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Relationship Between Fatty Acid Concentrations in Wine Yeasts and Sugar Fermentation at Different Temperatures*

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The effects of temperature and fermentation stage on the cellular concentrations of unsaturated and saturated fatty acids on the fermentation abilities of four different Saccharomyces cerevisiae wine yeasts were studied at three temperatures. The total unsaturated and saturated fatty acid concentrations were determined at six fermentation stages. Unsaturated fatty acid concentration decreased and the concentration of saturated fatty acids increased as fermentation proceeded. Saturated fatty acid concentration of the yeast cells correlated positively with high specific fermentation rates during the later stages of fermentation in contrast to the belief that fluid membranes with high unsaturated fatty acid concentrations enhance ethanol tolerance and, therefore, fermentation performance.

The cell membrane Saccharomyces cerevisiae encloses the cytoplasm and selectively allows nutrients to enter and metabolic products to leave the cell. Furthermore, cell wall components are deposited on this matrix during growth of the cell. It, therefore, affects growth and fermentation and is ultimately of interest to brewers and wine makers. Lipids are an integral part of cellular membranes and consequently changes in lipid composition are associated with changes in membrane characteristics such as fluidity and permeability (Rattray, Schibeci & Kidby, 1975), which have been reported to affect ethanol tolerance of yeast (Thomas, Hossack & Rose, 1978).

Factors which affect the lipid composition of micro-organisms are growth rate (Brown & Rose, 1969a), medium composition (Brown & Rose, 1969b), growth temperature (McMurray & Rose, 1971), dissolved-oxygen tension (Brown & Rose, 1969a) and glucose concentration (Johnson, Nelson & Brown, 1972). Lower growth temperatures for example increase the proportion of unsaturated lipids in yeast cells (Pfister, Hancock & Garrison, 1977). However, according to McMurray & Rose (1971), temperature variation does not significantly alter the fatty acid composition of the early exponential phase cells, but profoundly affects the synthesis of these compounds during the later exponential phase. Because unsaturated fats melt at lower temperatures than their saturated counterparts, the change in composition related to growth temperatures has been described as an "antifreeze" effect; the increased proportion of unsaturated fats ensures a more fluid membrane structure at lower growth temperatures which presumably helps maintain membrane functions (Patel & Lewis, 1982).

Fatty acids are structurally and functionally important and affect cell membrane function(s) to a great extent (Andreasen & Stier, 1954). The nature of the fatty acids (incorporated into phospholipids) affects the functions of certain proteins in the membrane (Pfister & et al., 1977). The functional role of lipids, their fatty acid composition and degree of unsaturation, are regarded as major factors in the transport effects of membranes and the realization of fermentative activities in micro-organisms (Sinensky, 1971). Unsaturated fatty acids (UFA) in yeasts and other eucaryotic organisms are produced by direct desaturation of a pre-existing saturated fatty acid by a desaturase system which requires molecular oxygen (Bloch et al., 1961). This desaturase enzyme system, however, becomes inactive when yeast cells grow in a medium containing unsaturated fatty acids (Radler, 1978).

This study was undertaken to determine the effects of temperature during fermentation on unsaturated and saturated fatty acid concentrations in four wine yeast strains and to establish whether there is a relationship between unsaturated or saturated fatty acid concentration and specific fermentation rate.

MATERIALS AND METHODS

Fermentations: The yeast strains, media and methods described by Venter et al. (1989) were used for the analyses and standardisation of yeast dry mass values to correspond with 12, 25, 50, 75, 100 g/l and 125 g/l sugar fermented (fermentation stages 1-6). Fermentation rates were previously determined by Venter et al. (1989) for the intervals 0-24, 13-37, 38-62, 63-87, 88-112 g/l and 113-137 g/l sugar fermented. These values were considered to indicate the activity of the yeasts at stages 1-6, respectively, and were used to determine specific fermentation rates (SFR) as g/l sugar fermented/day/100 mg yeast cells. Mean saturation index (SI) was also determined for all treatments using the following formula: SI = SFA / UFA

Gas chromatographic analysis of fatty acids: The method of Moss, Shinoda & Samuels (1982) was modified and used for fatty acid analysis. To 3 ml yeast suspension (50 mg to 200 mg yeast dry weight) in a 15 ml glass bottle was added 4 ml of methanol AR and 0.7 g NaOH, as well as 200
RESULTS AND DISCUSSION

For all three variables (UFA concentration, SFA concentration and fermentation rate) strain x temperature x stage interactions were indicated by the multivariate test (the sphericity test was rejected in all three cases) and separate analyses were therefore performed for each yeast strain. The approximate significance levels (SL) for UFA and SFA concentrations were respectively 0.7532, 0.0018 (WE 452), 0.5445, 0.2542 (WE 500), 0.5496, 0.1182 (WE 14), and 0.8492, 0.2509 (WE 372). In the UFA concentration analyses by strain, the sphericity tests were not rejected, but for the SFA concentration analyses, the sphericity test was rejected only in the case of WE 452.

