Red Beet Pigment Composition. Effects of Fermentation by Different Strains of Saccharomyces cerevisiae

M. DRDAK, R.C. ALTAMIRANO, A. RAJNIKAHOVA, P. SIMKO, J. KAROVICOVA, and D. BENKOVSKA

ABSTRACT

Pasteurized red beet juice was inoculated with seven strains of Saccharomyces cerevisiae after adjusting pH to 5.8. Fermentation was at 26°C. The decay of red beet pigments was spectro-photometrically determined and expressed as betanine. Average percentages of betanine, isobetanine, betanidine, isobetanidine and the saccharide components were monitored by HPLC. After 40 hr fermentation, the strains Oenoferm, 1090 and Tokaj 76D showed pigment retentions and sucrose concentrations (g.L⁻¹) of 62% and traces, 58% and 3.4, and 56% and 1.4 respectively, and were considered the most suitable.

Key Words: red-beets, pigments, acidity, fermentation, Saccharomyces.

INTRODUCTION

PRODUCTION of red beet concentrate and powder has been studied for many years and the many patents concerning it (Zhuk and Tsapalova, 1972; Kadzhoja, 1972; Oszlanyi and Rizhava, 1976; Chkeidze, 1974; Verhulst et al., 1977; Drdak and Nasca kova, 1985) have been used in the fermentative process. Pourrat et al. (1983), in fermentation with Aspergillus niger and Saccharomyces oviformis removed the sugars and lowered the percentage of vulgaxanthin. The objective of our work was to follow the influence of different strains of Saccharomyces cerevisiae on red beet pigment composition.

MATERIALS & METHODS

Preparation of samples

The samples were prepared as described earlier (Drdak and Nasca kova, 1985). This consisted of crushing the raw material, pressing, centrifuging the dispersed particles (3000 rev.min⁻¹) and fermenting.

In the fermentation, a strongly fermentative strain of mesophilic yeast, Saccharomyces cerevisiae (Oenoferm, Erbsliih Geisenheim), five new isolated strains (marked for working purpose as : 6C, 11C, 1090, 3A, 74F) characterized as alcohol resistant, suitable for secondary wine fermentation (Dept. of Biochemical Technology, Slovak Tech. University), and Saccharomyces oviformis (Tokaj 76D V 10-25-34 from the collection of KVUVV (Complex Research of Vine Cultivation and Wine Production, Bratislava) recommended in previous works (Drdak and Nascakova, 1984, 1985; Drdak et al., 1989) have been used. Oenoferm was revitalized according to the producer's instruction, and the other strains were grown on malt extract broth. The pasteurized juice was inoculated with 1 x 10⁹ vital cells/ mL after adjusting pH to 5.8. Fermentation was at 26°C in closed dark vessels and was monitored after 40, 60 and 180 hr to determine the percentage of individual pigments and of the main saccharide components (sucrose glucose and fructose).

Determination of pigments

For determination of pigments, a spectrophotometric method was used which did not require separation of individual pigments and enables betacyanines to be determined. (Nilsson, 1970).

HPLC of betacyanine pigments

For the separation and determination of betacyanine pigments, the method developed by Schwartz and von Elbe (1980) and a chromato-graph (Laboratorni Pirstroje, Prague) consisting of: two HPP4001 pumps, a GP1 Gradient Programmer, a UV VIS detector LCT 2563, a TZ 4620 recorder, and a LCI 30 loop injector were used. Operating conditions were: Column Sepharose 50X 3 cm (Teckla Ltd. Prague), flow rate 0.3 mL.min⁻¹ pressure 10 MPa; filter with maximum at 546 nm. Solvent A: CH₃OH / 0.05 M KH₂PO₄ (18:82 v/v) adjusted to pH 2.7 with H₃PO₄; Solvent B: H₂O / 3% CH₂OH (60:40 v/v). Gradient:

Table 1- Percentage of individual red beet pigments during fermentation

<table>
<thead>
<tr>
<th>YEAST</th>
<th>Fermentation time [hr]</th>
<th>Beta-</th>
<th>Isobeta-</th>
<th>Beta-</th>
<th>Isobeta-</th>
<th>DP</th>
</tr>
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<tbody>
<tr>
<td>OENOFORM</td>
<td>40</td>
<td>36.32</td>
<td>6.69</td>
<td>45.69</td>
<td>8.03</td>
<td>3.25</td>
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<tr>
<td></td>
<td>60</td>
<td>11.59</td>
<td>4.29</td>
<td>66.33</td>
<td>14.23</td>
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<tr>
<td></td>
<td>180</td>
<td>2.83</td>
<td>3.92</td>
<td>72.48</td>
<td>14.77</td>
<td>4.57</td>
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<tr>
<td>11C</td>
<td>40</td>
<td>41.46</td>
<td>8.78</td>
<td>38.13</td>
<td>8.36</td>
<td>3.25</td>
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<td>8.51</td>
<td>2.85</td>
<td>69.64</td>
<td>14.07</td>
<td>4.93</td>
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<tr>
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<td>180</td>
<td>7.84</td>
<td>2.10</td>
<td>70.61</td>
<td>14.27</td>
<td>5.15</td>
</tr>
<tr>
<td>1090</td>
<td>40</td>
<td>21.58</td>
<td>4.64</td>
<td>59.94</td>
<td>10.85</td>
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<td>60</td>
<td>10.63</td>
<td>3.75</td>
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<td>3.64</td>
<td>1.09</td>
<td>75.42</td>
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<td>53.15</td>
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<td>3.86</td>
<td>63.64</td>
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<td>10.49</td>
<td>2.80</td>
<td>65.70</td>
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<td>6.26</td>
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<td>3A</td>
<td>40</td>
<td>27.24</td>
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<td>67.00</td>
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<td>74F</td>
<td>40</td>
<td>32.44</td>
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<td>48.87</td>
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<td>66.04</td>
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<td>18.79</td>
<td>4.87</td>
<td>54.01</td>
<td>10.30</td>
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<tr>
<td>Pasteurized juice</td>
<td></td>
<td>81.18</td>
<td>17.12</td>
<td>50.80</td>
<td>12.30</td>
<td>1.69</td>
</tr>
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</table>

