Participation at the international conference ``MACROWINE 2016, Macromolecules and Secondary Metabolites of Grapevine and Wine''

Final Report to Australian Grape and Wine Authority

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Research Organisation: National Wine and Grape Industry Centre, Charles Sturt University

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1. Abstract

The principal activity of the travel was to attend the international congress Macrowine 2016. The opportunity to have an oral presentation gave us the chance to present at the scientific community part of our results related with the project NWG 1401, funded by AGWA. The exposure of the data opened a discussion with experts of the field of wine macromolecules. The discussion focussed mainly on the option for further experimentation. Moreover, the attendance to the Macrowine 2016 gave the opportunity to get in contact with potential collaborators all over the world. Several presented interest on the work that was presented at the congress and for future collaborations. Finally, the participation at the Macrowine 2016 was a good opportunity to keep up to date with the lastest discoveries in the field of wine macromolecules.

2. Executive Summary

Part of the AWGA funded (NWG 1401) research work was presented at the international congress Macrowine 2016 at the session ‘Macromolecules and metabolites’. From the general discussion after the oral presentation, it seems that the research was well received and of interest to the audience. Consequently, discussion was made on possible strategies to continue/complete further work with international collaborations with various research groups from overseas. Finally, the participation at the congress allows us to be updated with the latest findings in our field of research and related fields. The number and quality of oral and poster presentations was a good opportunity to keep up to date with research in the wine related field.
3. Details of the Travel


Macrowine is a prestigious international conference focused on the wine macromolecules. The 6th International Conference of Macromolecules and Secondary Metabolites of Grapevine and Wine (Macrowine 2016) was hosted in the French speaking part of Switzerland. It took place in Changins (Nyon, Vaud, Switzerland) from June 27 to June 30, 2016. The conference was organised jointly by CHANGINS (Viticulture and Enology) and the HES-SO University of Applied Sciences and Arts, Western Switzerland.

The congress was focused to understand the structure, evolution, role and physico-chemical interactions of vine and wine metabolites and macromolecules. There were four main topics:

- **Life in the vineyard**: influence of vineyard biological activity on the composition of grapes and wine. The aim of this session is to present relevant recent research results on the direct or indirect impacts of vineyard organisms on the vine and the resulting wine.

- **Macromolecules and metabolites**: analysis, chemistry and functionality in wine. The aim of this session is to present ongoing research on the physical, chemical and functional aspects to improve the understanding and knowledge of the winemaker and to provide him with more tools so as to make the right decisions.

- **Process**: influence of processing, technology and biotechnology on must and wine composition and sensory characteristics. The aim of this session is to present research results of the different enological processes transforming grapes into wine. They can be linked to biotechnology (e.g. yeasts, bacteria, enzymatic preparations), winery technology (e.g. treatments, filtration, membrane, stabilisation, gas management, impact of temperature) or analytical tools (e.g. automation, sensors, online measurements). Enological benefits, as well as the impact on wine chemistry and/or sensory characteristics of wine should be focused on.

- **Modulators of aroma and taste**: impact of macromolecules on sensory perception. The goal of this session is to present latest research in neurosciences, sensory analysis and analytical chemistry related to these aspects and allowing to explain changes in
sensory perception based on molecular interactions. Interactions in the wine matrix itself, as well as between wine and food during their consumption are of interest.

During the congress 38 oral and 157 poster presentations took place. 614 research scientists from the academic world, the industry and governmental organisations, coming from more than 40 different countries were co-authors, projecting the high scientific interest and level of the congress.

3.2. Attendance and oral presentation

The well organised and the high quality of the oral presentation made it quite easy to follow the congress from the beginning until the end. Below is a summary of several some of the oral presentations that have are of particular interest to my field of research.

The first session was the ‘Process’. The keynote speaker was Paul Kilmartin (University of Auckland) and his presentation was entitled ‘Controlling the effects of oxidation with machine harvested Sauvignon blanc grapes’. His work has a special interest for the group that I am part, under the supervision of Dr. Andrew Clark, first because machine harvesting is a common practice in Australia and secondly because oxidation processes are one of the main topics that we are working on. His research team demonstrated that for the Sauvignon blanc variety, the varietal thiols 3-mercaptohexanol and 3-mercaptohexyl acetate increase their concentration levels with the grape maceration provided by machine harvesting or grape freezing. Moreover, they found that the addition of antioxidants at harvest can be used effectively to lessen the effects of oxidation and maximise the formation of the varietal thiols, to suit the requirements of the target wine style. The use of elemental sulphur (S0) as a fungicide shows a direct correlation between S0 in juice and the varietal thiols in wine, however, they recommend controlling the use of the fungicide close to the harvest period in order to avoid the increase of the unpleasant reductive sulfur compounds in the final wine.

