-reducing sugars and/or of reversion sugars due to the heat treatment. Furthermore, sucrose inversion could also occur. It is interesting to observe that although there were changes in reducing and non-reducing sugars, the levels of total sugars remained the same throughout the process.

3.4. Levels of bioactive amines during concentrated orange juice processing

Among the twelve bioactive amines investigated, only three were detected in the samples: putrescine, spermidine and synephrine (Table 3). The original juice contained a total of 125.9 mg/L of amines. Putrescine was the prevalent amine (77%), followed by synephrine (20%) and spermidine (3%). The presence of these amines was expected as the first is an obligate precursor of spermidine, which is ubiquitous in the plant kingdom, playing critical roles in several processes, such as root growth, somatic embryogenesis, control of intracellular pH, flower and fruit development and response to abiotic stress (Adão & Glória, 2005; Tassoni et al., 2004; Vieira et al., 2007). One may wonder if the high putrescine levels might affect orange with the putrid flavour described in spoiled fish, however, the low pH characteristic of orange will keep the amines positively charged increasing its flavour threshold. Few sensory data on amines are available. This represents an area that deserves further attention (Glória, 2005).

According to Wheaton and Stewart (1970), synephrine is a typical bioactive amine in citrus. The presence of this amine in orange juice has previously been reported by several researchers (Pellati et al., 2005; Vieira et al., 2007; Wheaton & Stewart, 1970). These investigators also detected the presence of other amines not detected in this study, among them tyramine, octopamine, spermine, histamine and serotonin. The difference in amine profile could be related to orange varieties, cultivating practices, and hygienic and sanitary conditions during production, processing and storage (Vieira et al., 2007; Wheaton & Stewart, 1970).

The three amines were present in samples from every stage during the processing of concentrated juice. There were no significant differences on synephrine and putrescine levels throughout processing, suggesting that these amines are resistant to the conditions prevailing during the processing of concentrated orange juice. However, the levels of spermidine were significantly lower after the third stage of evaporation.

3.5. Proline levels

The amino acid proline was detected in every sample of orange juice analysed. The levels varied from 1.29 to 1.99 mg/L, which is similar to the literature values (Robards & Antolovich, 1995). There was a significant difference in proline levels (Tukey test at 5% probability) throughout processing (Fig. 2), with lower levels after filtration and in the first stage of evaporation. This result indicates that there can be loss of proline during filtration. The loss of proline during the first stage of the evaporation process, along with the loss of reducing sugars, suggests the occurrence of Maillard reaction at this stage (Blank, Devaud, Matthey-Doret, & Robert, 2003; Huyghues-Despointes, Yaylayan, & Keyhani, 1994; Moens, Evans, Looker, & Nimlos, 2004; Shinoda et al., 2005; Tressl, Rewicki, Helak, Kamperschroer, & Martin, 1985). In fact, del Castillo et al. (1999) observed a considerable loss of proline, as well as alanine, arginine and asparagine, during heat treatment of orange juice.

3.6. Correlation studies

Pearson correlation studies between levels of bioactive amines and proline and physicochemical characteristics indicated significant (p < 0.001) positive correlations between putrescine and total

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**Table 2**

Levels of reducing, non-reducing and total sugars of samples collected during different stages of concentrated orange juice processing.

<table>
<thead>
<tr>
<th>Processing stage</th>
<th>Levels of sugars (g/L)</th>
<th>Reducing</th>
<th>Non-reducing</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original juice</td>
<td>41.74±</td>
<td>36.87±</td>
<td>78.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(4.45)</td>
<td>(5.17)</td>
<td>(9.07)</td>
<td></td>
</tr>
<tr>
<td>Filtration</td>
<td>45.74±</td>
<td>30.76±</td>
<td>76.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(5.71)</td>
<td>(2.05)</td>
<td>(7.6)</td>
<td></td>
</tr>
<tr>
<td>Evaporation (1st stage)</td>
<td>37.17±</td>
<td>39.12±</td>
<td>76.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.42)</td>
<td>(2.48)</td>
<td>(2.65)</td>
<td></td>
</tr>
<tr>
<td>Evaporation (2nd stage)</td>
<td>37.04±</td>
<td>42.83±</td>
<td>79.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2.74)</td>
<td>(5.4)</td>
<td>(7.98)</td>
<td></td>
</tr>
<tr>
<td>Evaporation (3rd stage)</td>
<td>46.65±</td>
<td>23.79±</td>
<td>70.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3.84)</td>
<td>(4.19)</td>
<td>(1.47)</td>
<td></td>
</tr>
<tr>
<td>Blending</td>
<td>44.05±</td>
<td>26.14±</td>
<td>70.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(4.22)</td>
<td>(5.65)</td>
<td>(3.58)</td>
<td></td>
</tr>
</tbody>
</table>

