

99

**Annual Report**  
**February 1, 2000**  
**California Competitive Grant Program for Research in Viticulture Enology and**  
**American Vineyard Foundation**

**Project Title:**

Studies on the Interactions of Flavor Compounds with Nonvolatile  
Components of Wine

**Principle Investigator:**

Susan E. Ebeler  
Department of Viticulture and Enology  
University of California-Davis  
One Shields Avenue  
Davis, CA 95616  
530-752-0696/530-752-0380  
FAX: 530-752-0382  
Email: [seebeler@ucdavis.edu](mailto:seebeler@ucdavis.edu)

**Summary:**

Flavor and aroma are important factors in influencing food and beverage (i.e., wine) choices. However, knowledge of flavor concentration alone is not sufficient to determine the perceived sensory aroma intensity. This is due to the presence of interactions between flavors and nonvolatile food components which can alter flavor volatility and release. We used NMR techniques to study the mechanisms of binding between flavor compounds and polyphenols, the main nonvolatile constituents of wine. Our results showed that the interactions are dependent on the structure of both the flavor compounds and the polyphenols. The interactions are principally due to hydrophobic interactions between the aromatic rings of the flavors and the polyphenols. However hydrogen-bonding effects help to stabilize the complex and enhance the specificity. By understanding the mechanisms of these interactions and their effects on flavor perception we will be better able to optimize grape and wine composition and winemaking procedures to improve wine flavor.

**Goals and Objectives:**

The overall goal of this research is to understand how polyphenols, important nonvolatile constituents of grapes and wines, influence the volatility, intensity, and release of flavor aroma compounds in wines. Specific objectives will be to:

- a) explore the effects of polyphenol structure on polyphenol/odorant interactions, through use of model odorant and polyphenol compounds,
- b) measure changes in volatility of odorants in the presence of polyphenols, through application of sensitive gas chromatographic headspace procedures,
- c) correlate instrumental results with measurements of sensory intensity to understand the effect interactions may have on flavor perception, and
- d) understand the mechanisms of polyphenol and odorant interactions through utilization of NMR technology.

**Significant Results and Accomplishments to Date:**

This proposal is a continuation of a previously funded project. During previous years we focused on developing analytical methods (Gas Chromatographic and NMR) and sensory procedures for measuring odorant interactions in model solutions (Objective b and c). Results have been published (Mialon and Ebeler, 1997) and presented at national meetings of the ASEV (June 1996 & June 1998). An oral presentation outlining recent progress was presented at the National Meeting of the American Chemical Society in New Orleans, LA in August, 1999. Two manuscripts have been accepted for publication as a result of this research (see attached).

During the current funding period we have focused on Objectives a and d. This involved an investigation of the interaction between 2 polyphenols, gallic acid and naringin, and three flavor compounds, vanillin, 2-methylpyrazine, and ethylbenzoate. Proton spectra of each of the individual compounds were compared with those of the polyphenol/odorant mixtures prepared in both D<sub>2</sub>O and DMSO-d<sub>6</sub>. The proton signals of all three flavors exhibited upfield chemical shift changes with increasing polyphenol concentrations and with increasing temperatures (25, 45,

60°C). The upfield shifts are consistent with hydrophobic pi-pi interactions between the aromatic rings of the polyphenols and the odorants, as hypothesized.

Based on the chemical shift changes of flavor protons at different flavor/polyphenol concentration ratios, association constants,  $K_a$  were calculated ( $K_a = PF/P \times F$ ). Association constants ranged from  $51 \text{ M}^{-1}$  for gallic acid/vanillin mixtures at 25°C to  $12 \text{ M}^{-1}$  for gallic acid/ethylbenzoate mixtures at 60°C. These values are similar to published values for noncovalent binding of flavors to  $\beta$ -lactoglobulin. Structure of both the polyphenol and the odorant influenced the observed results. For example, although  $K_a$  decreased as temperature increased for all three flavors, the effects was most pronounced for vanillin and weakest for ethylbenzoate in either gallic acid or naringin mixtures. On the other hand, compared to naringin, gallic acid had a greater observed association (largest overall  $K_a$ ) with vanillin at 25°C. Gallic acid/vanillin mixtures were most influenced by changes in temperature.