Almudena Marrufo-Curtido, student of Vicente Ferreira (Universidad de Zaragosa) presented an interesting work. The work was the ‘Development and validation of a standardized oxidation assay for the accurate measurement of the ability of different wines to form “de novo” oxidation related aldehydes’. The achievement of “de novo” formation of aldehydes permits us to have a new perspective for the formation of the aldehydes through direct oxidation and their influence on wine organoleptic characteristics. This method can be
applied in our laboratory (NWGIC/CSU) and for this reason the presentation captured our attention.

The keynote speaker Vicente Ferreira, at the session ‘Macromolecules and metabolites’, made a brief presentation related with the origin and geneses of reduction and oxidation aromas. In a graphical way, he presented that oxidative and reductive aromas that often appear after bottling are due to pre-accumulated (before bottling) off-odors concealed under the form of non-volatile and odorless complexes. The aldehydes (accumulated mainly under the form of SO2 adducts) and the mercaptans (accumulated mainly under the form of Cu(II) complexes) can slowly release after the bottling and release oxidative and reductive off-odors respectively. This issue is of great interest for the Australian wine industry as the presence of reductive off-odors is something that concerns the wine producers. Also this is a topic that we focus in our group.

Gal Kreitman (Penn State University) present ‘Reaction mechanisms of copper and iron with hydrogen sulphide and thiols in model wines’. He show that Cu(II) was rapidly reduced by H2S and thiols (cysteine, 3-sulfanylhexan-1-ol and 6-sulfanylhexan-1-ol) to Cu(I). The addition of Fe(III) in combination with Cu resulted in the rapid reduction of Fe(III) by Cu (I) and the resulting Fe(II) redox cycled by reacting with oxygen. In our group we have found that the H2S can be present in the wine after the fining process. The present work complements our own work, that the presence of Fe and thiols prevented the removal of H2S by precipitation as insoluble CuS by promoting H2S oxidation and formation of polysulfides and consequently keeping both metals and sulphide species in wine after the copper fining process.

The quality of the poster presentations was quite good and covered a wide range of topics. Reporting them all in this report is not possible due to the large number of posters. Also the most significant findings to our research project (NWG1401) were the oral presentations outlined above.

On behalf of my research team, I presented part of our research project ‘Metal ion speciation: Understanding its role in wine development and generating a tool to minimise wine spoilage’ (NWG 1401; funded by Wine Australia). The oral presentation was entitled ‘Fractionation of copper and iron in wine: Assessment of potential macromolecule and sulfur binding agents’. This work was signed by Andrew Clark, Geoffrey Scollary and by me from CSU/NWGIC
and from Eric Wilkes, Paul Smith and Mark Smith from AWRI. The work that we presented is the following:

‘Copper and iron are known to substantially impact wine stability through oxidative, reductive or colloidal phenomena. However, the binding of metal ions to different wine components under wine conditions, and the impact of this binding on the ability of the metal ions to induce spoilage processes, is not well understood. This study surveyed a range of 50 red and white wines for an understanding of the variability of broad metal categories within the wines. Two techniques were used. The first is an electrochemical constant current stripping potentiometry technique (ccSP). A thin mercury film on a screen printed carbon electrode was utilised in a flow system for the direct analysis of labile copper in red and white wine by constant current stripping potentiometry with medium exchange. The second technique was based at the solid phase extraction (SPE) fractionation of wine with subsequent analysis of the metal content of each fraction by inductively coupled plasma–optical emission spectroscopy (ICP-OES). With the current technique the metals were fractionate into hydrophobic, cationic and residual forms. The hydrophobic form include the no-labile form of the metals and mainly the forms where the metals are strongly bind with other wine components. Tartaric acid metal complexes were sufficiently labile to be classified as cationic. The cationic fraction may also include metal protein complexes with sufficient positive charge to allow them to pass through the hydrophobic column and be exchanged on the cationic cartridge. The residual fraction, which consists of metal ions passing through both cartridges, would most probably be negatively-charged or neutral complexes of the metal ions that were not sufficiently hydrophobic to be retained by the hydrophobic column. Acidic polysaccharides, phenolic acids or other organic acids (eg: citric acid) are potential binding agents that may contribute to the residual fraction of the metal.