Analysis was performed after standardization of total soluble solids to 10.5 °Brix. Mean values ± standard deviation in the same column with different letters are significantly different (Tukey test, p < 0.05).

**Table 3**

Levels of bioactive amines in samples collected during different stages of concentrated orange juice processing.

<table>
<thead>
<tr>
<th>Processing stage</th>
<th>Amine levels (mg/L)</th>
<th>Putrescine</th>
<th>Synephrine</th>
<th>Spermidine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original juice</td>
<td>97.6±</td>
<td>24.7±</td>
<td>3.6±</td>
<td>125.9±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(74.5)</td>
<td>(9.14)</td>
<td>(0.18)</td>
<td>(14.4)</td>
<td></td>
</tr>
<tr>
<td>Filtration</td>
<td>100.4±</td>
<td>29.6±</td>
<td>5.0±</td>
<td>135.0±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(22.4)</td>
<td>(3.13)</td>
<td>(0.83)</td>
<td>(24.0)</td>
<td></td>
</tr>
<tr>
<td>Evaporation (1st stage)</td>
<td>91.5±</td>
<td>33.5±</td>
<td>4.7±</td>
<td>129.7±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(28.1)</td>
<td>(1.97)</td>
<td>(1.93)</td>
<td>(28.0)</td>
<td></td>
</tr>
<tr>
<td>Evaporation (2nd stage)</td>
<td>72.4±</td>
<td>30.2±</td>
<td>3.6±</td>
<td>106.1±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(10.7)</td>
<td>(2.31)</td>
<td>(0.24)</td>
<td>(13.1)</td>
<td></td>
</tr>
<tr>
<td>Evaporation (3rd stage)</td>
<td>80.7±</td>
<td>31.7±</td>
<td>3.7±</td>
<td>116.1±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(14.7)</td>
<td>(1.00)</td>
<td>(1.00)</td>
<td>(12.8)</td>
<td></td>
</tr>
<tr>
<td>Blending</td>
<td>117.4±</td>
<td>30.1±</td>
<td>5.5±</td>
<td>153.0±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(36.8)</td>
<td>(0.90)</td>
<td>(2.42)</td>
<td>(38.4)</td>
<td></td>
</tr>
</tbody>
</table>

Analysis was performed after standardization of total soluble solids to 10.5 °Brix. Mean values ± standard deviation in the same column with different letters are significantly different (Tukey test, p < 0.05).

**Fig. 2.** Levels of proline in samples collected throughout the processing of concentrated orange juice.
amines ($R^2 = 0.9754$) and between acidity and ratio ($R^2 = 0.9991$). A significant negative correlation was observed between pH and density ($R^2 = -0.9623$). These results suggest that total amine levels were mainly affected by putrescine levels.

4. Conclusion

In summary, during processing of concentrated orange juice, there were significant changes in soluble solids from 10.5 to 68.2 °Brix after the third stage of evaporation, decreasing to 64.5 °Brix after incorporation of fresh juice and essential oils. Throughout processing, there were significant changes in pH, acidity, density and reducing sugars. However, the addition of fresh juice and essential oils to the concentrated juice provided the components lost during processing. Losses in proline were clearly detected due to thermal pasteurization, probably due to reaction with reducing sugars during Maillard browning. Three amines were found in the samples throughout processing (putrescine, spermidine and synephrine). Losses of spermidine during heat treatment were evident; however, synephrine and putrescine were resistant to heat treatment, and remained unchanged throughout processing.

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References


