A Research Note

Conversion of Ferulic Acid to 4-Vinylguaiacol by Yeasts Isolated from Frozen Concentrated Orange Juice

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ABSTRACT

Yeasts were isolated from frozen concentrated orange juice, grown in Sabouraud dextrose broth at 25°C, and tested for the ability to cometabolize ferulic acid. Strains of Rhodotorula sp., Candida lambica, Trichosporon pullulans, and Candida intermedia decarboxylated ferulic acid nonoxidatively to an off-flavor compound, 4-vinylguaiacol. By decarboxylating naturally occurring ferulic acid, these and other yeasts have the potential to contribute to off flavors in improperly stored fruit juices.

Key words: Ferulic acid, off flavors, orange juice, yeasts, 4-vinylguaiacol

Off flavors and odors in improperly stored orange juice are caused by several volatile compounds; the most objectionable of these is 4-vinylguaiacol (4-hydroxy-3-methoxystyrene) (12, 14, 17). Even at concentrations of 50 to 75 μg/l, this compound causes detectable changes in the flavor of orange juice (17). 4-Vinylguaiacol is believed to arise from the spontaneous decarboxylation of ferulic acid (4-hydroxy-3-methoxycinnamic acid), a natural component of orange juice (12, 14). Commercially pasteurized single-strength orange juice, depending on the storage conditions, contains 2.9 to 9.5 mg/l of ferulic acid in both free and bound forms (12).

Orange juice and other fruit juices normally contain a variety of yeasts (4, 5, 16). Deak and Beuchat (5) have reported that the most abundant yeasts in concentrated orange juice are Candida stellata and Saccharomyces cerevisiae. Although strains from orange juice have apparently not yet been tested, several yeasts obtained from various culture collections have been shown to decarboxylate ferulic acid to 4-vinylguaiacol. Species with this ability include S. cerevisiae (1, 6, 7, 9), Dekkera bruxellensis (≡ Brettanomyces intermedium) (8), Hansenula spp. (1, 8), Candida intermedia (1), and Rhodotorula mucilaginosa (= R. rubra) (9, 10).

In an attempt to find out whether yeasts are a potential cause of 4-vinylguaiacol production in orange juice, we isolated several yeasts from frozen concentrated orange juice and tested them for the ability to decarboxylate ferulic acid.

MATERIALS AND METHODS

Yeasts were isolated from ten different brands of frozen concentrated orange juice by serial dilution on acidified potato dextrose agar (Edge Biologicals, Memphis, TN). Strains representing different morphological types were selected and identified by the keys of Barnett et al. (2).

To determine their ability to metabolize ferulic acid, yeast strains were grown in triplicate 125-ml Erlenmeyer flasks, each containing 30 ml of Sabouraud dextrose broth (Remel Laboratories, Lenexa, KS). The cultures were incubated at 25°C with shaking at 105 rpm. After 2 days, the cultures were supplemented with a solution of ferulic acid (Fluka Chimica, Buchs, Switzerland) that had been dissolved in N,N-dimethylformamide and filter-sterilized. The final concentrations in the medium were 13 mM ferulic acid and 85 mM N,N-dimethylformamide.

After the cultures had been incubated with shaking for another 24 h, the yeast cells were collected on filters. The dry weight of the cells averaged 91 to 229 mg per 30 ml. Each filtrate was extracted with three equal volumes of ethyl acetate, which was dried over anhydrous sodium sulfate. The ethyl acetate was removed in vacuo at 30°C and the residues were dissolved in methanol.

For qualitative analysis, the major metabolites were purified by thin-layer chromatography (TLC) on silica gel plates (J. T. Baker, Phillipsburg, N.J.) developed in benzene/acetone (9:1, vol/vol). UV/visible absorption spectra were obtained with a Shimadzu UV-2101PC spectrophotometer (Shimadzu Corp., Kyoto, Japan).

For quantitative analysis, a Shimadzu high-performance liquid chromatograph (HPLC) was used with a Shimadzu CR.501 Chromatopac integrator. Metabolites were separated on a Spherisorb 5 μm ODS-1 column (250 by 4.6 mm; MetaChem Technologies, Torrance, CA) with an isocratic mobile phase consisting of KH2PO4 buffer (10 mM, pH 3) and methanol (40:60, vol/vol). The flow rate was 1 ml/min and the UV detector was set at 264 nm. Metabolite concentrations were calculated with ε = 10,574 M−1 cm−1 (10).

Gas chromatography/mass spectrometry (GC/MS) and nuclear magnetic resonance (NMR) spectroscopy were used to identify metabolites. GC/MS was performed by electron ionization, using a...
RESULTS

Of the 109 yeast colonies that were isolated from frozen concentrated orange juice, six strains representing the different morphological types were identified. The isolates included Candida intermedia, C. lambica (anamorph of Pichia fermentans), Pichia anomala, Rhodotorula sp., Saccharomyces cerevisiae, and Trichosporon pullulans. Many of the strains, when grown in Sabouraud dextrose broth with 13 mM ferulic acid, produced a metabolite with a spicy odor.

