Warren J. Baker Endowment
for Excellence in Project-Based Learning
Robert D. Koob Endowment *for Student Success*

PROPOSAL NARRATIVE

I. Project Title

Malolactic Fermentation Sensor Development Project

II. Abstract

Malolactic fermentation (MLF) is a critical step in red winemaking and many white wines as it reduces unwanted acidity and alters the flavor and aroma profile to be more palatable and desirable. Lactic acid bacteria (LAB) convert malic acid into lactic acid and carbon dioxide in order to create a proton motive force, which helps to create energy for the metabolization of carbohydrates. Malic acid is not vital for the metabolization of carbohydrates and LAB will continue to survive once MLF is complete and continue to metabolize and the following microbial spoilage will cause the production of undesirable levels of volatile acidity (VA), a source of very undesirable characteristics, which will require spending money to rectify the wine, if such actions are cost effective. Thus, monitoring MLF is critical for winemakers to know when to arrest the fermentation and stop microbial spoilage. Currently, the most widely accepted method of monitoring MLF is by daily measuring of malic acid levels via the enzymatic and spectrophotometric method. This method is very accurate, but requires a skilled lab technician, around 30 minutes to run a set of samples and expensive lab equipment. As advanced technologies push into the wine industry, winemakers have begun to search for new high tech solutions for monitoring MLF. This has created a high demand for an automated sensor, making this project an important and much wanted step in winemaking technologies.

III. Introduction

This project combines enology and electrical engineering for the development of an automated sensor capable of monitoring malolactic fermentation (MLF). Malolactic fermentation is a heterofermentative process (Lerm et al. 2010). A heterofermentative process will often allow for easy changes in some of the physical properties of the wine. In the case of MLF, malic acid is converted into lactic acid and carbon dioxide and carbon dioxide is released into the atmosphere (Lerm et. al 2010). Indirectly measuring MLF is possible by measuring changes in carbon dioxide, density or displacement (B. Zoecklein, personal communication, 2016). As found on the ChemSpider website (sponsored by the Royal Society of Chemistry), the density of L-malic acid is 1.6+/-0.1 g/cu-cm and the density of L-lactic acid is 1.3+/-0.1 g/cu-cm (chemspider.com). A typical concentration of malic acid is 2 g/L. Specific gravity as density of a liquid is as compared to a linear calibration curve generated by interpolating between the

density of water and a higher density solution (typically unfermented grape must). The specific gravity change due to MLF in this wine would equal 0.00080 g/cm3. Cypress Semiconductor currently produces the Integrated Fermentation Control System (IFCS) which measures brix (sugar content) during alcoholic fermentation.

The IFCS (3 IFCS units will be provided at no cost by Cypress Semiconductor for the completion of this project) uses specific gravity to indirectly measure brix levels by bubbling air through fixed pressure tubes into grape must and measuring the change in pressure differential as the sugar converts to alcohol. The pressure tube technology is potentially adaptable as a sensor for MLF. The accuracy of the pressure tubes needs to be tested. The current accuracy is within 0.0019 g/cm3 during AF. An ideal measurement for an MLF sensor would be able to see a change in specific gravity as small as 5x10^-5 g/cm3 with an accuracy of +/- 5x10^-6 g/cm3.

AF is considered to be a 'noisy' process, in electrical engineering terms, due to the tumultuous nature of AF (M. Holst, personal communication, 2016). CO2 bubbles in the AF process cause instantaneous pressure perturbations of more than 0.11 g/cm3 (M. Holst, personal communication, 2016). MLF is considered to be less tumultuous as the process is slower and less CO2 is being released at a single time and should therefore produce less noise. This reduction in noise could make the pressure tube technology suitable for monitoring MLF. This project is aimed at proving a correlation between specific gravity and malic acid concentration and testing the accuracy of the pressure tube technology during MLF.

IV. Objective(s)

1) Test for correlation between specific gravity and malic acid concentration during malolactic fermentation.
2) Test sensitivity, compatibility and potential of pressure tube technology as a means of monitoring MLF.

V. Methodology

A half ton of Sangiovese grapes will be received, crushed, treated with lysozyme, split into 3 IFCS units at 32 gallons of grape must each and inoculated with EC 1118 to start alcoholic fermentation. Alcoholic fermentation will be completed under the supervision of and according to the methods of Dr. L. Federico Casassa at a partner winery in the San Luis Obispo region.

At the end of alcoholic fermentation, wine must from all three IFCS units will be mixed together and pressed off of the grape skins. The wine must will then be split into three equal portions, by volume, in the 3 IFCS units and inoculated with VP-41 to start malolactic fermentation.

Beginning with one sample taken before MLF inoculation, a sample will be taken from each IFCS unit at a depth consistent with the IFCS intake and immediately filtered to 0.45um and frozen in dry ice and placed in a freezer. This will be done daily at 9:00 pm.

The IFCS will take specific gravity readings twice daily, once at 8:00 pm and once at 10:00 pm and store the data points on the sensor control dashboard. They will later be averaged for the most accurate daily value.

On a weekly basis, samples will be thawed in a water bath, and measured for density/specific gravity, using an atomic scale, and malic acid concentration, using the enzymatic method, until malic acid concentration reaches zero.

The specific gravity data will be plotted against the malic acid concentration and be analyzed for a correlation. The IFCS data points will be compared with the malic acid concentration data points on a daily basis and tested for variance.