The use of the electrochemistry technique (Figure 1) shows that the predominant form of the copper is the non-labile, indicating that copper found in wine mainly bind with other wine components. The SPE fractionation (Figure 2) showed that the predominant copper form was the hydrophobic, confirming the results of the electrochemical technique, which the copper is mainly found in bind form in the wine. On the other hand, iron is mainly in cationic form for the white wines and in residual form for the red wines.
Figure 1. The analysis of labile copper in 50 white and red wines by flow medium exchange constant current stripping potentiometry. The error bars are the st.dev. (n=3).

Figure 2. The fraction of hydrophobic, residual and cationic forms of Cu by SPE and analysis by ICP-OES in 50 white and red wines. The error bars are the st.dev. (n=3).
Figure 3. The fraction of hydrophobic, residual and cationic forms of Fe by SPE and analysis by ICP-OES in 50 white and red wines. The error bars are the st.dev. (n=3).

In light of these results, the binding efficiency of specific classes of wine macromolecules and wine sulfur compounds for copper(II) and iron(II) was assessed, and related to the metal categories found in the surveyed wines. The wine macromolecules examined included isolated white wine protein, white wine polysaccharide, red wine polyphenols (including procyanidins and monomeric phenolic compounds), and white wine polyphenols. The binding was assessed by mixing the wine components with copper (II) (0.4 mg/l) and iron (II) (3 mg/l) in a model wine system (pH 3.2) in low oxygen wine conditions.

The results showed that in the wines surveyed the metal ions had significant variability in fractionation, with high proportions of bound copper (i.e., non-labile or hydrophobic) and iron distributed amongst a variety of forms (i.e., cationic, residual and hydrophobic). In the case of the wine macromolecules (Figure 4, 5 and 6) we could not find binding ability with metals except at the case of red wine polyphenol. It was found that the red wine polyphenol wine fraction demonstrated evidence of interaction with both copper and iron. Part of Cu was found as non-labile with the electrochemical technique and in hydrophobic form for both Cu and Fe by the SPE fractionation and analysis by ICP-OES.
Figure 4. The analysis of labile copper in model wine solutions at pH 3.2 and 400 μg/l Cu by flow medium exchange constant current stripping potentiometry. The error bars are the st.dev. (n=3).

Figure 5. The fraction of hydrophobic, residual and cationic forms of Cu by SPE and analysis by ICP-OES in model wine solutions at pH 3.2 and 400 μg/l Cu. The error bars are the st.dev. (n=3).
Figure 6. The fraction of hydrophobic, residual and cationic forms of Fe by SPE and analysis by ICP-OES in model wine solutions at pH 3.2 and 3 mg/l Fe. The error bars are the st.dev. (n=3).

However, the red wine polyphenol was contaminated during the extraction phase and complicated interpretation of the results. For that reason another trial was designed. The impact of pH on the binding of the metal ions was also established. The red wine polyphenolic compounds assessed at pH 4.0 included epicatechin, quercetin-3-glucoside, caffeic acid, gallic acid, ellagic acid, red wine tannin and red wine polyphenols (crude extract). Of these phenolic compounds only the red wine polyphenol (crude extract) exhibited binding capacity to Cu according to both the electrochemical and ICP-OES techniques. Alternatively, most of the phenolic compounds induced Fe to adopt the residual form over the cationic form. The red wine polyphenol also induced both residual and hydrophobic forms of Fe (Table 7, 8 and 9).
**Figure 7.** The analysis of labile copper in model wine solutions with red wine polyphenols and individual polyphenols at pH 4.0 and 400 μg/l Cu by flow medium exchange constant current stripping potentiometry. The error bars are the st.dev. (n=3).

**Figure 8.** The fraction of hydrophobic, residual and cationic forms of Cu by SPE and analysis by ICP-OES in model wine solutions with red wine polyphenols and individual polyphenols at pH 4.0 and 400 μg/l Cu. The error bars are the st.dev. (n=3).
Figure 9. The fraction of hydrophobic, residual and cationic forms of Fe by SPE and analysis by ICP-OES in model wine solutions with red wine polyphenols and individual polyphenols at pH 4.0 and 3 mg/l Fe. The error bars are the st.dev. (n=3).

A range of potential sulfur-binding agents (hydrogen sulfide, methanethiol, ethanethiol, glutathione, cysteine, 2-furanyl methanethiol, dimethylsulfide, carbon disulphide and 4-mercapto-4-methyl-pentan-2-one) were examined for the binding ability with metal ions. The study was assessed at pH 4.0 and 400 μg/l of Cu or 3 mg/l of Fe. Of the sulfur compounds examined, the only one which demonstrated sufficient binding to Cu was hydrogen sulfide. This was measured as electrochemically non-labile Cu and as 'hydrophobic' Cu by the SPE ICP-OES method. The other sulfur compounds examined but showed no binding to Cu or Fe, by the techniques adopted. Hydrogen sulfide showed no binding to Fe as assessed by the SPE and ICP-OES technique (Table 10, 11 and 12).
Figure 10. The analysis of labile copper in model wine solutions with different sulfur compounds at pH 4.0 and 400 μg/l Cu by flow medium exchange constant current stripping potentiometry. The error bars are the st.dev. (n=3).

Figure 11. The fraction of hydrophobic, residual and cationic forms of Cu by SPE and analysis by ICP-OES in model wine solutions with different sulfur compounds at pH 4.0 and 400 μg/l Cu. The error bars are the st.dev. (n=3).
Figure 12. The fraction of hydrophobic, residual and cationic forms of Fe by SPE and analysis by ICP-OES in model wine solutions with different sulfur compounds at pH 4.0 and 3 mg/l Fe. The error bars are the st.dev. (n=3).

The results demonstrate an important insight into the predominant forms of iron and copper ions in wine, and also insight into the main binders, especially from the perspective of wine macromolecules.

After the presentation some observations and questions were made by the colleagues. They suggested to fractioned the polysaccharide and work mainly with red wine polysaccharide instead of white wine. Some of them have observed that oligomeric polysaccharides bind with copper(II). We keen to confirm the binding ability of the different polysaccharide fractions with the use of our techniques (ccSP and SPE + ICP-OES). One other interesting question was related with the competition of the sulfur compounds between each other to bind with the metals. This question has sense mainly for H2S in combination with the other sulfur compounds. It would be interesting to observe if the other sulfur compounds can compete with H2S and decrease its ability to bind with copper.
4. Opportunities for international collaboration

The travel to the conference gave the opportunity strengthening relations with experts in the field of macromolecules and possible would permit a more comprehensive study of these types of interactions and their influence on wine quality. The group of Dr. Ferando Zamora (Rovira i Virgili University, Spain) has a vast experience in winemaking processes, in red wine polyphenols, polysaccharide and also white wine proteins. They are interested in collaborations. It can begin with exchange of PhD students that can help the visibility of CSU and the NWGIC outside from Australia. Also they are interesting in wine making techniques and how this can affect the accumulation of sulfur off-odor compounds in wine.

Dr. Vicente Ferreira (University of Zaragosa, Spain) also presented interest to collaborate with our group. His scientific activity has focused on the understanding of the chemical basis of flavor perception and mainly on the development of analytical methods for the screening and quantitative determination of many odorants. We are interested in the analytical methods utilized by Ferreiras’ group in order to analyze the sulfur and aldehydes present in wine and their impact on off-odors production. This collaboration can allow further insights into wine chemistry that at the moment we cannot achieve alone and allowed a deeper understanding of oxidative and reductive processes during wine bottling and aging.

Dr. Thierry Doco (INRA Montpellier, France), an expert in wine polysaccharides, indicated interest about the ability of wine macromolecules to bind with metal ions. He is interesting to fractionated wine polysaccharide and study their ability to bind with the metal ions. Finally, Dr. Cedric Saucier (University of Montpellier, France) was present and his research is focussed on the physical and chemical evolution (oxidation and colloidal stability) during ageing, and also the interactions of phenolic compound with other taste or aroma active substances. We discussed the possibility of working together in order to understand better the mechanisms of oxidation and reduction in the wine and their impact on wine quality.

All the above potential collaborations can help our current research and other projects to liaise with important research groups, located mainly in Europe. Moreover, one of the main topics of our research group at CSU/NWGIC is the understanding the oxidative/reductive processes that occurs in the win as indicated by metals. All these collaborations can help us enrich our knowledge and aid the Australian wine industry in producing higher quality wines.
5. Conclusions

In general terms, the outputs of the project AGT 1519 funded by Australian Wine and Grape Authority were covered successfully. The principal object that was the participation and the oral presentation of our work at the international conference Macrowine 2016 was completed without problems. The presentation caught the attention of the participants. The visibility of our center (CSU/NWGIC) and Australian wine research remains high profile and this will contribute to collaborations that will aid Australian wine industry research outputs. It was also important to attend the congress and become updated on the latest outputs in the wine research.

6. Acknowledgements

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