Using the calculated association constants, the van't Hoff relationship was used to derive enthalpy and entropy changes for each molecular complex. The absolute values for enthalpy changes were higher with gallic acid mixtures than with naringin. These enthalpy changes indicate that interactions between gallic acid and either vanillin or 2-methylpyrazine are more enthalpically favored as compared to interactions of these flavors with naringin. However, the reverse effect was observed with ethylbenzoate, where interactions with naringin were more enthalpically favored. These observed changes are also consistent with a hydrophobic interaction mechanism as the main driving force for flavor/phenol interactions in polar solutions.

In order to further study the spatial orientation of the flavor/phenol complexes, 1D NOE and 2D ROESY NMR experiments were performed. The 1D NOE difference spectra of a gallic acid/vanillin mixture revealed that the two compounds were chemically exchanged through hydrogen bonding. When the aldehyde proton of vanillin was saturated, the carboxyl proton and the three hydroxyl protons on gallic acid showed strong negative NOE enhancement (70-80%). This negative enhancement can be attributed to a well-interconnected matrix held together by hydrogen bonding. Gallic acid and 2-methylpyrazine mixtures also showed a similar result.

The 1D NOE result was supported by 2D ROESY experiments. The ROESY spectrum of gallic acid and vanillin mixtures in DMSO- $d_6$  revealed intermolecular cross-peaks between the aldehyde proton of vanillin and the three hydroxyl protons on gallic acid. It was not possible to observe evidence for NOE with gallic acid/2-methylpyrazine mixtures.

1D NOE difference spectra of naringin/flavor mixtures showed only positive enhancement (~10%) within intramolecular protons. Some protons of naringin are dipole-dipole coupled with each other indicating intermolecular associations between naringin molecules.

Results from the 1D and 2D studies indicate that hydrogen bonding interactions are also important for the formation of stable complexes as in the case of vanillin/gallic acid mixtures. In

addition, charge induced dipoles between flavor compounds and polyphenols (e.g., 2-methylpyrazine and gallic acid) can result in strong interactions.

From these results it is clear that both odorant and polyphenol structure can influence complex formation. Studies aimed at measuring the effect of these interactions on the volatility of the odorants (Objectives b) are currently underway and will continue into the next funding year.

#### **Presentations Resulting from this Work:**

- Oral presentation, ASEV National Meeting, Reno, NV, June, 1996. Abstract In: American Journal of Enology and Viticulture (1996) 47(3), 352.
- Poster presentation, ASEV National Meeting, Sacramento, CA, June 1998 . Abstract In: American Journal of Enology and Viticulture (1998) 49(4), 455.
- Oral presentation, American Chemical Society, New Orleans, LA, August 1999.

#### **Publications Resulting from this Work:**

Mialon, V. and S. E. Ebeler. 1997. Utilization of a time-intensity protocol for evaluating the effects of flavor/matrix interactions on retronasal aroma perception. *J. Sensory Studies*, 12: 303-316.

Jung, D.-M., J. S. de Ropp, and S. E. Ebeler. Study of interactions between food phenolics and aromatic flavors using one and two dimensional <sup>1</sup>H-NMR spectroscopy. *J. Agric. Food Chem.*, In Press.

Jung, D.-M., J. S. de Ropp, and S. E. Ebeler. Thermodynamic evaluation and nuclear overhauser effect study on intermolecular interaction between polyphenolics and flavors. In: *Flavor Release: Linking Experiments, Theory and Practice.*, D. Roberts, and A. J. Taylor, eds., American Chemical Society, Washington, DC. In Press.

#### **Research Success Statements:**

The information obtained from this work will lead to new insights into the way in which polyphenol interactions influence wine aroma intensity and temporal flavor perception. This work will provide winemakers with critical information that may be used to vary wine composition and processing conditions in order to optimize wine flavor. In addition, similar interactions influence wine color (i.e., copigmentation), haze formation, and astringency perception; therefore understanding these interaction mechanisms will be critical for a complete understanding of wine chemistry.

#### **Funds Status:**

A PhD graduate student (Da-Mi Jung) has been hired and will continue working through 2000-2001. Funds allocated during 1999-2000 will be fully spent by the end of the current funding period.