When the ethyl acetate extract of a culture of Candida lambica Y-54 was analyzed by HPLC, the elution profile (Fig. 1) showed two major peaks with retention times of 5.4 and 8.3 min. The first peak corresponded to ferulic acid. The metabolite eluting at 8.3 min was purified by TLC (Rf = 0.84) and found to have a UV/visible absorption spectrum with \( \lambda_{\text{max}} \) values at 211 and 264 nm, similar to those reported for 4-vinylguaiacol (9).

Other yeasts from orange juice were also tested for the ability to metabolize ferulic acid. Those that produced a metabolite with the properties of 4-vinylguaiacol included several strains of Rhodotorula sp., C. lambica, T. pullulans, and C. intermedia (Table 1). Rhodotorula sp. strains converted up to 80% of the ferulic acid to 4-vinylguaiacol (Table 1).

<table>
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<tr>
<th>Strain</th>
<th>4-Vinylguaiacol concn, mM (± 1 SD)</th>
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<tbody>
<tr>
<td>Candida intermedia Y-66</td>
<td>1.1 ± 0.2</td>
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<tr>
<td>Candida lambica Y-54</td>
<td>2.9 ± 1.0</td>
</tr>
<tr>
<td>Rhodotorula sp. 1-13</td>
<td>10.7 ± 0.5</td>
</tr>
<tr>
<td>Trichosporon pullulans 1-15</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>Abiotic control</td>
<td>0.0 ± 0.0</td>
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</tbody>
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* Cultures were incubated for 24 h in Sabouraud dextrose broth after the addition of 13 mM ferulic acid.

The mass spectrum of the metabolite, which showed a molecular ion \([M^+]\) at m/z 150 and fragment ions at m/z 135 \([M^+-\text{CH}_3]\), 107 \([M^+-\text{COCH}_3]\), and 77, was similar to the mass spectrum reported for 4-vinylguaiacol (9). The proton NMR spectrum (Table 2) confirms the identification of the metabolite as 4-vinylguaiacol. Differences from published values (15) for the chemical shifts of the -OH proton and the vinyl 2' a and 2'b protons could be accounted for by the use of acetone-\(d_6\) instead of CDCl₃.

DISCUSSION

These results demonstrate that several strains of yeasts naturally occurring in frozen concentrated orange juice were able to produce 4-vinylguaiacol from ferulic acid. Previous isolates that have been obtained from fruits and juices include Candida lambica and Rhodotorula mucilaginosa from orange juice (3, 11, 16); Rhodotorula glutinis from orange peel (16); R. glutinis, R. mucilaginosa, and Trichosporon sp. from grapefruit sections (13); and R. mucilaginosa from lemon concentrate and apple juice (5, 16). Strains of Saccharomyces cerevisiae, Pichia anomala (= Hansenula anomala), and other yeasts that had not been obtained from orange juice have already been shown to produce 4-vinylguaiacol from ferulic acid (1, 6, 7, 9).

We conclude that the off-flavor compound 4-vinylguaiacol can be produced from ferulic acid not only by abiotic means (12, 14) but also by species of yeasts found in frozen concentrated orange juice. Our results offer a possible explanation for

![Figure 1. HPLC elution profile of the ethyl acetate extract of Candida lambica Y-54 grown in Sabouraud dextrose broth containing ferulic acid.](http://meridian.allenpress.com/jfp/article-pdf/58/11/1260/1659824/0362-028x-58_11_1260.pdf)
the origin of this off-flavor compound in improperly stored citrus fruit juices. Further research will be needed to determine appropriate control measures.

**TABLE 2. Proton NMR chemical shifts and coupling constants of the 4-vinylguaiacol metabolite produced from ferulic acid by Candida lambica Y-54**

<table>
<thead>
<tr>
<th>Proton assignment</th>
<th>Chemical shift (ppm)</th>
<th>Coupling constant (Hz)</th>
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<tbody>
<tr>
<td>–OH</td>
<td>7.58</td>
<td></td>
</tr>
<tr>
<td>OCH₃</td>
<td>3.87</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7.09</td>
<td>J₃,₅ = 1.9</td>
</tr>
<tr>
<td>5</td>
<td>6.90</td>
<td>J₃,₆ = 8.2</td>
</tr>
<tr>
<td>6</td>
<td>6.78</td>
<td></td>
</tr>
<tr>
<td>1’</td>
<td>6.65</td>
<td>J₁’,₂a = 17.6</td>
</tr>
<tr>
<td>2’a</td>
<td>5.62</td>
<td>J₂a,₂b = 0.9</td>
</tr>
<tr>
<td>2’b</td>
<td>5.05</td>
<td>J₁’,₂b = 11.0</td>
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* Protons numbered 3, 5, and 6 are on the ring carbons. Those numbered 1’, 2’a, and 2’b are on the vinyl carbons; 1’ and 2’a are arranged cis to each other.

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**REFERENCES**