*DP = Degradation Products.

Table 2 - Changes in red beet pigment content during fermentation

<table>
<thead>
<tr>
<th>YEAST</th>
<th>Concentration of red beet pigment C (mg.L⁻¹ betanine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40 hr C R [%] 60 hr C R [%] 180 hr C R [%]</td>
</tr>
<tr>
<td>OENOFORM</td>
<td>374.5 62.0 343.2 55.4 227.8 36.8</td>
</tr>
<tr>
<td>11C</td>
<td>374.5 62.0 343.2 55.4 227.8 36.8</td>
</tr>
<tr>
<td>1090</td>
<td>337.8 57.7 314.8 50.8 199.5 32.2</td>
</tr>
<tr>
<td>6C</td>
<td>371.5 59.9 310.9 50.2 177.9 28.7</td>
</tr>
<tr>
<td>Tokaj76D</td>
<td>346.1 55.8 295.1 49.2 104.6 16.9</td>
</tr>
<tr>
<td>74F</td>
<td>339.2 54.7 300.2 48.4 84.1 13.8</td>
</tr>
</tbody>
</table>

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SUCCESSFUL PRODUCTION of red beet (Beta vulgaris var. rubra) pigment concentrate by fermentation, requires rapid initiation and progress of the fermentative process. Selection of a suitable yeast is based on this fundamental requirement. The yeasts with the suitable enzymatic system for a given composition of red beet juice (high portion of sucrose) must rapidly begin to utilize sugar with consequent CO2 production when the fermentation is carried out in a closed system. For selection of the strain, it is also necessary to take into consideration the changes in pigments resulting from the oxidative process, enzymatic activity and metabolites of yeasts. In spite of inactivation of enzymes in pressed beet juice before fermentation, a significant pigment decrease occurred from the decomposition process of main pigments during fermentation (Drdak et al., 1989).

The samples were monitored after 40, 60 and 180 hr for individual pigments and saccharides. The last reading was not important from the point of view of concentrate production. It was made so the effects on the pigment, of different fermentative conditions, and further influences of enzymes on the content of pigment could be followed. It also helped determine if an equilibrium state was reached among the forms of pigment in a given medium.

The portion of individual pigments, mainly betanine, isobetanine, betanidine and isobetanidine (Table 1) was expressed as relative percentage on the basis of the total area of peaks in the HPLC chromatograms. Degradation products were shown as individual peaks in the chromatograms. The pigments were collected and their identities determined on the basis of their Rf values, absorption spectra and the sequence of elution. (Schwartz and von Elbe, 1980).

In general, for each individual strain, different percentages of individual compounds remained after fermentation. Also note the different portions of degradation products and the different levels of cleavage of glucoside from betanine produced during fermentation. Similarly, the appearance of isocompounds was different and the proportion changed during fermentation. From the data we could not determine if it was due to enzyme production or was a result of chemical hydrolysis after fermentation. The different pH values after 180 hr fermentation were: Oenoferm, 4.6; 11C, 4.3; 1090, 4.7; 3A, 4.2; 6C, 4.3; Tokaj 76D, 4.2; and 74F, 4.2.

It is interesting to compare the decrease of red beet pigments determined spectrophotometrically and expressed as betanine concentration [mg L-1]. In Table 2 the measured values and retention R [%] are shown. With regard to the requirement to be finished in 40-48 hr, the strains 11C, 6C and Oenoferm or 1090, and 3A were comparable. The activity of the strains Oenoferm, 11C, 6C, 76D and 3A could also be compared on the basis of pigment retention and average percentage of initial red pigment content.

The percentage of pigment in the total solids of pigment concentrate or powder was greatly influenced by the resultant saccharide concentration (Table 3). The samples were monitored at the same time for betacyanines. The average determined values refer to the different rates of sucrose inversion caused by D-fructofuranosidase and to the different rates of utilization of glucose and fructose. On the basis of our criteria for selection of the strains, we recommend Oenoferm, 1090, and 76D as suitable strains for fermentation of beet juice. This recommendation was a compromise between the relation between pigment retention and sugar utilization.

REFERENCES


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