Reduction of bitter component of pomelo juice by chemical treatment and immobilized enzyme

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Effect of pH, total soluble solids (TSS), β-cyclodextrin, immobilized enzyme on removal of naringin and on quality of pomelo juice was studied. The maximum reduction in naringin content (0.34 mg/mL) was observed in juice, treated with immobilized naringinase compared to control (1.1 mg/mL). Change of pH from 3.5 to 4.5 reduced naringin content from 31.8% (pH 3.5) to 40.9% (pH 4.5). However, lowering of bitter principle was 49% for 30°B and 43.6% for juice of pH 4.5 & TSS 30°B. Minimum changes in physico-chemical parameters were observed with immobilized enzyme treated juice. Effect of these treatments on the activity of enzymes were also studied. Minimum amylase activity was observed with immobilized enzyme treated sample 18.9 U to that of control 32 U. Change in pectin methyl esterase (PME) activity was not so significant with an increase of pH. Maximum reduction of PME activity was observed with sample of 30°B TSS. However, retention of peroxidase activity was maximum for β-cyclodextrin treated sample 2.1 K_{1}×10^3/mL compared to control 2.7 K_{1}×10^3/mL. Treatment with immobilized enzyme gave an acceptable debittered pomelo juice.

Excessive bitterness in some processed citrus juices adversely affects their marketability. Therefore, a larger portion of processed juice obtained from some non-conventional fruits can not be popularized due to bitterness. Adsorptive removal of bitter principles and tirable acid from citrus juices has been reviewed by Johnson and Chandler. Different methods reported to remove these bitter components from citrus juices include treatment of the juice with enzymes, with immobilized bacteria and enzyme and with insoluble polymers. Other methods reported include raising of pH of the juice, addition of sweetening agents to reduce their bitterness.

Pomelo (Citrus grandis) is an indigenous minor fruit of commercial value. Bitterness is the main problem of pomelo juice. Naringin is one of the main bitter compounds in the juice. However, no information is available on the suitability of debittering techniques for pomelo juice and development of such technology could help processing of this indigenous tropical fruit. So, different methods have been explored to reduce bitterness and results of such studies are presented in this communication.

Experimental Procedure

Materials and Methods

Pomelo fruits were procured from the local market, washed under running water, and cut into two halves. Then juice was extracted by screw type extractor and treated separately as follows:

(i) the pH of juice was raised to (a) 3.5, (b) 4.0 and (c) 4.5 with sodium bicarbonate.
(ii) juice was mixed with sugar syrup (70°B) to achieve final TSS of (a) 20°B, (b) 25°B and (c) 30°B.
(iii) the pH of juice was adjusted to 4.5 by sodium bicarbonate and TSS to (a) 20°B, (b) 25°B and (c) 30°B.
(iv) the juice was treated with β-cyclodextrin monomer at 4% by vigorous mixing, followed by vacuum filtration through Buchner’s funnel.
(v) the juice was passed through a bioreactor packed with immobilized naringinase.

Preparation of crude naringinase

Naringinase was obtained by solid state fermentation using Aspergillus oryzae. For this purpose sterilized wheat bran containing 50% moisture was inoculated with 20% (v/w) spore suspension (1×10^8 spores) of A. oryzae and incubated at 30±2°C for 7 days under stationary condition for enzyme secretion. The mold bran was soaked in distilled water (Bran-water, 1:4) kept at 5°C overnight, squeezed through cheese cloth, centrifuged at 10,000 rpm for 15 min and the supernatant was used as a source of crude naringinase.
Partial purification of naringinase
The crude naringinase after 5 fold concentration was precipitated using chilled acetone and used for the immobilization.

Immobilization of enzyme
The above enzyme concentrate was immobilized in calcium alginate using a standard method\(^1\). The juice passed through the bioreactor packed with immobilized enzyme with a residence time of 5 h at 28±2°C. The untreated juice served as a control.

All the treated juices and control were preserved with 250 ppm SO\(_2\) at ambient temperature for physico-chemical and biochemical analysis. The total soluble solids (TSS) were determined with the help of a hand refractometer (Erma, Tokyo). Total titrable acidity, reducing sugars, ascorbic acid and cloud were determined by standard AOAC methods\(^16\). Naringin, furfural and total phenol were determined by methods described by Ranganna\(^17\). Protein was estimated as per Lowry et al.\(^18\). Peroxidase activity was measured by the method of Joslyn & Zuegg\(^19\). Amylase activity was measured according to methods of enzymology\(^20\). PME activity was measured by titrimetric method.

Results and Discussion
Physico-chemical characteristics of pomelo showed peel, 27.4; seed, 2.9; pulp, 68.2; juice recovery (pulp basis), 33 and refuge, 38.8%; TSS, 9.4°B; pH 3.3, 16.6 mg ascorbic acid/100 mL juice and 1.2 mg naringin/mL.