In the case of fermentation rate only the WE 500 sphericity test was not rejected (SL = 0.1546). The analysis of fermentation rate by yeast strain showed stage x temperature (quadratic) interaction, although there was a general tendency suggesting that the nature of the interaction could be simplified to stage x temperature (linear). Venter et al. (1989) showed that each yeast strain had different fermentation rate patterns, with higher specific fermentation rates at the higher temperatures.

The rate of the decrease in UFA concentration (Fig. 1) and the variation in SFA concentration (Fig. 2) per 100 mg cells from the first (12 g/l sugar fermented) to the sixth stage (125 g/l sugar fermented) differed significantly between temperatures for all four yeast stains (data not shown). The UFA and SFA contents of yeast cells are considered to be individual characteristics of strains as indicated by the temperature x strain x stage interaction.

The decline in the UFA concentration of the yeast cells as the fermentations progressed can be ascribed to growth of the cells under non-aerated conditions in the absence of exogenous sterols and unsaturated fatty acids (Venter et al., 1989). Occasional high values at advanced fermentation stages could be ascribed to experimental errors. The yeast dry mass increased considerably over the first three stages, but little or no yeast growth was observed over the final three stages (Venter et al., 1989). The larger decreases in UFA concentration with yeast strains WE 452 and WE 500 than with strains WE 14 and WE 372 from the first to the second fermentation stage (Fig. 1) were the result of their larger increases in dry mass during these stages. (Venter et al., 1989). The overall tendency of lower UFA concentrations at higher temperatures could therefore be ascribed to a corresponding higher growth rate of yeast cells, resulting in a dilution of UFA as these compounds are not produced under anaerobic conditions (Fig. 1).

Yeast strain WE 14 had the highest specific fermentation rates (SFR) at the first and second fermentation stages at 15°C and 20°C, followed by WE 452, WE 500 and WE 372 (Venter et al., 1989). However, at the sixth fermentation stage yeast strain WE 500 had the highest SFR of the four yeast strains at all three temperatures. The UFA concentrations showed a similar trend at 10°C with WE 500 being higher than WE 452 and WE 372 but at 15°C and 20°C no significant differences could be detected (Fig. 1 & Table 1). At this (the sixth) stage SFR values of WE 452 and WE 500 tended to be higher than...
Fatty acid concentrations in wine yeasts

FIGURE 1
Unsaturated fatty acid concentration in cells of *Saccharomyces cerevisiae* strains WE 452, WE 500, WE 14 and WE 372 during fermentation at three temperatures.

that of WE 14 and WE 452 at 10°C and 15°C, albeit not always significant (Table 2). However, at 20°C yeast strains WE 372 had the highest cell UFA concentrations (Fig. 1 & Table 1), but the lowest SFR (Venter et al., 1989), whereas WE 452 and WE 500 had lower UFA concentrations with the highest SFR. Statistical analysis showed that a negative correlation ($r = -0.50$) was found between the UFA concentration and the SFR during the sixth fermentation stage. Thus, a high UFA content alone does not ensure a high fermentation rate; it rather seems that a low UFA concentration correlates well with high SFR at 20°C.

The SFA concentrations of strain WE 14 increased as the fermentation proceeded (Fig. 2). However, in the case of strains WE 452 and WE 500 the SFA concentrations decreased considerably from the first to the second fermentation stage, whereafter they increased. The final SFA concentrations of the latter two strains were considerably higher than those of WE 14 and WE 372 (Table 2).

TABLE 1
Mean unsaturated fatty acid concentration in four yeast strains at three temperatures during the sixth fermentation stage.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>WE 452</th>
<th>WE 500</th>
<th>WE 14</th>
<th>WE 372</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.91b</td>
<td>1.51a</td>
<td>1.07a</td>
<td>0.98b</td>
</tr>
<tr>
<td>15</td>
<td>0.89b</td>
<td>0.85b</td>
<td>0.98b</td>
<td>0.75b</td>
</tr>
<tr>
<td>20</td>
<td>0.68b</td>
<td>0.69b</td>
<td>0.61b</td>
<td>0.84b</td>
</tr>
</tbody>
</table>

Means, within rows, followed by the same letter do not differ significantly ($P \leq 0.05$)

*S. Afr. J. Enol. Vitic., Vol. 10 No. 2 1989*
When the SFA concentrations shown in Fig. 2 were compared with the SFR values of Venter et al. (1989), it was found that the former did not affect the latter during fermentation. However, at the final stage a positive correlation \((r = 0.87)\) was found between the SFA concentrations and SFR of the four yeast strains. These results confirmed those of Nagar-Legmann & Margalith (1987) who showed that a fast-fermenting strain of \textit{Saccharomyces cerevisiae} had higher SFA and free sterol concentrations than \textit{Saccharomyces mellis} T 453 [reclassified as \textit{Zygosaccharomyces cerevisiae} (Von Arx, 1978)], which was a slow fermentor. Otoguro et al. (1981) indicated that the membranes of \textit{Saccharomyces cerevisiae} require certain amounts of SFA and that membrane functions (energy metabolism, transport, etc.) are impaired by SFA starvation. The importance of SFA in the membrane should therefore not be underestimated.

