Cellulases are an important family of enzymes found in many plant, bacterial, fungal, and yeast species. Plants encode cellulases [EC 3.2.1.4] that catalyze the cleavage of the internal 1,4-β-glucan glucosidic bond, which is an essential component of their cell walls. These enzymes are distributed in plant food products in different stages of fruit development, including abscission, fruit softening, vascular differentiation and senescence. A new fluorescent assay system has been developed to monitor cellulase activity in continuous assay. 

Methods

- Crushed Pinot Gris and Pinot Noir grape samples, including skin, flesh, and seeds, picked at varying
  developmental stages from vineyard blocks were mixed, stored for 5 minutes, and cell wall solubilized
  using sodium hypochlorite (0.025 U/ml) and cellulase (25 U/ml). Samples were centrifuged for 6 minutes at
  7500 rpm. Cellulase activity was determined for initial cellulase inhibition

Figure 2: Initial Velocity vs. Brix

\[ y = -6 \times 10^{-6}x + 0.0002 \]

Potential Cellulase Inhibitors Present in Grapes

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Ki Values (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucosamine</td>
<td>0.4</td>
</tr>
<tr>
<td>Grupe</td>
<td>0.5, 1.4, 7.4</td>
</tr>
<tr>
<td>Sodium hypochlorite</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Discussion and Conclusions

We measured cellulase activity in grape juice samples from Pinot Noir and Pinot Gris grape varieties
and found that activity is strain-specific in grape samples tested. However, the presence of a cellulase inhibitor or
inhibitors was found in both Pinot Noir and Pinot Gris grape varieties correlating to ripening and variety. Previous
publications have shown a positive correlation between cellulase activity and berry abscission [1]. It is thought that
this cellulase activity is localized primarily in the berry cell wall, middle lamella, and parietal bodies of discusion zone cells where the Glu is attached to the stem. This may explain why mature berries have exhibited less cellulase inhibition. The berry-mature cellulase activity may be localized to the abscission zone and inhibited in other areas of the berry.

We believe that the cellulase inhibition assay may be a convenient, high-throughput method to monitor
grape ripening. A linear relationship between ripening (measured using the typical BRX and TA analyses) and cellulase inhibition was found.

Our data indicates that the cellulase inhibitor or inhibitors in the grape samples is most likely a small molecule as the samples assayed showed no protease activity (data not shown) [2]. SDS-PAGE (Figures 4 and 5) did not show a correlation between protein levels and BRX values.

Cellulase inhibitors have also been reported in grape leaves. Porter and Schwartz identified these cellulase inhibitors to be tannins. Coleus blumei is known to be present in grape skin and seed, thus the grape samples tested where in contact with their skin and seeds possibly providing a source of the inhibitory tannin. Our new objective will be to measure tannin levels in these same samples.

References: