Developing Innovative Agri-Products Initiative
Research Project Final Report
Contribution Agreement -Vote 10 Funding

Project Title:	DIAP04803 – Integration and Implementation of Applied Science Toward the Development of a Sustainable, High-Value Wine Industry in British Columbia
Start Date (yyyy-mm-dd):	2010-07-01
Expected End Date (yyyy-mm-dd):	2013-02-28
Actual End Date (yyyy-mm-dd):	2013-02-28
Principal Investigator (PI):	Dr Cédric Saucier

Short Executive Summary of report:

Polyphenols from grapes are responsible for the color and taste of red wines. They also provide
unique nutritional and health benefits to humans when consumed in moderation. Amongst these
compounds, tannins are particularly important for the taste and the stability of red wines. The goal of our activity within the project was to provide new tools that will allow rapid and objective measurements of tannin quality, enhancing innovativeness and competitiveness. By using liquid mass spectrometry coupled with mass spectrometry we have discovered new tannin monomers present in grape seeds. We also have demonstrated the impact of tannin on other sensory attributes such as color and aroma compounds.

A. Research Progress and Accomplishments (to date in relation to expected milestones and deliverables / outputs)

- Include brief summary of:
  - Introduction, literature review, objectives, milestones and deliverables / outputs.
  - Approach / methodology (summary by objectives).
- Include results and discussion (overview by objectives and milestones), next steps and references.

Introduction

Proanthocyanidins, known as flavan-3-ols polymers or condensed tannins, are mixtures of four different monomeric units: (+)-catechin, (-)-epicatechin, (-)-epigallocatechin and gallocatechin gallate . Proanthocyanidins are extracted from grape skins and seeds during the maceration and fermentation processes. Grape seeds are important sources of monomeric units consisting of both (+)-catechin, (-)-epicatechin and epicatechin gallate, whereas the monomeric unit (-)-epigallocatechin is located in skin only [1-2]. In red wine, (+)-catechin, (-)-epicatechin and their derivatives are the main compounds that are responsible for the astringency [3-7], the bitterness, the structure and the aging of wines [8].

Results

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Objective 1: New markers and fast methods for phenolic analysis

By using innovative mass spectrometry methods (UHPLC-Q-TOF) we have discovered 14 flavan-3-ol monoglycosides in grape seeds and red wines. Flavan-3-ol monoglycosides, having four aglycons (+)-catechin, (-)-epicatechin, (-)-epigallocatechin and epicatechin gallate monomeric units, are detected for the first time in Vitis vinifera L. cv. Merlot grape seeds and wine. These compounds were analyzed in red wine, seed and skin extracts by electrospray ionization quadrupole time of flight mass spectrometry (Q-TOF MS) in negative mode. Fragment ions derived from retro-Diels Alder (RDA) condensation, heterocyclic ring fission (HRF), benzofuran forming fission (BFF) and glycoside fissions were detected in targeted MS/MS mode. The comparative study of red wine, seeds and skins showed evidence that these compounds in wine originate from grape seeds, as they were not detected in skins. Our method allows for the identification of these glycosylated compounds based on their exact mass, retention times and their specific fragmentation pattern. However, exact glucose position on the monomeric units cannot be determined. Hemisynthesis of authentic standards and analysis grape seed during ripening is in progress. We will also try to correlate the total concentration of these new compounds with seed tannin content.

Objective 2: Impact of tannins on sensory attributes

A sensory experiment was done in collaboration with Margaret Cliff at PARC Summerland. We have explored the sensory and analytical effects of adding grape seed extract (GSE; 0.0, 0.5, 1.0, 2.5 and 5.0 g/L) to a commercial red wine. Total phenol, color intensity and hue analyses were conducted. Sensory profiling, using 12 trained judges, evaluated the intensity of astringency, fruity and woody/earthy aromas, and red color of the wines. Special care was taken to avoid perceptual biases among the sensory attributes, by conducting the astringent, aromatic and color determinations independently of one another. Analyses of variance were used to evaluate the sensory effects, while regression analyses were used to relate the mean sensory attributes to the GSE concentrations. Positive linear regressions were observed between GSE and astringency ($R2 = 0.841$), woody/earthy aroma ($R2 = 0.933$) and color ($R2 = 0.925$), while a negative linear regression was observed for fruity aroma ($R2 = 0.911$). The presence of GSE significantly enhanced the woody/earthy aroma and suppressed the
fruity aroma. Practical applications: We have demonstrated that GSE not only influenced the mouthfeel of a wine, but also the color and aroma. Because the perceived sensory attributes (astringency, color, fruity and woody/earthy) are highly correlated ($0.801 < R < 0.982$) and dependent on the type of wine and GSE, winemakers are advised to conduct in-house trials prior to tannin adjustments in the cellar. We have shown that sensory changes can be successfully modeled using linear regression to allow winemakers to predict the change in aroma, color and astringent
attributes, associated with the addition of GSE.