Different physico-chemical parameters as a function of treatments are summarized in Table 1. Reduction in TSS (°B) and reducing sugars was observed with pH adjusted juice. Maximum transmittance was observed with immobilized enzyme treated juice. This appears to be due to cloud destabilizing enzyme and filtration during passage of juice through the column. Loss of ascorbic acid was observed in each treatment. Minimum loss was observed with β-cyclodextrin treated sample. There was a reduction in naringin content in all the treatments. The maximum being achieved in juice treated with immobilized enzyme (0.3 mg/mL), indicating it as the most effective technique for debittering. An appreciable reduction in naringin content also took place in juice of pH 4.5. However, the lowering of the bitter principle into TSS adjusted juice is apparently due to dilution effect.

Some important enzymes associated with fruit and vegetables are pectin methyl esterase (PME), peroxidase and amylase. Their activity results in the separation of pulp as well as loss of cloud, and breakdown of starch.

Figure 1 shows the effect of different treatments on peroxidase activity. Peroxidase activity was the same for samples of pH 3.5 and 4.0, and increased slightly at pH 4.5. It was 1.2, 1.2 and 1.5 K\(_{10^3}\)x mL at pH 3.5, 4.0 and 4.5, respectively compared to 2.7 K\(_{10^3}\)x mL for control. Decrease in peroxidase activity was observed with an increase in brix of juice and the activity varied from 66 (20°B) to 51% (pH 4.5, 30°B) compared to control (100%). Treatment with immobilized enzyme decreased peroxidase activity.

<table>
<thead>
<tr>
<th>Parameters (°B)</th>
<th>Control</th>
<th>pH adjusted to</th>
<th>Brix adjusted to</th>
<th>pH 4.5 and Brix adjusted to</th>
<th>Treatment with β-cyclodextrin</th>
<th>Imm. Enz.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS (°B)</td>
<td>9.4</td>
<td>9.2</td>
<td>9.3</td>
<td>9.3</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>pH</td>
<td>3.3</td>
<td>3.5</td>
<td>4.0</td>
<td>4.5</td>
<td>3.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Reducing sugars (%)</td>
<td>3.4</td>
<td>2.1</td>
<td>3.3</td>
<td>3.3</td>
<td>8.7</td>
<td>9.0</td>
</tr>
<tr>
<td>NEB (440 nm)</td>
<td>0.64</td>
<td>0.66</td>
<td>0.69</td>
<td>0.69</td>
<td>1.13</td>
<td>1.17</td>
</tr>
<tr>
<td>Furfural (515 nm)</td>
<td>0.39</td>
<td>0.33</td>
<td>0.46</td>
<td>0.52</td>
<td>0.51</td>
<td>0.52</td>
</tr>
<tr>
<td>Total phenol (765 nm)</td>
<td>0.77</td>
<td>0.76</td>
<td>0.76</td>
<td>0.76</td>
<td>0.53</td>
<td>0.43</td>
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<tr>
<td>Haze (660 nm)</td>
<td>11.13</td>
<td>16.80</td>
<td>17.99</td>
<td>17.95</td>
<td>12.04</td>
<td>12.37</td>
</tr>
<tr>
<td>Titrable acidity (%)</td>
<td>1.32</td>
<td>1.26</td>
<td>1.21</td>
<td>1.19</td>
<td>1.02</td>
<td>0.92</td>
</tr>
<tr>
<td>(as citric acid)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naringin (mg/mL)</td>
<td>1.10</td>
<td>0.75</td>
<td>0.67</td>
<td>0.65</td>
<td>0.73</td>
<td>0.71</td>
</tr>
<tr>
<td>Ascorbic acid (mg/100 mL)</td>
<td>14.85</td>
<td>12.64</td>
<td>11.55</td>
<td>11.37</td>
<td>12.39</td>
<td>11.64</td>
</tr>
</tbody>
</table>

NEB – Non Enzymatic Browning
significant with an increase in pH from 3.5 to 4.5 and it was 10.82, 10.78 and 10.75 PEUx10⁴/mL for samples of pH 3.5, 4.0 and 4.5 respectively. Reduction of PME activity was observed with an increase in TSS (°B). It was 8.92, 8.76, 7.55 PEUx10⁴/mL for 20, 25 and 30°B samples, respectively.

Treatment with β-cyclodextrin reduced the activity of PME, 74% activity was retained with β-cyclodextrin treated juice compared to control (100%). Retention of PME activity was higher in juice treated with immobilized naringinase than that of β-cyclodextrin treated juice, but less than that of control juice. For immobilized enzyme treated juice and control, PME activity was 10.85 and 12 PEUx10⁴/mL, respectively.

Figure 3 depicts the effect of different treatments on the activity of amylase. For TSS adjusted juice amylase activity was less than other samples. This may be due to (i) dilution of protein, and (ii) increase in pH. Minimum amylase activity (18.9 U) was observed in immobilized enzyme treated juice compared to 32U in control juice.

**Conclusion**

It may be concluded that treatment of pomelo juice with immobilized enzyme is better for the removal of its bitterness and making juice acceptable in taste, as evaluated by a panel of 10 tasters.

**Acknowledgement**

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**References**

Notes

16 AOAC Methods of Analysis, 10th Edn (AOAC, Washington, DC), 1965.