Annual Report
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Project Title:

Kinetics of flavor formation during grape juice fermentations

Principal Investigator:

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Summary:

Using solid phase microextraction coupled with gas chromatotgraphic analysis, we are able to "continuously" monitor ester production throughout grape juice fermentations. In previous studies we used this technique to monitor differences in production of acetate and fatty acid ethyl esters that could be related to the progression of the fermentation. In addition a multi-peak pattern of ester production was observed which had not previously been reported. During the past year (2001-2002) our studies showed that:

- Ethanol concentrations did not have a significant effect on measured concentrations of most esters studied using the SPME technique. However, at ethanol levels greater than 5%, measured concentrations of ethyl decanoate were significantly decreased. This may indicate that SPME analysis underestimates concentrations of this ester as fermentations proceed and ethanol concentrations increase.
- Carbon dioxide flow rates at levels approximating those occurring at the height of fermentation had only a minimal effect on measured ester concentrations. These results suggest that SPME sampling provides an accurate picture of total ester concentrations throughout fermentation, even when volatilization rates are expected to be high (i.e,. logarithmic yeast growth).
- Yeast inoculation level did not significantly impact the concentrations or production profiles of the ethyl esters and acetate esters studied, except for ethyl acetate. The reason why ethyl acetate production responds differentially to yeast inoculum levels is unknown.

Objectives of Proposed Research:

The overall goal of this research is to develop a kinetic model for batch alcoholic fermentations that will describe the overall production of important wine flavor compounds during yeast fermentations. The model can be used to describe the effects of yeast inoculum level, fermentation temperature, and CO_2 production on flavor composition of wine.

Summary of Major Research Accomplishments and Results:

Experiments during the past year have focused on developing a better understanding of the variables that influence ester production and volatilization during fermentations. The esters of interest were the acetate esters, ethyl acetate, isoamyl acetate, and, hexyl acetate, and the ethyl esters , ethyl hexanoate, ethyl octanoate, and ethyl decanoate. Our initial studies have focused

on the following three variables and their effects on production of these esters: ethanol concentration, CO_2 flow rates, and yeast inoculum level.

Ethanol

Measurement of volatile flavor compounds such as esters using the SPME technique is dependent on partition equilibria of the volatile compound between the headspace and the solution (i. e., the wine or fermenting must) and between the headspace and the SPME fiber. Because ethanol can influence the air/headspace partitioning we evaluated the effects of different ethanol levels on measured response for the different esters studied. Our results show that except for ethyl decanoate, ethanol did not have a significant effect on measured response for these esters. This indicates that in general, the SPME method provides an accurate estimate of ester concentration throughout the fermentation, even as ethanol concentrations change. However, when monitoring ethyl decanoate levels during fermentation, concentrations of this ester may be underestimated as ethanol concentration of the must increases above 5%. Calibration of the SPME response using standards of higher ethanol concentration is necessary for accurate measurement of ethyl decanoate.

Carbon Dioxide

Because CO_2 production during fermentation could influence both volatilization of esters into the headspace, as well as partitioning and/or volatilization of esters from the headspace into the SPME fiber, we evaluated a range of CO_2 flow rates on ester response. CO_2 gas was bubbled through the closed 1L fermentors at various flow rates and the ester concentrations measured using the standard procedures. The results indicated that at flow rates simulating those that would be expected to occur in the fermentors at the peak of fermentation (~30 mL/min), a slight decrease (<20%) in measured ester concentration was observed relative to when no CO_2 flow was present. However the response at higher flow rates (>50 mL/min) was not different from when no CO_2 flow was present. Higher CO_2 flow rates would be expected to increase ester volatilization into the headspace; whether CO_2 flow would influence the volatilization of compounds sorbed onto the SPME fiber is unknown. It is possible that at low/moderate flows (i.e., ~20-40 mL/min) increased ester volatilization of sorbed molecules from the SPME fiber occurs relative to when no CO_2 is present, resulting in a slight decrease in measured ester response. At high flow rates (>50 mL/min), volatilization from the fiber may be countered by increased volatization into the headspace from the solution so that an "equilibrium" situation is obtained and no net effect on measured ester concentration is observed.

Yeast inoculation level

In order to begin to understand the effects of fermentation rate on ester production we fermented Chardonnay juice with two inoculum levels of Premier Cuvee yeast. The inoculation dose represented the standard level recommended by the manufacturer (0.25 g/L) and a dose 2 times the recommended level (0.5 g/L). Fermentations were carried out in controlled temperature water baths at 18°C. The fermentation with the higher inoculation level had a slightly shorter lag phase and completed the fermentation in 200 hours compared to 250 hours for the standard inoculation level. Preliminary results indicated that for most esters analyzed there was little or no effect on the production profile or concentrations of the esters. At the higher inoculation level however, ethyl acetate concentrations began to increase sooner and much more rapidly compared to the standard inoculation. Final ethyl acetate concentrations were not different for the two inoculation levels. Further work is needed to verify these results.

The fermentations performed previously by Vianna and Ebeler (2001) took almost twice as long to reach dryness as those in the current study. Therefore, while only minimal effects of fermentation rate were seen in the current study, a wider range of rates should be studied in order to fully characterize the effects of fermentation rate on ester formation.

Interestingly, the general ester production profiles observed in this study were slightly different than those observed previously by Vianna and Ebeler (2001): a multi-peak profile of ester production was previously observed which was not observed here. While both studies used the same yeast (Saccharomyces bayanus) the Chardonnay juices were not from the same vintage year. Therefore further work is clearly needed in order to evaluate the effects of yeast and juice nutrient characteristics on the formation of esters and other flavor compounds.

Outside Presentations of Research:
None

Research Success Statement:

This work is providing information about the dynamic changes that occur in flavor production through out grape juice fermentations. By identifying important variables that may influence ester formation (e.g., fermentation rate, yeast species and inoculation rate, fermentation temperature, etc.) we will ultimately be able to provide winemakers with information and tools that they can use during the winemaking process in order to optimize and control the flavor of the final wine.

Funds Status:
Funds will be expended by the end of the fiscal year.

References Cited:
E. Vianna and S. E. Ebeler. 2001. Monitoring ester formation in grape juice fermentations using solid phase microextraction coupled with gas chromatography-mass spectrometry. *J. Agric. Food Chem*., **49**, 589-595.