Annual Report
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Project Title: The Chemical Evolution and Preservation of Color in Red Wine Aging.

Principal Investigator:
Andrew L. Waterhouse
Department of Viticulture and Enology
University of California
Davis, CA 95616-8749
email: alwaterhouse@ucdavis.edu
phone: 530-752-4777, fax: 530-752-0382

Objectives and Experiments Conducted to Meet Stated Objectives

Prepare and purify tritium-labeled malvidin-3-glucoside
- Optimize conditions for tritium incorporation
- Purify malvidin-3-glucoside from byproducts
- Find conditions for product stability

Addition of labeled malvidin-3-glucoside during fermentation
- Conduct small-scale fermentation

Additions of labeled malvidin-3-glucoside to wines and allow aging reactions to occur.
- Modify variables that will affect wine aging

Separate the different chemical fractions to determine the fate of the labeled malvidin
- Optimize conditions to separate different wine fractions
- Reduce interference to account for tritium in each fraction

Analyze the effects of the different treatments, i.e. proportion of anthocyanin retained, losses to colorless products, polymeric color, transformed pigments, etc.

Identify unknown labeled products

Analytical methods to calculate red colored polymeric structures (SO_2 bleaching, HPLC and Adams' assay).

Summary of Major Research Accomplishments and Results (by Objective)

Prepare and purify tritium-labeled malvidin-3-glucoside: malvidin-3-glucoside has been labeled with tritium *via* enzymatic synthesis. Petunidin-3-glucoside was the original anthocyanin and it was methylated with catechol-*O*-methyl transferase producing malvidin-3-glucoside. In addition to the desired product a labeled isomer was also produced. In order to conduct valid experiments it was necessary to purify malvidin-3-glucoside in enough quantities for the experiments.

The purification starts with a solid phase extraction process. This step eliminates most of the tritiated methyl donor agent that did not react and reduces the amount of tritium. The purification continues with a reverse phase-HPLC system. It was found that the type of column was very important because it changed the order of elution between the isomers. Once the conditions for synthesis and purification were established, enough labeled material was obtained for the experimentation with wine.

Stability of the product (an anthocyanin) was affected by oxidation, and it was found that the dilute solutions of purified anthocyanin rapidly degrades. The best option was to synthesize and purify the product right before it was used. Alternatively, the conditions after the solid phase extraction are also good to preserve the material. These conditions include high methanol percentage as solvent, low pH, a high phenolic concentration from the starting material (petunidin-3-glucoside), storage at 5°C and exclusion of oxygen.

Addition of labeled malvidin-3-glucoside during fermentation: previous experiments and scientific literature have shown an increase in anthocyanin concentration followed by a sharp decrease. The initial increase is attributed to the extraction of the pigments from skins. The following decrease has not been fully explained. We conducted a small-scale fermentation in triplicate and we added labeled malvidin-3-glucoside when the anthocyanin concentration was the highest (at day 4). After that we have sampled the fermentation every day until it was finished and froze the samples for later analysis. In addition we stored the pomace to account for possible losses due to absorption.

Additions of labeled malvidin-3-glucoside to wines and allow aging reactions to occur: 2001 Cabernet Sauvignon wine was obtained from a winery in Napa and used in the experiments. Wine was adjusted to pH 3.6 and filtered. The variables studied are pH, temperature, tannin concentration, co-pigmentation and oxygen.

- pH: wine was adjusted to three different pHs (3.1 , 3.6 ,and 4.1)
- temperature: compares wine stored at three different temperatures (5, 20 and 35°C)
- tannin concentration: compares the base wine with wine that contains an extra amount of tannins extracted from seeds.
- co-pigmentation: compares the base wine with wine where chlorogenic acid was added. This compound increased the co-pigmentation value.
- oxygen: compares the wine exposed regularly to oxygen with wine under anaerobic conditions.

Separate the different chemical fractions to determine the fate of the labeled malvidin: It was necessary to develop an analytical technique that combines the fractionation of phenolic compounds by size and the quantification of radioactive material. We have optimized a normal-phase HPLC method in order to maximize the amount of radioactive material injected. In addition, we corrected for interferences that prevent the efficient quantification of tritiated material (know as quenching). In order to correct for quenching, we constructed a quench curve with known amounts of tritium added to it. First samples analyzed shown that tritiated malvidin-3-glucoside has been incorporated into polymeric structures. Therefore, it is direct evidence that anthocyanins incorporate into tannins.

Publications and reports resulting from Project

Labeling of malvidin-3-glucoside has been accepted for publication in the Journal of Agricultural and Food Chemistry.

Results obtained from the addition of labeled malvidin-3-glucoside to wines will be presented during the 2002 ASEV annual meeting in Portland. The presentation will discuss the effect of different variables on chemical incorporation of malvidin-3-glucoside into polymeric structures and color during wine aging.

Concise Summary of Current Years Results

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During the last year we have been able to synthesize and purify labeled malvidin 3-glucoside (the major pigment in red wine). Our report on the novel procedure to prepare this radiolabeled substance has been accepted for publication. The amount of material produced was enough to start a series of experiments that study the fate of malvidin-3-glucoside during wine aging. The variables that are being varied are pH, temperature, tannin concentration, co-pigmentation and oxygen. In order to analyze the new products we have developed an analytical technique that combines the fractionation of polymeric compounds with tritium quantification.

We have also taken advantage of labeled malvidin-3-glucoside to study the fate of malvidin-3-glucoside during wine fermentation. In this case we have conducted small-scale fermentation where the labeled material has been added when the maximum concentration of anthocyanins occurred (approximately at day 4).