VI. Timeline

October 2016: crush grapes and begin alcoholic fermentation
October/November 2016: complete alcoholic fermentation and begin malolactic fermentation. Take daily samples and begin testing samples weekly.
December 2016: complete malolactic fermentation and testing of samples
January 2017: Analyze data and complete report
February 2017: Disseminate report

VII. Final Products and Dissemination

- Completion of Cal Poly Senior Project course for Wine & Viticulture students
  - Includes a paper detailing the results of the project
- An article to be submitted in pursuit of being published in the American Journal of Enology and Viticulture
- Assessment and recommendations on pressure tube technology as MLF sensor to be presented to Cypress Semiconductor
- During the project, additional avenues of dissemination will be explored.

VIII. Budget Justification

Winemaking supplies, including inoculations and grapes:	$824.86
Analysis, includes enzymatic assays and disposable equipment:	$423.71
Publication Costs (AJEV):	$0
Total:	$1,248.57

IX. Works Cited

Works cited and in text citations were completed to the specifications of the American Journal of Enology and Viticulture.

Butzke, C.B.. 2002. 2000/2001 Survey of Winery Laboratory Proficiency. Am. J. Enol. Vitic. 53:163-169

Lerm, E., L. Engelbrecht, and M. du Toit. 2010. Malolactic Fermentation: The ABC's of MLF. S. Afr. J. Enol. Vitic. 31:186-212

Volschenk, H., H.J.J. van Vuuren, and M. Viljoen-Bloom. 2006. Malic Acid in Wine: Origin, Function and Metabolism during Vinification. S. Afr. J. Enol. Vitic. 27: 123-136

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PROPOSAL BUDGET

Student Applicant(s): Timothy Holst Braden Bautista	
Faculty Advisor: Dr. L. Federico Casassa	
Project Title: Malolactic Fermentation Sensor Development Project	**Requested Endowment Funding**
Travel *subtotal*	**$0**
Travel: In-state	**$0**
Travel: Out-of-state	**$0**
Travel: International	**$0**
Operating Expenses *subtotal*	**$ 1,248.57**
Non-computer Supplies & Materials	$1,248.57
Computer Supplies & Materials	**$0**
Software/Software Licenses	**$0**
Printing/Duplication	**$0**
Postage/Shipping	**$0**
Registration	**$0**
Membership Dues & Subscriptions	**$0**
Multimedia Services	**$0**
Advertising	**$0**
Journal Publication Costs	**$0**
Contractual Services *subtotal*	**$0**
Contracted Services	**$0**
Equipment Rental/Lease Agreements	**$0**
Service/Maintenance Agreements	**$0**
TOTAL	**$1,248.57**

CAL POLY
SAN LUIS OBISPO
Wine & Viticulture

Wine and Viticulture Department
College of Agriculture, Food & Environmental Sciences

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Dear reviewers,

It is my pleasure to endorse with this letter the proposal entitled "Malolactic fermentation sensor development project" by Mr. Timothy Holst and Mr. Braden Bautista. Both Tim and Braden are enrolled as undergraduates in the Wine and Viticulture Department of Cal Poly San Luis Obispo, with concentrations in viticulture and enology, respectively. The Wine and Viticulture department includes nearly 300 undergraduate majors, making Cal Poly among the largest Wine and Viticulture programs in the country.

Both Tim and Braden will be enrolled in one of the classes I will teaching during summer (WVIT 442 – Sensory Evaluation of Wine). Braden will subsequently take two of my enology classes (WVIT 405 and WVIT 406). Their passion and enthusiasm about wine chemistry and sensory analysis were evident to me from the moment of our first interaction. It is worth mentioning that Tim is a viticulture concentration major and he has decided nevertheless to pursue a project that is enology-oriented to "broaden his horizons" as he explained to me. In addition to that, both Tim and Braden have demonstrated both analytical skills and a clear understanding of the basis of the scientific method, which makes them, in my view, a perfect fit for this endowment aimed at fostering undergraduate research.

The project that Tim and Braden have decided to pursue is aimed at developing an automated sensor to effectively and efficiently monitor the time-course of malolactic fermentation (MLF) in wines. The monitoring of MLF is critical for winemakers to know when to arrest the fermentation and stop microbial spoilage. Currently, the most widely accepted method of monitoring MLF is by daily measuring of malic acid levels, which is expensive, labor-intensive and lengthy. This has created a high demand for an automated sensor, making this project an important and much wanted step in winemaking technologies.

We, as a Department, have a Pilot Winery plant in which we can effectively execute the project, including all the necessary winery equipment to process grapes (crusher-destemmer; positive displacement pumps; vertical presses; wine tanks of variable volume; bottling line). Our facility has also a dedicated spectrophotometer. Lastly but perhaps more importantly, the successful completion of this project will allow both Tim and Braden to complete two of their required classes (WVIT 461 – Senior Project I and WVIT 462 – Senior Project II).

I remain open to expand upon the information above and look forward to working with Tim and Braden in the near future.

Respectfully,

L. Federico Casassa, Ph.D.
Assistant Professor of Enology and Sensory Analysis
Wine & Viticulture Department
California Polytechnic State University, San Luis Obispo
College of Agriculture, Food & Environmental Sciences
Phone: (805)-756-2751
Email: lcasassa@calpoly.edu