Next steps/Perspectives:

Perspectives: Proposal for the new BCWGC project is on the phenolic content in white wines in relation to antioxidant and aroma properties.

References

[1] M.O. Downey, J.S. Harvey, S.P. Robinson. Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. Aust. J. Grape Wine Res. 2003, 9, 15.

[2] J.M. Souquet, V. Cheynier, F. Brossaud, M. Moutounet. Polymeric proanthocyanidins from grape skins. Phytochem. 1996, 43, 509.
[3] D. Rossetti, J. H. H. Bongaerts, E. Wantling, J. R. Stokes, A. M. Williamson. Astringency of tea catechins: More than an oral lubrication tactile percept. Food Hydrocolloids. 2009 ,23, 1984.
[4].E.C. Bate-Smith. Haemanalysis of tannins: The concept of relative astringency. Phytochem. 1973, 12, 907.
[5].E. Monteleone, N. Condelli, C. Dinnella, M. Bertuccioli. Prediction of perceived astringency induced by phenolic compounds. Food Qual. Prefer. 2004, 15, 761.
[6].S. Kallithraka, J. Bakker, M. N. Clifford. Effect of pH on Astringency in Model Solutions and Wines. J. Agric. Food Chem. 1997, 45, 2211.
[7]. R. Gawel, P. G. Iland, I. L. Francis. Characterizing the astringency of red wine: a case study. Food Qual. Prefer. 2001, 12, 83.
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[9] Kennedy JA, Ferrier J, Harbertson JF, des Gachons CP (2006) Analysis of Tannins in Red Wine Using Multiple Methods: Correlation with Perceived Astringency. Am J Enol Vitic 57:481-485
[10]. Kennedy JA, Jones GP (2001) Analysis of Proanthocyanidin Cleavage Products Following Acid-Catalysis in the Presence of Excess Phloroglucinol. Journal of Agricultural and Food Chemistry 49:1740-1746
[11].Mercurio M, D., Smith P, A. (2008) Tannin Quantification in Red Grapes and Wine: Comparison of Polysaccharide- and Protein-Based Tannin Precipitation Techniques and Their Ability to Model Wine Astringency. J Agric Food Chem 56:5528-5537

B (I). Funded Collaborators (Co-PI, AAFC, other federal scientists)

- Include the name of scientist / organization.

B (II). Acknowledgement of non-funded collaborators (who provide support, e.g. access to other laboratory or other facilities and equipment input / advice / guidance / assistance, etc).

- List the participants who support your project but are not receiving any funding through the program.
- Include name of scientist / organization.

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C. Variance Report (if applicable, describe how the work differs from the proposed research)

- Include changes to objectives and project work plan / budget, changes to the team, other constraints.

Besides the analysis of tannin and their sensory properties, some important new molecules were discovered that may change how grape phenolic ripening is measured. To our knowledge, it is the first time that new wine phenolic molecules are discovered in Canada and we are grateful to AAFC to make that discovery possible.

D. Impact Assessment (if applicable, describe how the variance factors above will impact project continuation)

- Include changes to the objectives, changes to the project work plan / budget, changes to performance (i.e. meeting targets).

The main targets have been reached but further data collection and more sensory validation is needed to allow technology transfer,

E. Achievements (include only those related to this project)

- Include innovations, publications / conferences, technology transfer, capacity building, success stories, media, recognition and other outputs.

Publications:

1. Delcambre, A., Saucier, C. Identification of new flavan-3-ol monoglycosides by UHPLC-ESI-Q-TOF in grapes and wine. *J. Mass Spectrom.*, 2012, 47 , 6 , 727-736.
2. Cliff, M.A., Stanich, K., Edwards, J.E, Saucier, C.T. Adding grape seed extract to wine affects astringency and other attributes. *J. Food Quality*, 2012, 35, 4, 263–271.

Conferences:

- Invited presentations (by Cédric Saucier):

1. University of California, Davis. October 10th 2012. Mass spectrometry of grape and wine tannins:
Challenges and discoveries.
2. 13th Annual Enology & Viticulture Enology. Penticton, BC, July 16-17, 2012. New

polyphenols in grape and wine as potential seed markers in wine.
3. University of Adelaide. February 16th 2012. High resolution mass spectrometry of tannins in grape and wine.
4. 12th Annual Enology & Viticulture Enology. Penticton, BC, July 18-19, 2011. Analytical and Sensory Evaluation of Red Wine Tannins.
5. University of Adelaide. February 10th 2011. Wine and grapes polyphenols :Chemical transformations and taste

- Oral Presentations at international congress (Bordeaux, California).

Training of HQP : 1 Post-doc (Adéline Delcambre) and one PhD (50% on the project, will finish in 2015)

F. Lessons learned (self-evaluation of project)

Important success was achieved. We need to work further on the validation and industry transfer. Discussion with the Industry suggests to also include white phenolic aspects which we have proposed in a follow up AIP project currently under review.

C. SAUCIER	April 13th 2013	
PI Name	**Date**	**Signature**