Furthermore, it was obvious that higher saturation index (SI) values were generally obtained at the higher temperatures and these values generally increased further as the fermenta-

**TABLE 2**
Mean saturated fatty acid concentration in four yeast strains at three temperatures during the sixth fermentation stage.

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>WE 452</th>
<th>WE 500</th>
<th>WE 14</th>
<th>WE 372</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.52ab</td>
<td>2.62a</td>
<td>1.23b</td>
<td>1.38ab</td>
</tr>
<tr>
<td>15</td>
<td>2.90a</td>
<td>3.00a</td>
<td>1.97a</td>
<td>1.96a</td>
</tr>
<tr>
<td>20</td>
<td>3.16a</td>
<td>3.92a</td>
<td>2.99a</td>
<td>1.43b</td>
</tr>
</tbody>
</table>

Means, within rows, followed by the same letter do not differ significantly \((P \leq 0.05)\)

Fatty acid concentrations in wine yeasts

TABLE 3
The mean saturation index for four yeast strains at six fermentation stages at three temperatures.

<table>
<thead>
<tr>
<th>Sugar fermented (g/l)</th>
<th>WE 452</th>
<th>WE 500</th>
<th>WE 14</th>
<th>WE 372</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10°C 15°C 20°C</td>
<td>10°C 15°C 20°C</td>
<td>10°C 15°C 20°C</td>
<td>10°C 15°C 20°C</td>
</tr>
<tr>
<td>12</td>
<td>0.88 1.16 1.20</td>
<td>1.31 1.23 1.40</td>
<td>0.77 0.99 0.88</td>
<td>0.84 0.95 0.92</td>
</tr>
<tr>
<td>25</td>
<td>0.80 1.36 1.00</td>
<td>0.89 1.01 1.52</td>
<td>0.85 0.94 1.51</td>
<td>1.26 1.19 1.60</td>
</tr>
<tr>
<td>50</td>
<td>1.32 1.42 2.05</td>
<td>1.27 1.45 1.92</td>
<td>1.01 1.14 1.94</td>
<td>1.51 1.56 1.72</td>
</tr>
<tr>
<td>75</td>
<td>1.32 1.95 2.88</td>
<td>1.50 2.52 2.12</td>
<td>1.25 1.50 1.83</td>
<td>1.48 2.01 2.39</td>
</tr>
<tr>
<td>100</td>
<td>1.35 2.16 3.68</td>
<td>1.40 2.32 3.86</td>
<td>1.20 1.60 2.80</td>
<td>1.41 2.35 3.03</td>
</tr>
<tr>
<td>125</td>
<td>1.67 3.26 4.65</td>
<td>1.73 3.49 5.68</td>
<td>1.15 2.02 2.30</td>
<td>1.41 2.61 4.04</td>
</tr>
</tbody>
</table>

*Mean saturation index = Saturated fatty acid conc. / Unsaturated fatty acid conc.

The results suggest that high membrane fluidity is incompatible with high SFR during the later stages of fermentation. It has been suggested that high membrane fluidity may be involved in the entrapment of ethanol molecules within the hydrophobic domain of the membrane, interfering with its free movement out of the cell (Legmann & Margalith, 1986).

At the sixth fermentation stage the yeast cells contained relatively low concentrations of ergosterol (Venter et al., 1989) and UFA (Fig. 1). Watson & Rose (1980) postulated a mechanism whereby membrane fluidity could be balanced through synthesis of phosphatiidinositol and phosphatidylserine containing a higher proportion of saturated fatty-acyl residues, strategically located in the membrane and thereby maintaining a degree of rigidity in that domain.

CONCLUSIONS

Fermentation temperatures affected the fermentation rate, UFA and SFA concentrations of different yeast strains to a great extent. High specific fermentation rates at the later stages of fermentation were correlated with high SFA concentrations. It appears to be in contrast with the findings of other workers i.e. that fluid membranes containing relatively high concentrations of UFA, might interfere with the movement of ethanol out of the yeast cell. A high intracellular concentration of ethanol will impair fermentation capabilities of yeast. On the other hand, results from this study in synthetic media indicate that high concentrations of SFA might well render yeast cells more tolerant to ethanol than their unsaturated counterparts, enhancing fermentation rates in the presence of ethanol during the later stages of fermentation.

LITERATURE CITED


